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Evaluation of same day diagnosis of TB microscopy in comparison to the spot-morning-spot method and knowledge, attitude and practice of health personnel towards the use of the same day diagnosis of TB in selected health institution in Addis Ababa, Ethiopia.

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Acronyms

AFB: Acid Fast Bacilli

BFM: Bright filed microscopy

DOTS: Directly Observed Treatment, Short-Course

FM: Fluorescent Microscopy

HIV: Human Immunodeficiency Virus

LED: Light Emitting Diode

LED-FM: Light Emitting Diode Fluorescence Microscopy

LM : Light Microscopy

LMIC : Low- And Middle Income Countries

LJ : Lowenstein - Jensen

MVP: Mercury Vapor

MGIT: Mycobacteria Growth Incubator Tube

MM : Morning- morning

M-TB: Mycobacterium Tuberculosis

PHC: Primary Health Care

PTB: Pulmonary Tuberculosis

SOP: Standard Operation Procedure

SPSS: Statistical Package for Social Science

TB: Tuberculosis

WHO: World Health Organization

X-spot: Spot sample after one hour of the first sample

ZN : Ziehl Neelsen

Operational Definitions

Tuberculosis: A bacterial infection caused by *Mycobacterium tuberculosis*. The disease usually affects the lungs (pulmonary) but can spread to other parts of the body in serious cases (extra-pulmonary).

Smear Positive Case: The presence of TB bacteria in a patient's sputum (sample of mucus or phlegm from a patient's respiratory tract) when examined under the microscope.

Smear Negative Case: Absence of TB bacteria in a patient's sputum (sample of mucus or phlegm from a patient's respiratory tract) when examined under the microscope.

Directly Observed Treatment Short Course: Watching the patient take his/her medication to ensure medications are taken in the right combination and for the correct duration.

Patient Delay: The length of delay between the onset of symptoms and patients first visit to health care.

Health Service Delay: The length of delay between health care visit and the diagnosis of tuberculosis.

Conventional: The usual practices of accepted standards of taste

The Same-day-diagnosis: Microscopy of two consecutive sputum specimens done on the same day.

Diagnosis defaulters: A patient who has been started giving at least four consecutive sputum for diagnosis and who has been interrupt giving sputum for diagnosis.

Precision: The quality of being reproducible in results or performance

ABSTRACT

Back ground: the need to collect serial sputum specimens over multiple patient visits results in a protected diagnostic process with rates of patients with high rates of patient dropout. Recent studies on SMS method of examination PTB reported that the first two specimens have high smear positivity in line with this WHO changed its policy to minimize the number of sputum specimens from three to two.

Objective: The main objective of this study was to evaluate the same day diagnosis of TB microscopy in comparison to the spot-morning-spot methods and KAT of lab personnel, GP, HO and Nurse to wards the use of the same day diagnosis of TB in selected health institutions in Addis Ababa.

Material and method: Across sectional study were conducted in 16 conveniently selected private clinics, governmental health centers, public hospitals and private hospitals, federal police and armed force hospital from September – December 2012.All patients who were avail themselves in the selected health institutions for the diagnosis of MTB and those health personal who were worked in each health facility. The diagnosis was performed using ZN sputum smear microscopy and light-emitting diodes fluorescent microscopy (LED-FM) technique. A structure and pre-validate questionnaires was used to collect data for KAP survey. Chi-square was used to assess the associations' different variables of techniques and health personals KAP study.

Result: A total of 209 participant enrolled, 43(21%) were identified culture positive,39 (18.7%) were detected by the same day approach and 40(19.1%) by the standard approach ($p>0.05$).on the other hand LED-FM and ZN microscopy were identified 39(18.1%) and 48(23%) respectively. Sensitivity was (88.4%) for ZN microscopy and (95.3%) for LED-FM, it was (99.4%) and (95.9%) for specificity.

More over the mean and median knowledge, practice and attitude score of laboratory personal about the same day diagnosis was 4.07, 5.96, 7.67 and 5, 5, 8 respectively. Majority (61%),(73%) and (97%) of Laboratory personal had good knowledge, practice and positive attitude while the mean and median knowledge score was 4.7 and 4 respectively, but the mean and median attitude and practice was 6.65, 6.4 and 8.7 respectively .majority (57.7%),(63.4%) and (72.4%) of other health personal had poor knowledge, positive attitude and good practice respectively.

using same day approach together with LED-FM would increase the smear detection rate, reduce work load, TAT, patient and health facility cost and transmission of TB. Hence, it is essential to address the advantages and dis advantages of the conventional approaches, LED-FM and same day diagnosis of TB to raise KAPs` of health personnel.

Therefore it is necessary to give in-service and off service training for health personnel towards the use of LED-FM, conventional approach and the same day approach in the diagnosis of TB

Chapter one

1. INTRODUCTION

1.1. Background

Tuberculosis is a curable and preventable disease .It is an infectious bacterial disease caused by *Mycobacterium tubercle*, an Acid Fast Bacilli as Koch stated and it is spread by aerosolization of droplet nuclei bearing *Mycobacterium tuberculosis* particles released from the lung of patient with cavitary pulmonary or laryngeal disease . According to WHO TB is the second most common cause of deaths due to infectious disease, and current trends suggest that TB will still be among the 10 leading causes of global disease burden in the year 2020(1,2).

Mycobacterium tuberculosis causes tuberculosis and is a very important pathogen of humans. Tuberculosis kills more people than ever, with the increasing number of HIV-infected individuals . The fact sheet of global tuberculosis is stunning with one third of the world's population currently infected and new infections occur every second, resulting in new tuberculosis infection in 1% of the world population annually. It is projected that newly acquired infections between 2002 and2010 would be 1 billion persons, of these, 150 million will get sick and 36million will die of tuberculosis. In Sub-Sahara African countries, the incidence of tuberculosis has doubled since the early 1980s (2,).

In developing countries, diagnosis of pulmonary tuberculosis depends primarily on the identification of acid-fast bacilli (AFB) using Ziehl-Neelsen sputum smear microscopy, a technique more than 100 years old. The sensitivity of this method varies and depends upon collection of sufficient sputum, proper preparation of smears, good staining technique, careful examination of smears, and availability of a good microscope. Several methods have been tried to improve smear microscopy for AFB (3).

TB case detection must be improved through the optimal use of existing diagnostic tools. The optimization of sputum microscopy services, often the only TB diagnostic services possible at primary health care (PHC) level in low- and middle income countries (LMICs), is urgently

needed. Smear microscopy has several limitations, including poor sensitivity, being labour intensive, and requiring skilled microscopists. Furthermore, the need to collect serial sputum specimens over multiple patient visits results in a protracted diagnostic process with high rates of patient dropout. Recent studies examining the yield of serial sputum specimens, usually collected as spot-morning-spot, have reported that the majority of patients with smear-positive PTB are identified by the first two sputum specimens and the World Health Organization (WHO) has recently changed its policy in this respect, reducing the minimum number of sputum specimens examined for each patient from three to two. This will result in reduced laboratory workloads in many settings, with the potential of improving the quality of sputum microscopy. Case detection may thus be expected to increase in locations where the number of new cases detected through improved microscopy quality exceeds the 2% to 5% of cases missed by not examining the third specimen (4).

Direct sputum smear microscopy is the most widely used test for the diagnosis of pulmonary tuberculosis (TB), available in most primary health care laboratories at health center level. The majority of laboratories use conventional light microscopy to examine Ziehl-Neelsen stained direct smears, documented to be highly specific in areas with a high prevalence of TB, but with varying sensitivity (20-80%).Fluorescence microscopy (FM) has been documented to have higher sensitivity (10%)than conventional ZN microscopy, and examination of fluorochrome-stained smears takes less time. Uptake of FM microscopy has, however, been hampered by high cost due to expensive mercury vapour light sources, the need for regular microscope maintenance, and the requirement for a dark room (5).

Low-cost ultra-bright light-emitting diodes (LEDs) with a long lifespan could replace expensive mercury vapour lamps and enable the development of microscopy systems that are substantially less expensive than conventional FM, offering the possibility for widespread use of LED-based FM in resource-limited settings. In view of these potential advantages, several companies have developed inexpensive, robust LED microscopes or LED attachments for routine use in high-burden countries. Preliminary data suggested that LED microscopy is feasible and as accurate as standard FM and field evaluation studies had been completed in several countries (6).

Compared to conventional mercury vapour fluorescent microscopes, LED microscopes are less expensive, require less power and are able to run on batteries, the bulbs have a very long half-life and do not pose the risk of releasing potentially toxic products if broken. LED microscopes are reported to perform equally well without a dark room. These qualities make LED microscopy feasible for use in resource-limited settings, having the potential to bring the benefits of fluorescent microscopy (improved sensitivity and efficiency) where needed most (5)**.**

The gold standard for TB diagnosis is the cultivation of *M. tuberculosis*. It can be performed on a variety of specimens, such as sputum and bronchial washings, and also other non-pulmonary samples. It is much more sensitive than microscopy and it allows the recovery of the bacteria for other studies, such as drug susceptibility testing and genotyping. Despite routine use throughout the developed world, TB culture remains unavailable in most high-burden countries, largely due to expense and infrastructure requirements. However, a number of high-burden countries have now developed laboratory capacity to perform TB culture and automated systems using liquid media are now available that may reduce the corresponding human resource requirement (8).

Conventional culture that uses a growth medium made from egg or agar is five to ten times more costly per sample than smear microscopy. Modern liquid media and accurate growth detection systems improve the sensitivity and greatly shorten the time needed for growth to be seen. The mycobacteria growth incubator tube (MGIT) is one of the most studied new culture methods. The mean time for detection of growth of mycobacteria in MGIT was short and ranged from 8 days to 16 days, including in HIV-infected tuberculosis patients, as compared with 20 days to 26 days in conventional culture (Lowenstein-Jensen) media (10).

Conventional case-finding approaches usually involve microscopy examination of 'spot-morning' sputum specimens (in countries with a two-specimen system) or examination of ‘spot-morning-spot' sputa (in those with a three-specimen approach). The majority of sputum results are therefore only available on the second or third day that the patient presents to the health service. Recent research has investigated the diagnostic accuracy of conventional case-finding strategies compared to an approach where two consecutive sputum specimens ('spot-spot')are

examined on the same day (so-called 'front-loaded' or 'same-day-diagnosis'), and also assessed whether patient drop-out from the diagnostic pathway can be reduced as a result(11). Hence we planned to implemant the same –day diagnosis inplace of spot-morning-spot methods in order to improve TB case detection through the optimal use of existing diagnostic tools inline with WHO recommandation in Ethiopia.

1.2. Statement of the problem

The global distribution of TB cases is skewed heavily toward low income and emerging economies. Africa, and more specifically Sub-Saharan Africa, faces the worst TB epidemic since the advent of the antibiotic era. These occur predominantly - approximately 6 million to 8 million- in the economically most productive 15 to 49-year-old age group (2).various factors such as delay in seeking treatment, ignorance towards the modes of spread of the disease and treatment default could contribute to the currently high caseload of TB and its mortality and morbidity (11).

1.2.1. Tuberculosis in Ethiopia

Ethiopia ranks seventh among the world's 22 high-burden countries with TB. According to WHO's Global TB Report 2010, the country had an estimated 44,398 TB cases in 2009, with an estimated incidence and prevalence rate of 300 and 470 cases per 100,000 populations respectively. Similarly case detection was 50% for all forms of TB. Among all new TB cases 30% were smear positive, 35% smear negative, 34% extra pulmonary and ,<1% smear unknown cases. In addition among re-treatment cases 2,259 (64%) were relapse cases, treatment after failure 381(11%), treatment after default 478(13%) and 56,040 had both TB and HIV (12).

Ethiopia's National TB and Leprosy Control Program began to implement DOTS strategy for TB control in 1991. While treatment is integrated into general health services and DOTS geographical coverage is 95 percent, due to the limited health infrastructure in the country, only approximately 60 to 70 percent of the population has access to DOTS services. The DOTS detection rate remains low, at 34 percent, compared with WHO's target of 70 percent detection. The limited diagnostic capacity for TB in the country remains a challenge to improving case detection rate (14).

Sputum smear microscopy is the principal method of diagnosing pulmonary tuberculosis (PTB) in resource poor settings like Ethiopia, however the sensitivity of microscopy is influenced by numerous factors, such as the prevalence and severity of the disease, the quality of specimen collection, the number of mycobacterium present in the specimen, the method of processing

(direct or concentrated), the staining technique, and the quality of the examination (microscope operator expertise, and time spent for smear examination (15).

Direct sputum smear microscopy is the most widely used test for the diagnosis of pulmonary tuberculosis (TB), available in most primary health care laboratories at health center level. The majority of laboratories use conventional light microscopy to examine Ziehl-Neelsen stained direct smears, documented to be highly specific in areas with a high prevalence of TB but with varying sensitivity (20-80%). Besides being labour-intensive, direct sputum smear microscopy may have considerable patient costs and inconvenience associated with the need to submit multiple sputum specimens over a period of up to three days. A number of TB control programmes have reported high rates of initial patient default (16).

It has been shown conclusively that good-quality microscopy of two consecutive sputum specimens identifies the vast majority (95–98%) of smear-positive TB patients and the diagnostic accuracy of conventional case-finding strategies in comparison with an approach in which two consecutive sputum specimens ('spot-spot') are examined on the same day (so-called 'front-loaded' or 'same-day') and whether patient drop-out from the diagnostic pathway can be reduced as a result (17).This study was, therefore, designed to determine the importance of the same-day-smear microscopy in the diagnosis of TB in Ethiopia in place of conventional smear microscopy.

1.3. **Literature review**

In 2009, the strength of the evidence base for a "same-day-diagnosis" approach (microscopy of two consecutive sputum specimens done on the same day) was assessed by the World Health Organization following standards appropriate for evaluating both the accuracy and patient/public health impact of new interventions. The evidence revealed that there was sufficient generalizable evidence that a same-day-diagnosis approach is equivalent, in terms of diagnostic accuracy, to conventional case-finding strategies by microscopy. However, significant organizational and programmatic changes would be required to optimize the advantages of a same-day diagnosis, which includes ensuring that laboratory results are received at the health facility and patients start treatment on the same day. WHO recommends that countries that have successfully implemented current WHO policy for a two-specimen case-finding strategy consider a switch to the same-day-diagnosis approach, especially in settings where patients are likely to default from the diagnostic process. Countries that are still using the three specimen case-finding strategy should consider a gradual change to the same-day diagnosis approach, once WHO-recommended external microscopy quality assurance systems are in place and good quality microscopy results have been documented (18).

A study which was conducted in India showed that among 513 patients, 40 defaulted on the second day. Of the total number of patients recruited, 124 (24.2%) were smear-positive. Using the 2-day protocol, 121 patients (97.6%) were identified as smear positive, whereas with the 1-day protocol 118 patients (95.2%) were identified as smear-positive (P = 0.3). Of the patients who defaulted, seven (17.5%) were smear positive. Comparison of the variation in results indicated that collection of a morning sample on the second day provided no significant benefit over collection of a second spot sample on the first day (19).

A four cross-sectional surveys were conducted in Yemen, Nepal, Nigeria, and Ethiopia. Sputum specimens were collected as spot-morning-spot plus one additional specimen one hour after the first spot (X-spot). The yield of two (spot-Xspot or spot-morning) or three (spot-morning-spot or spot-X-spot-morning) specimens was compared. 216 patients had ≥ one positive smear. Of these, 210 (97%) were identified by the spot-morning-spot, and 210 (97%)

were identified by the spot-Xspot-morning specimens. Spot-morning identified 203 and spot-Xspot specimens 200 patients, respectively, (P >.1). The time, number of visits and patients' costs to complete smear microscopy could be reduced by frontloading the collection of sputum specimens (20).

A retrospective study conducted in Rwanda showed that among 364 suspects fulfilling the inclusion criteria were studied over the 2-years period for AFB; made up of 207 (56.9%) male and 157 (43.1%) females. The age ranges was 5 - 80 years with a mean of 32.3 years . The overall prevalence of sputum smear positive cases were 17.3% (63 0f 364) and most of the positive patients were within the age range 15 – 44 years. The highest percentage of TB was seen in the age group of 15 - 24 years compared with the lowest percentages in the age group below 14 years and above 45 years.The prevalence rates of smear-positive pulmonary TB for 2007 and 2008 were 17.9% and 16.7% respectively. A total of 63 (17.3%) suspects were found to have at least one positive smear and 61 (17%) fulfilled the case definition (at least two positive smears). Of these, 56 (88.9% of those with one or more positive smears and 92% of those who fulfilled the case definition) were detected from the first specimen and 7 (11.1%) were positive on the second specimen but not the first. The third specimen did not have any additional diagnostic value for the detection of AFB as shown in (21).
A study which was conducted in Cameroon showed that from a total of 799 suspects were screened using the MM strategy, identifying 223 smear-positives, and 808 suspects with the SMS strategy, yielding 236 smear-positives. Of the MM, 256 were culture-positive, of whom 195 (76%) were smear-positive. For SMS, these figures were respectively 281 and 206 (73%), a non-significant difference. The MM and SMS strategies also detected respectively 28 and 30 smear-positive cases not confirmed by culture. No cases were lost to treatment with either strategy (22).

Astudy conducted in Hawassa showed that out of of 4099 (26%) of the suspects had at least one positive smear with 3753 (91.6%) of the first specimens being positive. A further 303 (7.4%) were negative in the first specimen but had a positive second specimen and 42 (1%) suspects had two negative specimens followed by a positive third smear. The value of the third sputum is negligible as 99% of the cases were identified from the first and second specimens.

Reducing the number of specimens to two or even one would have multiple advantages in countries where laboratories are usually over-burdened and are not easily accessible to the population. Submission of two specimens on the same day could improve compliance in submitting samples and collecting results as the number of diagnostic visits would be reduced without significant loss of sensitivity (23).

Also a study conducted in Bushullo Major Health Centre (BMHC) in the Southern Region of Ethiopia out of 243 suspects were enrolled; 52 had confirmed PTB: 49 (94%) were detected by the same-day method and 51 (98%) by the standard method($P > 0.05$). The same-day approach would reduce the number of visits required for diagnosis, save resources for the health system and the patient, and ultimately improve case detection in poorer countries (24).

In a systematic review Karen Steingart and co-workers reported that fluorescence microscopy of auramine-stained smears gave a similar specificity and on average a 10% higher sensitivity than Ziehl-Neelsen staining in 18 studies analysed, with a higher sensitivity in 16 of the 18 studies. Additionally, microscopic examination of auramine O stained smears requires less time than smears stained with the Ziehl-Neelsen method. Another potential advantage of fluorescence microscopy is the requirement of only one or two sputum specimens rather than three to reach an acceptable level of performance, thereby shortening and improving the diagnostic process (25).

One study compared front-loaded and conventional strategies, using LED fluorescent microscopy, in a subset of patients (n=2,303) enrolled in the randomised controlled trial mentioned above. Using the two-specimen collection strategy, the sensitivity of front-loaded LED microscopy (68%; 95%CI 62% - 74%) did not differ significantly from that of conventional fluorescent microscopy (72%; 95%CI 66% - 77%). Specificity of front-loaded LED microscopy (95%; 95%CI 93% -96%) was also not statistically different when compared to conventional fluorescent microscopy (94%; 95%CI 92% - 95%). Findings were similar for the three-specimen collection strategy: Sensitivity of front-loaded LED microscopy (75%; 95%CI 69% - 80%) did not differ significantly from that of conventional fluorescent microscopy (74%; 95%CI 6%8 - 79%). Specificity also did not differ between the two

methods, LED specificity being 92% (95%CI 91% - 94%) and that of conventional fluorescent microscopy being 93% (95%CI 91% - 94%). This difference was smaller when the proportion of patients with three smears was considered (94.2% in the frontloaded and 92.7% in the standard scheme, difference 1.5%, 95%CI 0.3%-3.7%). When three-specimen front-loaded LED microscopy was compared with a two-specimen frontloaded LED approach, sensitivity did not differ statistically although specificity was slightly lower (26).

A total of 1357 pulmonary and 917 extra-pulmonary specimens were examined during the study. LED FM had 78.3% sensitivity and 92.0% specificity against mycobacterial culture when using pulmonary specimens, and 34.0% sensitivity and 88.8% specificity for extra-pulmonary specimens. The mean time per smear examination was 2.48 min for ZN vs. 1.41 min for LED FM. Several biases in study design and operation identified during analysis, which are likely to lead to underestimates of LED FM accuracy (27).

Of the 221 sputum specimens evaluated, 36 (16.3%) were positive for Mycobacterium tuberculosis by culture. Sensitivity and specificity documented for the different modalities were 84.7% and 98.9%, respectively, for the LED assessment; 73.6% and 99.8%, respectively, for the MVP assessment; and 61.1% and 98.9%, respectively, for light microscopy. Kappa values for interreader variation were 0.87 for the LED assessment, 0.79 for the MVP assessment, and 0.77 for light microscopy. The mean time to read a negative smear was 1.4 min with fluorescence microscopy and 3.6 min with light microscopy, reflecting a time savings of 61% with fluorescence microscopy(7).

Two hundred thirty-three of 464 (50%) patients had culture-positive TB. There was no difference in sensitivity between single-specimen and two-specimen strategies when smears were examined with LM (55 vs. 56%; difference, -1%; 95% confidence interval [CI], -5 to +2%) or LED FM (61 vs. 64%; difference, -3%; 95% CI, -7 to +1%). LED FM was more sensitive than LM with both the single-specimen (61 vs. 55%; difference, 6%; 95% CI, 2-10%) and two-specimen strategies (64 vs. 56%; difference, 8%; 95% CI, 3-12%). Findings were similar among the HIV-infected patient subset (n = 321 patients) (28).

Astudy conducted in kenya from 1398 suspects enrolled, 993 (71%) had a complete diagnostic work-up involving three sputum specimens for ZN and FM, culture and chest X-ray (CXR). Irrespective of whether ZN or FM was used on one, two or three smears, the overall diagnostic process detected 92% culture-positive cases. Different strategies affected the ratio of smear-positive to smear-negative TB; however, FM was more sensitive than ZN ($P < 0.001$). FM performance was not affected by the patient's HIV status. The cost per correctly diagnosed smear-positive case, including savings, was 40.30 US dollars for FM on two specimens compared to 57.70 US dollars for ZN on three specimens (29).

Prospective study was carried out in Bangabandhu all samples were stained by both ZN stain and Auramine stain and as a gold standard all were cultured on Lowenstein-Jensen Media. On evaluation of all sputum samples were found negative by ZN method but by auramine stain 16%, 20%, 20% cases were found positive by conventional fluorescence microscopy (CFM), LED and culture respectively. LED fluorescence microscopy is more useful test to distinguish the smear negative cases. It also provide an effective guideline to make decisions regarding judicious use of anti tubercular drug therapy (35).

A study conducted in Abuja showed that out of Two hundred and twenty-four patients with chronic cough had 135/672 (20%) positive on-the-spot smears and 47/224 (21%) positive morning smears. The same-day and internationally recommended approaches identified 44 and 45 of the 78 patients with positive cultures, respectively. 106/194 (55%) patients were HIV positive. Only 9-11% of their smears were positive compared with 30-32% for HIV negatives ($P < 0.01$) (36).

1.4. Significance of the study

Recent studies examining the yield of serial sputum specimens, usually collected as spot-morning-spot, have reported that the majority of patients with smear-positive PTB are identified by the first two sputum specimens and the World Health Organization (WHO) has recently changed its policy in this respect, reducing the minimum number of sputum specimens examined for each patient from three to two.If specimens were collected at the time of consultation (1st on-the-spot) and the morning of the following day (morning sample), the spot-morning specimens would still require a minimum of two visits, which is the minimum required by the spot-morning-spot scheme currently used in most diagnostic centers of LMICs. In addition, the spot-morning and spot-morning-spot schemes still examine a substantial proportion of samples the second day of the diagnostic process. If the process could be the same-day-smear "front-loaded", that is, if all or the majority of sputum collections were conducted the first day of the diagnostic process, this may reduce the number of visits required and reduce patient drop-out, particularly if results could be made available the same day(30).
Therefore, implemanting the same-day-smear microscopy in place of the conventional smear microscopy in Ethiopia could be used

- As a document for policy makers to establish the same day diagnosis of TB supporting the recommendations given by WHO
- To provide information on the advantage of the same-day smear microscopy and gap of conventional method
- As a base line for researchers to conduct further research

1.5. Objective

1.5.1. General Objective

To evaluate the same day diagnosis of TB microscopy in comparison to the spot-mornig–spot methods and KAP of lab personnel,GP,HO and Nurse to wards the use of the same-day diagnosis of TB in selected health insttutions in Addis Ababa, Ethiopia.

1.5.2. Specfic objectives

- To compare the same day diagnosis with spot-mornig–spot diagnosis
- To estimate precision of smear microscopy in the same-day diagnosis
- To evaluate LED fluorescence microscopy in the same day diagnosis of TB microscopy
- To assess KAP of lab personnel,GP,HO and Nurse to wards the use of the same-day diagnosis of TB

1.6. Hypothesis

The same-day-diagnosis of sputum for PTB has no difference when compared with the current conventional case- finding approach.

Chapter two

2. Materials and Methods

2.1. Study Design and Study Period

A cross sectional study was conducted from September to December , 2012 in the selected 16 governmental, private and uniformed health hospitals.

2.2. Study setting

This study was conducted in Ras Desta Hospital, Zeweditu Hospital, Yekatit 12 Hospital, Minilik II Hospital, st.pol Hospital, St. Peter Specialized TB Hospital, Gulelie Semen mazegaga health center, Arada health center and Baleteshachew Health Center conveniently selected from governmental health institutions, Hayat, Betazata, St. Geberel hospitals from private hospitals, Teklehaymanot and Aqlesia from private clinic. In addition in two conveniently selected police and armed force hospitals which are participated in EQA and found in Addis Ababa

2.3. Study population

Patients who were come in those selected health institutions for the diagnosis of PTB and those health personals who are working in TB clinics and TB laboratory in selected health institutions will be included in the study population**.**

2.4. Sampling methods and Techniques for the study

A study site were selected from those health facilities that have both TB clinics (DOT), run direct microscopy for AFB regularly and LED-FM and participate in EQA, from those study sites will be selects conveniently from region. In addition, patients who have visited those health facilities, and health personals that are working in those facilities, voluntary and give their consent to participate were included in the study.

2.4.1. **Sample size determination for smear microscopy**

sample size of the study population were all patients who give sputum sample for TB microscopy included conveniently from all sites of the study setting with in the study period.

N.B: the study is method comparison, the maximum sample size in method comparison is from 100-200 because of this we select a total of 220 samples with its non response rate for this study.

2.4.2. **Sample size determination for KAP study**

Since similar study sites were used for both Patients and KAP analysis and the prevalence not specifically known for KAP, we use 50 % to calculate the sample size, so the sample size was:

$$N = \frac{(Z\alpha/2)^2 * (1-p) * (p)}{(d)\ 2}$$

$$N = \frac{(1.96)^2 * (1-0.5) * (0.5)}{(0.05)^2}$$

N =384

Where N = minimum sample size

Zα/2 = 1.96 at 95% Confidence Intervals (CI)

P = 50 % prevalence of KAP

d= margin of error 0.05 at 95% CI

With 10 % non response rate N =422

2.5. Inclusion and Exclusion Criteria

2.5.1. Patient inclusion and exclusion criteria

Those patients attending in the selected health facilities who were examined in OPD and those new, and re-treatment patients who are normally send for AFB direct microscopy , patient's able to produce adequate amount of sample of mucopurulent sputum suspected for M.TB were included in the study but those patients who are extra pulmonary TB, unable to produce adequate amount of sample of mucopurulent sputum suspected for M.TB, and patients who are on treatment were excluded.

2.5.2. **Inclusion and exclusion criteria for KAP**

Medical doctor, Healthofficers and Nurses working in TB clinic, diagnosing TB patients and Laboratory personal working in Tb laboratory were included for KAP studies.

2.6. Study variables

2.7.1. Dependent variable

- Yield
- Precision
- knowledge, attitude and practice (KAP) to wards the same day diagnosis

2.7.2. Independent variables

- Age
- Sex
- Field of study
- Degree of Qualification
- Experience

2.7. Data collection for KAP study

A questionnaire-based assessment on Knowledge, Attitudes and Practices of health personnel on the same day diagnosis of TB were conducted at selected health facilities from September to December 2012.A structured and pre-tasted questionnaire was used to collect data. Interviewers and supervisors were trained for two days. Crosschecking was conducted in sample facilities for consistency. Verbal consent was obtained from each respondent. Questionnaires were Includes information on the socio demographic characteristics of the respondent, Knowledge, Attitudes, and Practice towards the same day diagnosis of TB that was stated on the annex part. Prior to the administration of the questionnaire, the subjects were briefed on the objectives of the study.

For each TB knowledge questions a score of one was given to a correct answer and zero score for incorrect and 'do not know' responses. Question on the knowledge part was rated and a total score was obtained. The median score was, thereafter, computed. Those with a total score equal to or below the median were classified as having poor knowledge, while those above the median were considered having good knowledge. For attitude and practice section frequency table were computed and practice section associated with knowledge level

2.7.1 Sample Collection for Microscopy Diagnosis

A sputum sample was collected from patients who were come for AFB diagnosisin in those health facilities. During collection four consecutive sputum sample at least 5-10 ml sputum samples was collected in clean, sterile, leak-proof, screw caped wide-mouth (35mm in diameter and volume capacity of 28-50 ml), one use disposable containers with easily labeled wall mark including date of collection, laboratory serial number , age and sex after careful instruction given to patients(31).

2.8. *Laboratory methods*

Spot and after one hour x-spot sample was collected at the first day and morning and spot sample was collected at the second day .from this sample smear was prepared for ZN microscopy and LED_FM technique and stained using AFB reagent and auramine O phenol method reagent respectively. All positive and negative samples was transported to EHNRI TB laboratory to confirm the result using Lj medium and biochemical taste

2.8.1 Direct smear preparation

From each spot and x-spot sample of three smears for reproducibillty check and from a total of four sample from each one smear of two sets of direct smears were prepared by taking a small portion of the purulent part of the sputum with an applicator stick, and smearing it on a microscope slide, which will then dried in the air and fumed on a hot plate. One set was stained with ZN and the other set was stain with the auramine O phenol method (31).

2.8.2. Principle of auramine o staining

Mycobacteria retain the primary stain even after exposure to decolorizing with acid alcohol, hence the term "acid-fast". A counter-stain is employed to highlight the stained organisms for easier recognition. Potassium permanganate is used as counter-stain and it helps prevent non-specific fluorescence. With auramine staining, the bacilli appear as slender bright yellow luminous rods, standing out clearly against a dark background. The identification of the *Mycobacteri*a with auramine O is due to the affinity of the mycolic acid in the cell walls for the fluorochromes. In fluorescent microscopy, light rays of shorter wave length pass through smear stained by a fluorescent dye, such as auramine O, which have the property of absorbing light rays of shorter wave length and emitting light rays of longer wave length. LED is used as a source of light and by means of suitable filter only light rays of shorter wave lengths are allowed to emerge and these rays are used for microscopy (32).

2.8.3. Principles of Lowenstein-Jensen Medium

L-Asparagine and Potato Flour are sources of nitrogen and vitamins in Lowenstein-Jensen Medium. Monopotassium Phosphate and Magnesium Sulfate enhance organism growth and act as buffers. Glycerol and the Egg Suspension provide fatty acids and protein required for the metabolism of mycobacteria. The coagulation of the egg albumin during sterilization provides a solid medium for inoculation purposes. Sodium Citrate and Malachite Green are selective agents to prevent growth of most contaminants and allow early growth of mycobacteria. (33)

2.8.4. Inoculation and Incubation of Culture media

Primarily condensed moisture observed at the bottom of culture medium slants were removed before inoculation. Inoculation for primary isolation, identification, and susceptibility testing of Mtb was done at BL-2 cabinet using pipettes. Each slope was inoculated 150 µl of the centrifuged sediment, distributed over the surface. Two slopes of LJ medium were inoculated per specimen. But preparation of inoculums for susceptibility testing was according to the McFarland standards of inoculums preparation procedure stated in the annex part (33).

All cultures were incubated at 35°-37°C until growth is observed and cultures were discarded as negative after eight and six weeks for primary isolation and susceptibility testing respectively. Inoculated media was preferably be incubated in a slanted position for at least 24 hours to ensure even distribution of inoculums. Thereafter, if incubator space is needed, bottles were placed upright. Caps were tightened to minimize evaporation and drying of media (33).

2.9. Result Interpretation

2.9.1. Direct microscopy reporting

Smears were examined using a light/electrical microscope scanning 100 oil immersion fields before reporting a smear as negative or positive. Acids Fast Bacilli in specimens were red rods shaped, 1 to 10 micro meter long and 0.2 to 0.6 micrometer wide but they also appear coccoide or filamentous (long, slender, even branching) but back ground and other cells stained blue. Grading system is according to WHO standard manual of direct smear microscopy of MTB that stated in annex part (33).

2.9.2. Auramine O smears reporting

The fluorescence microscope was the Primo-star plus Transmitted-Light Microscope with an attached LED. The film was examined with a 40x objective and a 10x eyepiece. The tubercle bacilli were seen as yellow luminous organisms. When fluorescent bacilli have been detected the smear was re-stained with ZN stain for confirmation under oil immersion examination. For this study, 2+ and 3+ were classified as multibacillary and 1+ as paucibacillary. Doubtful reports was considered negative (32).

2.9.3. Culture reading

Egg based LJ was examined for growth twice a week for the first four weeks starting on day 3 post inoculation, and thereafter, once a week until the eighth week. All specimens showing growth in culture were confirmed as AFB by smear microscopy of the colonies and reported immediately as "culture positive for *Mycobacterium* pending identification". Mtb bacilli, in primary isolation, they hardly show any visible growth during the first week of culture. On egg-based media they produce characteristic non-pigmented colonies, with a general rough, white creamy and dry appearance simulating breadcrumbs. Contaminated cultures and rapidly growing *Mycobacterium* (colonies yielded in less than 7 days) were removed and repeated sample processing from the sediment (33).

Mtb colonies were well developed within 3 to 4 weeks as white creamy appearance on LJ media; results were reported immediately after detection and cultures were kept up to 8 weeks before discarding if no growth is detected at weekly examination (33).

2.10. Quality control

Positive and Negative control specimens and smears were used in every batch of culture and staining. Blinded reading of the slides was done by another group of four independent technicians. An experienced technician independent from the two groups, read an arbitrary 10% positive and 5% negatives slides that are selected randomly. In addition to this, the same technician re-read all the smears with discrepant results. All reagents were prepared in accordance with standard operating procedure (SOPs) used at the laboratory. For KAP survey also ten percent of the target population was asked to answer the questionnaires for validation. The final questionnaire were revised based on the pre-validate questionnaires and preliminary data.

2.11. Statistical analysis

Data was double entered using EPI-INFO version 3.5.1 and further statistical analyses were made in SPSS version 16. Chi-Square was used to assess the associations of different variables with methods, health personals KAP and health personals Knowledge with the same day diagnosis of TB microscopy. $P < 0.05$ was statistically significance.

The sensitivity, specificity, positive and negative predictive values of the sputum smear examinations including their 95% confidence intervals (CI) were calculated by using the sputum culture results as the "gold standard." $P < 0.05$ was considered being statistically significant.

2.12 .Ethical clearance

The research proposal was revised by the department of Medical Laboratory Science, and evaluated and reviewed by the Research Committee of the department and ethically approved. Before the start of field work official letter of co-operation were written to EHNRI, Addis Ababa health bureau, private health facility, Army hospital and federal police hospital for the

purpose of sample collection and laboratory testing from AAU/MF, department of medical laboratory science. There were high degree of confidentiality during data collection and informed consents were obtained from each study subject.

2.13. Dissemination of results

The findings of this study were presented to school of medical laboratory and the result will be disseminated to the study area. The findings will also be disseminated to different organizations (governmental and non-governmental) that had a contribution to improve and prevent the wide spread of *Mycobacterium Tuberculosis*. Findings were also be presented in different seminars and workshops to disseminate and submitted for possible publication.

Chapter three

3. RESULTS

3.1. Socio-demographic characteristics of the study population

A total of 220 patients with suspected pulmonary tuberculosis were enrolled from September, 2011 to December, 2012 in three health centers, two private clinics and 11 hospitals which are participating in EQA. Socio-demographic characteristics such as age and sex were obtained for all study subjects and analyzed (Table 1). The mean age was 39 years with a range of 13-78. 148 (70.8%) were males. with a male to female ratio of 2.1: 1.

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Table 1: Distribution of Socio –demographic characteristics of patients by age and sex (n = 220), September 2011-December 2012 Addis Ababa, Ethiopia

Variables	Frequency	Percent (%)
Age group		
10-20	07	3.3
21-30	37	17.7
31-40	89	44.6
41-50	46	20
≥51	31	14.8
Sex		
Male	148	70.8
Female	61	29.2

3.2. Results of SS, SMS, SSM and SM approach by ZN staining using culture as gold standard.

A total of 220 pulmonary tuberculosis suspected patients were included in the study. Among these, 11 patients did not fulfill the inclusion criteria and were excluded. From these 209 patients, a total of 836 sputum specimens were collected. Four sputum samples from each patient were collected for spot, extra-spot, morning and second day spot sample. SMS samples were analyzed by each health facility laboratory; spot and extra-spot sample analyzed by principal investigator.

Out of total 209 cases, it was observed that 43(20.6%) cases were diagnosed as pulmonary tuberculosis by culture method as a gold standard. SMS and SM showed the nearest result as 40(19.1%) positive cases, in which 1 case was found as false positive. On the other hand SS and SSM detected 39(18.7%) and 40(19.1%) cases positive, respectively (Figure 1). Both missed 1 case each. The difference in the case-yields between SS with SMS, SSM and SM approaches were 0.4%, 0.9% and 0.45%, respectively the case detection of each approach was almost found to be similar.

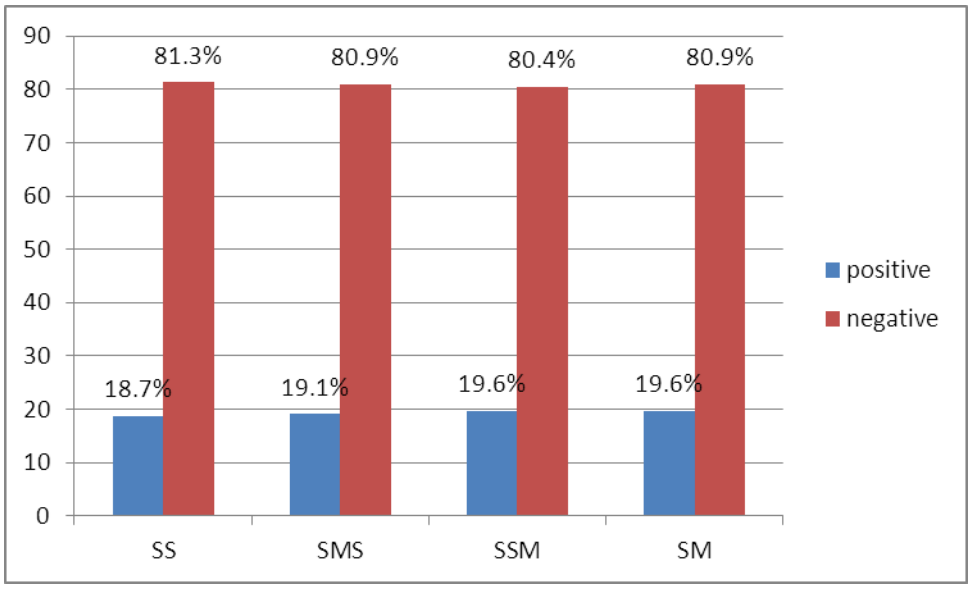

Fig 1. Distribution of microscopy TB positive rate using different spaceman collection approach SS, SMS, SSM and SM approach by ZN staining and culture for mycobacterium.(SS=spot and extra-spot, SMS=spot-morning-spot, SSM=spot-extra-spot-morning, SM=spot-morning

3.3 Comparison of same day (spot, extra-spot) and spot-morning-spot (SMS) sampling method using ZN

Morning sputum specimens of 209 (100%) of the patients enrolled were cultured, and 43 were found to be culture-positive. The analysis was conducted on 209 patient's sputum sample using ZN as shown in (Table 2). Out of the 43 culture positives, first spot and extra-Spot (SS) and spot-morning-spot (SM) identified 38 (88.4%) and 39 (90%) smear positives, respectively. Two percent of the cases were recognized as false positive by both approaches. Both SS and SMS approaches missed 11.6% and 9.3% of the cases, respectively. All 165 culture negative samples were found to be smear negative by both approaches. The sensitivity, specificity, PPV, NPV and accuracy using culture as a gold standard were 88.4%, 99.4%, 97%, 97.3%, 97.1% for SS, while 90.4%, 99.4%, 98%, 97.5%, 97.6% were for SMS approaches, respectively (Table 2).

This study indicates that the sensitivity and specificity of Spot, extra-Spot (SS) was non-inferior to the sensitivity and specificity of spot-morning-spot (SMS) approach.

Table 2 . Comparison of spot, extra-spot (one day sampling) and spot, morning and spot (two days sampling) sampling method by ZN staining method using culture as a golden standard , September 2011-December 2012 Addis Ababa, Ethiopia

	Culture(n=209**)**						
Approach	**Positive**	**Negative**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV∗**	**NPV£**
Same day(SS•)							
positive	38	1	88.4	99.4	97	97.3	97.1
negative	5	165					
Standard(SMS◊)							
positive	39	1	90.4	99.4	98	97.5	97.6
negative	4	165					

∗ = positive predictive value, £ = negative predictive value, ◊=spot- morning- spot sample, ●=pot, extra-spot sample.
(Sensitivity, specificity, PPV, NPV and accuracy are expressed in percentage)

3.4. Evaluation of LED-fluorescence microscopy and ZN smears microscopy based on the same day diagnosis using spot and extra-spot samples.

A total of 209 pulmonary tuberculosis suspected patients were participated in the study. Out of the study subjects a total of 418 spot and extra-spot sputum specimens were collected for our study. Morning sputum specimens of 209 (100%) of the patients enrolled were cultured, and 43 were culture-positive. The analysis was conducted on 209 patient's spot, extra-spot sputum sample (same day diagnosis) using ZN stain bright field microscopy and AO stained LED-FM as shown in (Table 4). First spot and extra-Spot (the same day sampling) 39 and 48 were identified positive by ZN bright field microscopy and AO stained LED-FM respectively, of 418 sputum sample. From a total of 43 culture positive patients, 88.4% were identified as positive, 2% were false positive and 11.6% were missed by ZN bright filed microscopy. By AO LED-FM, 95.3% were identified positive, 16.2% were false positive and 5% were missed. The sensitivity, specificity, PPV, NPV and accuracy yielded with LED-fluorescent microscopy using culture as a gold standard were 95.3%, 95.9%, 85.4%, 98.8%, 95.7%, respectively. While for ZN stained bright field microscopy were 88.4%, 99.4%, 97.3%, 97.1%, 97%, respectively (Table 3).

Table3. Evaluation of LED-fluorescence microscopy in comparison to ZN smear microscopy based on the same day diagnosis(spot, extra-spot) sample using culture as a golden standard (n=209) , September 2011-December 2012 Addis Ababa, Ethiopia

Method	Culture		Sensitivity	Specificity	PV♦	NPV●	Accuracy
	Positive	Negative					
ZN stained(BFM◊)							
positive	38	1					
negative	5	165	88.4%	99.4%	97.3%	97.1%	97%
AO stained(LED-FM∗)							
positive	41	7					
Negative	2	159	95.3%	95.9%	85.4%	98.8%	95.7%

◊= bright filed microscopy, ♦= positive predictive value, ●=negative predictive value ∗= light emitting diode fluorescent microscopy

3.5. Health Personnel Knowledge, Attitude and Practice towards the same day diagnosis

3.5.1. Socio Demographic Characteristics of Health Personnel's

A total of 246 GP, HO, Nurses working in the study sites participated in this study. Of these, 114(46.3%) were males with a male to female ratio of 2.2: 1. The mean age of the respondents was 31 years ranging from 25 to 45 years old and more than half 165(67.1%) of respondents were B.Sc. holders, the remaining 55(22.4%), 26(10.6%) were M.D. and diploma holders, respectively. Similarly, 63% (155) had one and less than one year working experience in Tb clinic and 37 % (91) had over two years working experience. (Table 5).Although 141 laboratory personnel working in the study sites participated. Of these, 52(36.9%) were females with a

female to male ratio of 2.2: 1. The median age of the respondents was 31 years ranging from 25 to 44 years old and more than half of respondents were B.Sc. holders 81(64.5%), the remaining 2(1.4%), 58(41.1%) were M.Sc. and diploma holders, respectively. Similarly, 19(13.5%) had one and less than one year working experience in laboratory and 122(86.5%) had more than two years working experience in laboratory (Table 4).

Table 4: Socio –Demographic Characteristics of Health Personnel in Selected Health Facility, September 2011-December 2012 Addis Ababa, Ethiopia

	GP,HO and Nurse		Laboratory personnel	
Variable	**frequency**	**percent**	**frequency**	**percent**
Age group				
25 – 29	95	38.6	56	39.7
30 – 35	76	30.9	38	27
36 – 40	64	26	40	28.3
≥ 41	11	4.5	7	5
Sex				
Male	114	46.3	89	63.1
Female	132	53.7	52	36.9
Education				
Diploma	55	22.4	58	41.1
BSc	165	67.1	91	64.5
MD	26	10.6	_	_
MSc	_	_	2	1.4
Work experience				

1 year and				
< 1year	155	63	19	13.5
2 years	12	4.9	7	5
3 years	19	7.7	9	6.4
4 years	3	1.2	33	23.4
5 years and				
> 5 years	57	23.2	73	51.8

3.5.1.1. Same Day Diagnosis Awareness and Sources of Information of Nurse, HO, and GP

From 246 respondents, 168 (68.3 %) have heard about the same day diagnosis of TB. were The source of information were TV, radio or internet for 65 (38.7%), news paper /magazine for 42(25%), person to person communication (friends) for 28(16.7%), teachers for 24 (14.3%) of the respondents. The remaining respondents said that billboards were their source of information. Among respondents, only 68.3% tried to search for information about the same day diagnosis of TB and 31.7% were not well informed about the same day diagnosis of TB (Table 5). The three best source of information mentioned by respondents were TV, internet or radio, friends and news paper/magazine.

Table 5. GP,HO, and Nurse source of information about the same day diagnosis, September 2011-December 2012 Addis Ababa, Ethiopia

Variables	Frequency	Percent
TV, radio or internet	65	38.7
Billboard	9	5.4

Newspaper/magazines	42	25
Friends/relatives	28	16.7
Teachers	24	14.3

3.5.1.2. GP,HO, and Nurse General Knowledge about the same day diagnosis of TB

Both sputum microscopy and X-ray were recognized by 77.2% of the study participants as tool of pulmonary tuberculosis (PTB) diagnosis. The remaining 22.8% of the participants knew sputum microscopy as the only tool of PTB diagnosis. The sputum collection time (schema) for conventional smear microscopy method and number of samples required in the conventional smear microscopy method for PTB were recognized by 80.1% and 56.4% of the respondents, respectively.

Only 29.7% of the respondents were aware that the time of sample collection, high result reporting time, work load and high cost for health facility and patients were the limitations of the SMS smear microscopy method. But 32.9%, 16.7%, 14.2% and 6.4% of the respondents recognized that time of sample collection, high result reporting time, work load and high cost for health facility and patients was the limitation of SMS smear microscopy, respectively.

Out of the total number of respondents, only 18.8% were aware of the same day diagnosis that is doing spot, X-spot with in one day and giving the result within the same day, but 51.6% were aware of spot, X- spot is the sample collection time (schema) in the same day diagnosis . Reduced TAT, work load and diagnosed defaulters were recognized by 47.2% of the respondents as advantages of the same day diagnosis of TB.

Time of sample collection, work load, delay of result and number of sample as causes of diagnosis defaulter were recognized by 63% of respondents, followed by time of sample collection (13.4%), and number of sample collection (3.3%). At the same time, 19.1% and 1.2% of the participants were aware that the quality of the test and work load was the cause of diagnosis defaulter, respectively (Table 6).

The overall respondents' knowledge score regarding the same day diagnosis, tools used to diagnose TB, number of sputum samples required for diagnosing TB, sputum collection time for the conventional method, sample collection time of the same day sampling method, advantages of the same day diagnosis, limitation of SMS sputum sample for the diagnosis of TB and the cause of diagnosis defaulter was computed (maximum of 8 scores). The mean and median knowledge score of respondents was 4.07 and 4, respectively. Using median score as cut off, majority (57.7%) had poor knowledge score (Table 6).

Table 6. GP,HO, and Nurse Respondent's general knowledge about the same day diagnosis of TB, September 2011-December 2012 Addis Ababa, Ethiopia

Variables	Frequency	Percent
Tools used to diagnose TB		
Sputum smear microscopy	56	22.8
Both sputum microscopy & x-ray	190	77.2
Number of much sputum sample required for diagnosing TB		
One	27	11
Two	18	7.3
Three	197	80.1
Four	4	1.6

Sputum collection time for the conventional method		
SMS	135	54.9
Spot , x-spot	1	0.4
MSM	63	25.6
SMM	21	8.5
MS	26	10.6
The same-day diagnosis		
Doing SMS sample	84	35
Doing tow spot samples within tow days	46	19.2
Doing S,X-S and giving result in the next day	32	13.2
Doing SMS within one day	33	13.8
Doing S,X-S with in 1 day and giving the res	45	18.8
In one day		
Sample collection time of the same day sampling method		
S, X-S	127	51.6
spot, m, x-spot	9	3.7
MS	14	5.7
I don't know	96	39
The advantages of the same day diagnosis		
Reduce turn around time	59	24
Reduce diagnose defaulters	23	9.3

Radiuses work load	11	4.5
I don't know	37	15
Reduce TAT, work load and diagnose default	116	47.2
Limitation of SMS sputum sample for the diagnosis of TB		
Time of sample collection	81	32.9
Has high result reporting time	41	16.7
Work load	35	14.2
Have high cost for health facility and patients	16	6.5
All are limitation of SMS samples	73	29.7
The cause of diagnosis defaulter		
Time of sample collection	33	13.4
Number of samples	8	3.3
Time of sample collection, work load,		
Delay of result and number of samples	155	63
Work load	3	1.2
Quality of the test	47	19.1
Knowledge score		
Good	104	42.3
Poor	142	57.7

SMS =spot-morning-spot, SSM=spot-spot-morning, SM =spot-morning, SMM-spot-morning-morning

3.5.1.3. Attitudes and Practices of GP , HO, and Nurse

In assessing the attitudes and practices of respondents, 16.7%(41) study subjects strongly believe that the same day diagnosis had equal output to SMS in the diagnosis of PTB, those who believe accounted 43.5% (107), and those who responded that they don't strongly believe and not believe that the same day diagnosis equal to SMS accounted 7.7%(19) and 19.1%(47), respectively. The rest responded neutral.

About 29.7%(73), 26%(64) and 42.7%(105) of respondents strongly believed, also 45.1%(111), 33.7%(83) and 28%(69) of the respondents believed that the same day diagnosis reduce TAT, work load, health facility and patient cost, respectively. Only 16.3%(40), 11.4%(28) , 8.1%(20) and 1.2%(3), 9.8%(24), 3.7%(9) respondents did not believe and did not strongly believe, respectively, but 7.7%(19), 19.1%(47) and 17.5%(43) respondents respectively not taking sides on the advantages of the same day diagnosis. (Table 7).

For the variable 'Diagnosis delay was the cause of diagnosis defaulter', 39.4 %(97) believed strongly, 25.6 %(63) believe and 20 %(51) did not believe. The remaining 14.2 % (35) were neutral to the idea.

Majority of respondents were very seriously worried about the sputum sample collection time for the diagnosis of PTB of spot morning spot schema.

About 18%(46), 20.3%(50), 17.9%(44) and 40.2%(99) respondents did always, some time, rarely and never work in TB clinic, respectively .Additionally, 59.9%(145) of the respondents did always order the conventional smear microscopy, 4.1%(10) of the respondents never ordered the conventional AFB, but 58.5%(144) of the respondents submitted laboratory requests with complete information, but 5.3%(13) did not (Table 7).

Majority 52.1% (125) of the respondents mentioned that patients collect their sputum always in the open air, but 20.4% (49) of the respondents had no idea where patients collact sputum.

The overall respondents' attitude and practice score was computed (maximum of 10 scores) for both attitude and practice. The mean and median attitude and practice score of respondents was

6.65, 6.4 and 8.7, respectively. Using median score as cut off, majority 63.4% and 72.4% had positive attitude and good practice score (Table 7)

Table 7: GP, HO and Nurse ` Attitude and Practice health personnel about the same day diagnosis of TB, September 2011-December 2012 Addis Ababa, Ethiopia

Variables	Frequency	Percent
The same-day diagnosis reduce TAT		
I strongly agree	73	29.7
Agree	111	45.1
Neutral	19	7.7
Disagree	40	16.3
I strongly disagree	3	1.2
The same day diagnosis has equal to SMS		
I strongly believe	41	16.7I
believe	107	43.5
Neutral	32	13
I don't believe	47	19.1
I don't strongly believe	19	7.7
Same-day diagnosis reduce the work load		
I strongly believe	64	26
I believe	83	33.7

Neutral	47	19.1
I don’t believe	28	11.4
I don’t strongly believe	24	9.8
Diagnosis delay is a cause for diagnosis defaulter		
I strongly believe	97	39.4
I believe	63	25.6
Neutral	35	14.2
I don't believe	51	20.7
Same-day diagnosis reduces health facility and patient cost		
I strongly believe	105	42.7
I believe	69	28
Neutral	43	17.5
I don't believe	20	8.1
I don't strongly believe	9	3.7
Worry on time of sample collection		
Very serious	125	52
Some what serious	47	19
May or may not be serious	20	8.1
Not very serious	15	6.1
Serious	36	14.6
Working in TB clinic		

Always	46	18.7
usually	7	2.8
Some times	50	20.3
Rarely	44	17.9
Never	99	40.2
Order AFB for your patient		
Always	65	26.4
Usually	65	26.4
Some times	62	25.2
Rarely	12	4.9
Never	42	17.1
SMS smear microscopy laboratory result back to OPD with in one day		
Always	49	19.9
Usually	9	3.6
Some times	26	10.6
Rarely	55	22.4
Never	107	43.5
Order the conventional AFB		
Always	145	59.9
Usually	37	15
Some times	39	15.9

Rarely	15	6.1
Never	10	4.1
Lab requests are submitted with complete information		
Always	144	58.5
Usually	84	34.1
Some times	5	2
Rarely	13	5.3
Patients produce sputum		
Always in the open air	125	52.1
Usually in the latrines	24	10
Some times in the waiting room	16	6.7
Rarely in the laboratory	26	10.8
I don't know	49	20.4
Attitudes and Practices score		
Positive	156	63.4
Negative	90	36.6
Good	178	72.4
Poor	68	27.4

OPD=out patient department, SMS=spot-morning-spot, AFB=acid fast bacilli, Tb =tuberculosis, TAT=turn around time

3.5.1.4. Univariate analysis of characteristics associated with low knowledge score among health personals.

Multivariate logistic analysis was conducted on independent variables that significantly associated ($p < 0.05$) with low knowledge score. The findings revealed that lower educational status, participant professional status and lower work experience were associated with low knowledge score , but age and sex were not associated (Table 8).

Table.8. Univariat analysis of characteristics associated with low knowledge score among health personals working in study sites , September 2011-December 2012 Addis Ababa, Ethiopia

Variables	poor knowledge	Odd ratio	95% CI	P- value
Profession				
Nurse	62	46.768	8.118-269.431	0.000
HO	42	22.422	4.310-116.644	0.000
Gp	1			
Age				
25-34 years	99	1.816	0.458-7.204	0.396
35-44 years	42	2.215	0.560-8.763	0.257
> or = 45 years	1			
Work experience				
0-2 years	102	0.274	o.118-0.633	0.002
>or = 3 years	41	1.130	0.334-3.819	0.845
Sex				
Male	63	1.207	0.727-2.004	0.468
Female	79	1.317	0.837-2.015	0.476

Educational status				
Diploma	26	0.328	0.145-0.744	0.008
BSc	101	15.893	2.418-104.876	0.004
MD	5			

3.5.2. Laboratory personnel Knowledge, Attitude and Practice towards the same day diagnosis

3.5.2.1. Laboratory personal General Knowledge about the same day diagnosis of TB

Out of the total number of 141 respondents, 68.8% (97), 80.9 %(114) recognized the sample collection schema and number of sputum sample required for the conventional AFB smear microscopy, but 31.2% (44),19.2%(27) of the respondents were not familiar with the conventional sample collection algorithms and number of sputum required for AFB diagnosis.

Only 29.5%(3), 5%(7), 54.6%(77) of the respondents recognized about the same day diagnosis, sample collection time of the same day diagnosis sampling methods and the advantages of the same day diagnosis, but the remaining of the respondents had no knowledge about it.

Majority of the respondents 95%(134),70.2%(99) recognized the method of staining for AFB examination, but 5%(7), 29.8%(42) of the respondents had poor knowledge (table 9).

The overall respondents' knowledge score regarding sputum collection time for conventional method, number of sputum required to diagnose TB, the same day diagnosis of TB microscopy, sample collection time for the same day sampling method, the advantage of same day diagnosis, familiar method of staining for AFB and the main reason that AFB examination was done were computed (maximum of 8 scores). The mean and median knowledge score of respondents was 4.07 and 5 respectively. Using median score as cut off, majority (61%) had good knowledge score (Table 9)

Table 9. Laboratory personnel general knowledge about the same day diagnosis of TB, September 2011-December 2012 Addis Ababa, Ethiopia

Variables	Frequency	Percentages
Sputum collection time for the conventional method		
Spot-morning-spot	97	68.8
Morning-spot-morning	28	19.9
Morning-spot	16	11.3
Number of sputum required to diagnose TB		
Two	7	5
Three	114	80.9
I don't know	20	14.2
The same-day diagnosis of TB microscopy		
Doing spot-morning-spot sample	44	39.3
Doing two spot sample with in two days	7	6.2
Doing spot-morning-spot with in one day	28	25
Doing spot, X-spot sample with in one day & gevining result in the same day	33	29.5
Sample collection time of the same-day sampling method		
Spot-morning-spot	94	66.7
Spot and X-spot	7	5
I don't know	40	28.4

The advantage of the same day diagnosis		
Reduce TAT◊	17	12.1
I don't know	7	5
Reduce diagnosis defaulter	40	28.4
Reduce TAT and work load	77	54.6
Familiar method of staining AFB do you use		
Gram stains	7	5
Ziahl-Nelson	134	95
Main reason that AFB♣ examination is done		
For diagnosis and follow up	99	70.2
For screening latent TB♦	42	29.8
Knowledge score		
Good	86	61
Poor	55	39

♣=acid fast bacilli, ♦ =tuberculosis, ◊=turn around time,

3.5.2.3. Attitudes and Practices of laboratory personal

In studying the attitudes and practices of laboratory personnel , 86 %(121) study subjects believed that smear microscopy was the cheapest and quickest method to diagnose PTB, but 14 %(20) the respondents did not accept this. Also 36.2 %(51), of the respondents were agreed on the ability of giving AFB result, but 63.8 %(90) were not agreed.

About 38.3% (54), 51.1% (72) 5) of respondents believed that the same day diagnosis reduce the work load and increase smear detection rate, respectively. However, 61.7% (87) and 41.8% (59) of the respondents did not believe that the same day diagnosis reduce work load and increase smear detection rate, respectively. Additionally, 7.1% (10) of the respondents had no idea on the increase smear detection rate.

Out of the total respondents, 27.7 %(39) always performed smear examination, 14.2% (20) usually perform AFB microscopy, but 58.2% (82) performed smear microscopy sometimes. Besides, 44.7% (63), 19.9% (28) and 19.1% (27) used standard grading systems for reporting AFB results, but 0.7%(1) and15.6 %(22) used rarely and never used, respectively. (Table10).

Majority of the respondents 81.6 %(115) always used new slides for AFB examination, 11.3% (16) used usually re used slides, but 7.1% (10) of the respondents uses most of the time new and sometimes re used slides.

About 67.7%(95), 87.9%(124) respondents believed and agreed that participating in EQA improves TB laboratory quality and implementing the same day diagnosis in EQA participating TB laboratory in more important, 32.4%(46), and12.1%(17) of the respondents did not believe and did not agree on the importance of participating in EQA to improve TB laboratory quality and implementing same day diagnosis in EQA participating TB laboratory is more important (table 10).

The overall respondents' attitude and practice score was computed (maximum of 10 scores) for both attitude and practice. The mean and median attitude and practice score of respondents was

5.96, 7.67 and 5, 8 respectively. Using median score as cut off, majority 73% and 97.9% had positive attitude and good practice score (Table 10)

Table10:`Attitude and Practice of laboratory personnel about the same day diagnosis of TB, September 2011-December 2012 Addis Ababa, Ethiopia

_ Variables	Frequency	Percent
Smear microscopy is the cheapest and quickest method to diagnose TB		
I strongly believe	63	45
I believe	58	41
Neutral	10	7
I don't believe	10	7
Give AFB result to patient in one day		
I strongly agree	42	29.8
I agree	9	6.4
Dis agree	48	34
I strongly dis agree	42	29.8
Reducing number of sample and complete diagnosis of TB in one day		
I strongly agree	28	19.9
I agree	7	5
Neutral	14	9.9
Dis agree	49	34.8
I strongly dis agree	43	30.5
Same day diagnosis reduce the woke load		
I strongly believe	26	18.4
I believe	28	19.9

I don't believe	64	45.4
I don't strongly believe	23	16.3
Same day diagnosis increase smear detection rate		
I strongly believe	20	14.2
I believe	52	36.9
Neutral	10	7.1
I don't believe	37	26.2
I don't strongly believe	22	15.6
Participating in EQA improve TB laboratory quality		
I strongly agree	92	65.2
I agree	32	22.7
Dis agree	17	12.1
Implementing the same-day diagnosis in EQA participating TB laboratory is more important		
I strongly believe	26	18.6
I believe	69	49
I don’t believe	16	11.4
I don't strongly believe	30	21
Perform sputum smear examination		
Always	39	27.7
Usually	20	14.2
Some time	82	58.2
Standard grading system for reporting AFB results		
Always	63	44.7
Some times	28	19.9

Usually	27	19.1
Rarely	1	0.7
Never	22	15.6
Slide used for AFB examination in the facility		
Always new	115	81.6
Usually re used	16	11.3
Most of the time new some times reused	10	7.1
Attitudes and Practices score		
Positive attitude	103	73
Negative attitude	38	27
Good practices	138	97.9
Poor practices	3	2.1

4. Discussions

4.1. Evaluation of the same day diagnosis

The diagnosis of a tuberculosis infection is not only reliant on clinical findings, but also on laboratory analysis. Since the late 19th century, laboratories have relied on a sputum smear test to diagnose AFB in pulmonary specimens. The mainstay of TB laboratory diagnosis has been the sputum smear. Since pulmonary infections with TB cause a chronic cough, sputum is an excellent medium in which to identify AFB. Once smears are made, slides are stained using a fuchsin-based staining system such as the Ziehl-Neelsen (ZN) method, and then observed using a traditional light microscope under 100x oil immersion (21).

Direct sputum smear microscopy is the most widely used test for the diagnosis of pulmonary tuberculosis (TB), available in most primary health care laboratories at health center level. The majority of laboratories use conventional light microscopy to examine Ziehl-Neelsen stained direct smears, documented to be highly specific in areas with a high prevalence of TB but with varying sensitivity (20-80%). Besides being labour-intensive, direct sputum smear microscopy may have considerable patient costs and inconvenience associated with the need to submit multiple sputum specimens over a period of up to three days. A number of TB control programmes have reported high rates of initial patient default as a result (27).

This study examined the different sputum sample collection approaches, comparing different microscopy techniques in the diagnosis of PTB based on 209 participant sputum specimens collected as spot-morning-spot plus an additional specimen collected one hour after the first spot (extra-spot or x-spot). Based on this, the yield of SS, SM, SMS and SSM was compared. The result of spot-morning-spot (SMS), spot-morning(SM) and spot-x-spot-morning showed 40(19.1%) positive cases. On the other hand, spot and X-spot (SS) identified 39(18.7%) positive case with 1(2%) missed case. The difference in case–yield between SS with SMS, SSM and SM approaches was o.4%, 0.9% and 0.45%, respectively.

The detection capacity of each approach was almost similar with the study conducted in Ethiopia, Nepal, Nigeria, and Yemen by Andy Ramseay *et.al.2009.* However, using two sample in one day (one day sampling method) with good EQA activity and patient instruction on sample collection could be more advantageous than two day sampling method in reducing patient cost, health facility cost, work load, turnaround time and increased TB diagnosis.

Also this study revealed that 18.7 %(39) were identified as smear positive, 81.3 %(170) were smear negative by same day diagnosis (SS). The conventional algorithm (SMS) identified 19.1 %(40) as smear positive and 80.9 %(169) as smear negative; while 21 %(43) were culture positive of the 100 %(209) participants. It was found that in case of the same day diagnosis there was agreement in 38(88.4%) cases, and 1 (2%) false positive, whereas for the conventional algorithm there was agreement in 39(90.7%) cases, and 1(2%) false positive comparing with 43 culture positive samples. Also 5(11.6%) cases were missed and detected as false negative by the one day approach (SS). On the other hand, the two day approach (SMS) missed 9.3 %(4) and detected as false negative using culture as gold standard. The sensitivity, specificity, accuracy PPV, and NPV were 88.4%, 99.4%, 97%, 97.3%, 97.1% and 90.4%, 99.4%, 98%, 97.5%, 97.6% for the same day diagnosis and the conventional algorithm, respectively using culture as a gold standard. The difference of sensitivity, specificity, accuracy, PPV and NPV between them was 2%, 0%, 1%, 0.2% and 0.5%, respectively. It shows that in this study there is no significant difference in smear positivity rate, sensitivity and specificity between the one day sampling and two day sampling methods in TB diagnosis .

Several recent studies have evaluated same-day smear microscopy performed using two specimens collected 1 hour apart and found the strategy to be as sensitive as smear microscopy performed using standard 2-day specimen collection (6, 24, 26, and 36). These were somewhat similar to our study findings.

To reduce the high direct and indirect patient costs and inconvenience associated with multiple health facility visits, patient cost and failure to complete smear evaluation our findings suggest that using a one day sputum collection (a spot, x-spot sputum specimen) with implementing strong EQA could be sufficient for the diagnosis of PTB. This would reduce work load, improve turnaround time, increase smear detection rate and reduce transmission of TB.

4.1.1. Evaluation of LED-FM and bright filed microscopy in the same day diagnosis

The backbone of TB diagnosis worldwide continues to be smear microscopy. Thus, increasing the sensitivity of smear microscopy could have a large impact on global TB case detection rates. As a result there have been several initiatives to optimize smear microscopy including changes in specimen collection procedures, specimen processing, and microscopy techniques (4).

Recent technical developments have the potential to improve some of the shortcomings of the smear diagnosis of TB. These include the development of illumination systems based on LEDs (light-emitting diodes), which resulted in LED fluorescence microscopy (LED-FM) becoming commercially available. Furthermore, the World Health Organization (WHO) recently modified its guidelines for the diagnosis of PTB and reduced the number of AFB required to declare a smear as positive (from 10 to 1 AFB), the minimum number of specimens needed for diagnosis (from three to two), and the number of positive smears required to classify a patient as having smear-positive TB (from two to one smear) (6). There was also an initiative from our country's side to use LED fluorescent microscopes in selected sites which are found nationwide (personal communication).

In this study on evaluation of the microscopic techniques by comparing them with the gold standard culture technique, it was found that in case of ZN stain there was agreement in 38(88.4%) cases, and 1 (2%) false positive whereas for Auramine-O (AO) stain there was agreement in 41(95.3%) cases, and 7(16.3%) false positive by LED comparing with 43 culture positive sample. In this study, with ZN stain 5(11.6%) cases were missed and detected as false negative. On the other hand, auramine stain LED fluorescent microscopy missed only 2(4%) and

detected as false negative using culture as gold standard. The sensitivity, specificity, PPV, NPV and accuracy were 95.3%,95.9%,85.4%,98.8%,95.7% and 88.4%,99.4%,97.3%,97.1%, 97% for LED-fluorescent stained microscopy and ZN stained bright filed microscopy, respectively .

The difference of sensitivity, specificity, PPV, NPV and accuracy between them was 6.9%, 3.5%, 11.9%, 1.7% and 1.3%, respectively. Our result shows that LED-fluorescent microscopy was more sensitive than bright field microscopy. The correlation between LED-fluorescent microscopy and ZN stained bright field microscopy based on spot, extra-spot sample and culture as gold standard method.This proves that AO stain examined by LED is a better method for its close comparability to the gold standard technique. These were almost comparable with other several studies (28,35).

However, the need for rapid smear results and effective treatment of the most infections TB cases remains paramount. The efficacy of LED fluorescence microscopy proved to be much higher than conventional fluorescent microscopy and bright field microscopy and comparable to that of culture. In this study, Auramine O (AO) stained sputum smear has been found to improve significantly the sensitivity, predictive value of negative test, percentage of false negative and efficiency. Therefore, LED microscopy of sputum by AO staining can be used effectively along with ZN stain for the diagnosis of pulmonary tuberculosis by implementing good EQA practice.

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4.1.2. Knowledge, Attitude and Practice

A questionnaire based survey was conducted among health personnel and laboratory personnel working in the selected sites for the same day diagnosis of PTB in selected health facility in Addis Ababa.

This study demonstrated that majority (57.7%) of respondents had low knowledge score. This study also showed that 38.7% and 25% of respondents heard about the same day diagnosis for the first time from electronics media and news paper/magazine, respectively. This indicates that electronic media and news paper/magazines could act as successful means of disseminating information about the same day diagnosis in the health and laboratory personnel .

Both sputum microscopy and X-ray were recognized by 77.2% of the study participants as tool of PTB diagnosis. The remaining 22.8% of the participants knew sputum microscopy as the only tool of PTB diagnosis. The sputum collection time (schema) for conventional smear microscopy method and number of sample required in the conventional smear microscopy method for PTB were recognized by 80.1% and 56.4% of the respondents, respectively.

Only 29.7% of the respondents were aware that the time of sample collection, high result reporting time, work load and high cost for health facility and patients were the limitations of the SMS smear microscopy method.

Majority of the respondents know both sputum smear microscopy and x-ray are tools of PTB diagnosis. Furthermore,80. 1% and 56.45% of the respondent recognize the sample collection time and number of sputum samples required for the conventional (two days) method of smear microscopy. But 32.9%, 16.75%, 14.2% and 6.4% of the respondents knows that time of sample collection; long result reporting time, work load and high cost for health facility and patients was the limitation of spot-morning-spot smear microscopy, respectively.

Similarly, out of the total number of respondent only 18.8% knows the same day diagnosis was doing two spot sputum sample or spot and x-spot (extra spot sample after one hour of the first spot sample) in one day and giving the result with in the same day(the first visit) . But 51.65 were aware of the sample collection time (schema) in the same day diagnosis. Also 47.2 % of the respondent knows that reduce TAT, work load, diagnose defaulter, patient and health facility cost and improve smear detection rate are advantage's of the same day diagnosis.

Majority of the respondents 81.6 %(115) always used new slide for AFB examination, 11.3% (16) used usually re used slides, but 7.1%(10) of the respondents uses most of the time new and sometimes re used slides

From total laboratory personnel 0.7 %(1) and15.6 %(22) were used rarely and never used, the standard grading system respectively. .

In general laboratory personnel had good knowledge Comparing with other health personnel regarding the conventional , the same day diagnosis approach and different diagnostic techniques of TB.

4.1.3. Limitation of the study

- Lack of literature on the KAP on the same day diagnosis.

4.2. Conclusions and recommendations

The diagnosis of tuberculosis in high-burden settings relies on sputum smear microscopy and requires multiple patients' visits to the health facilities. This approach could be improved if most specimens were collected the first day of consultation. This study reports the smear microscopy findings of 209 adults with chronic cough participating in 16 health facilities in Addis Ababa. Sputum specimens were collected as spot morning- spot plus one additional specimen one hour after the first spot (X-spot or extra-spot). The yield of two (spot and X-spot) or three (spot-morning-spot) specimens was compared. Of these, (18.6%) were identified by the spot-morning-spot, and (19.1%) were identified by the spot, X-spot specimens. The findings of this study has shown that the time, number of visits , work load, turnaround time and patients' costs to complete smear microscopy could be reduced by one day (same day or frontloading) collection of sputum specimens.

Furthermore, LED based Fluorescence Microscopy (FM) was shown to be more sensitive than ZN for diagnosis of pulmonary tuberculosis. Hence, LED microscopy of sputum by AO staining can be used effectively along with ZN stain for the diagnosis of pulmonary tuberculosis, Using this method (LED) in clinical laboratories with the one day sampling approach could help process large specimen numbers and increase the smear detection rate thereby decreasing false negative smears. However, because FM was shown to have false positive results for bloody samples and samples from patients on anti-TB. It is recommended that the positive slides should be checked by ZN. The use of FM could help detect more patients and avoid the daunting task of waiting for culture service and results which usually may take 3 to 8 weeks.

Low knowledge score about the same day diagnosis was more evident among Nurses, HO. Low knowledge score also associated with lower educational status, participant professional status and lower work experience were associated with low knowledge score. Therefore it is important giving appropriate on jobs and off jobs training and establishing proper sources of information, education and communication pathway that improve the level of Knowledge.

4.2.1. Recommendations

- Service providers should be able to initiate or refer patients for treatment on the same day of consultation
- Giving training for health staff responsible for requesting sputum smear ,doing and instructing patients on sputum collection has to be strengthening
- Responsible bodies and health institution with their partners should give training for laboratory staff unfamiliar with fluorescent microscopy techniques.
- Guide line on the use of both LED-FM and ZN microscopy in TB diagnosis should be developed at the same time.
- In service or off service training on TB diagnosis using LED-FM, same day diagnosis and facilitating electronic media should be given to create awareness.
- Large scale study on the same day diagnosis and KAP of the Nurses , HO MD and laboratory personnel should be designed and done.

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Annex I: Information read to be respondent

My name is Sisay Kebede;i am MSc student of Addis Ababa University, Faculty of Medicine, School of Medical Laboratory Science. And the aim of the study isevaluation of the same-day diagnosis of TB microscopy in comparison to the spot-morning-spot diagnosis on TB pationts and KAP assessment on health profesionalson selected health facilities in Addis Ababa Region.

The study will be conducted through analysis of sputum sample by ZiehlNelseen and *LED-FM* methods. The information you provide will be used to improve TB diagnosis and to design appropriate public health interventions for future. Your answers will not be released to anyone and will remain anonymous. Your name will not be written on the questionnaire or be kept in any other records. Your participation is voluntary and you may choose to stop the interview at any time. Your participation do not have any influence for your service that you want to use .In addition in your participation in the study do not have any invasive procedure, only give fourconsecutive sputum samples as recommended by the health personnel and each questionnaire only take 5-10minutes. At the end of the study the results will be desteribute to the health biuro and the concerned body.For the successes of our study, we will be asking to give correct answer for respective questions.

Thank you for your assistance.

N.B: If you want to request additional information about the study, you will call by those phone numbers Contact address of PI, 0911 184800

Annex II:Consent Form

I_____ here by giving my consent for giving four consecutive sputum samples as recommended by health personnel for microscopy diagnosis of M.tbandto answer questions. I understand there is no serious invasive procedure at the beginning as well as at the end of the study. I understand this study will be used not only for me but also for other TB positive patients. I know Anti-tuberculosis drugs are available at those health facilities. I believe that at the end of study the result also explain for concerned body only for the purpose of the study.

Signature _____ Date_____

Thank you in helping with this important study

N.B: If you want to request additional information about the study, you will call by those phone numbers Contact address of PI, 0911 184800

Annex III; Amharic Version of Consent Form

Annex IV: Questionnaires

Section- I: KAPquestionnaires for health personnel (HO,GP ,Nurs)

[This section is preferably filled in by laboratory personnel working in TB clinic]
N.B [please read the instruction and asks verbal consent to answer the question]
Dear Colleague.

My name is_____ I am here to conduct MSc research, entitled "Evaluation of the same-day diagnosis of TB in comparison to spot-morning spot method and assessment of knowledge, aptitude and practice on health personnel in same selected health institute in Addis Ababa" Your facility is one among randomly selected health facilities to participate in this study. We would appreciate your assistance in responding to this questionnaire. The information you provide me is confidential and will not be shared with any one else without your consent. No one including your supervisor will know what you tell me.
The information you provide me is extremely important and valuable, as it will help the NTP and the private health facilities in understanding areas and ways of collaboration for the rapid expansion of TB care. However, you have all the right not to respond to questions.

Part II: KAPquestionnaires for health profisionals

Name of the health facility_____ QRE Code no_____

Sub city _____ Kebele _____

Date_____/_____/_____

Part –I : General questions

101.What is your educational status?

1.Diploma
2. BSc.
3.MD
4.MD+Speciality

102. What is your profession?

a)specialist
b)GP
c)HO
d)Nurse
e)Others

103. How many years do you work in TB clinic?

1. Less than or equal to one year
2. Two years
3. Three years
4. Four years
5. Five and more than five years.

Part-II: Knowledge level questions

201. Have you ever heard of the same day-diagnosis of TB microscopy?

Yes

No

202. If yes, what was the source of information?

1. TV, Radio or internet
2. Billboards
3. Newspapers/magazine
4. Friends/Relatives
5. Teachers

203 . If TB diagnosis is done in your facility, what tool (in addition to patient history and physical examination) do you use to diagnose suspects?

1. Both sputum microscopy and X–ray.
2. X-ray
3. Sputum smear microscopy
4. CBC
5. Gram stain

204 . How many sputum specimen(s) is/are required to diagnose pulmonary TB?

1. One
2. Two
3. Three
4. Four
5. I don't know

205 . Would you please tell me the sputum collection time (schedule) for the conventional method at the time of the diagnosis?

1. Spot-morning-spot
2. Spot-X-Spot
3. Morning-spot-morning
4. Spot-morning-morning
5. Morning-spot

20 6 . Do you know the sample collection time of the new WHO policy (the same-day sampling method) on the diagnosis of Tb microscopy?

1. Spot-morning-spot
2. Spot and X-Spot
3. Morning-spot
4. Sputum smear not done
5. I don't know.

20 7 . What is the same-day diagnosis of Tb microscopy?

1. Doing spot-morning-spot sample
2. Doing two spot sample with in two days
3. Doing spot-X-spot sample and giving result in the next day
4. Doing spot-morning-spot with in one day
5. Doing spot-X-spot sample with in one day and gevining the resualt in the same day

208 . Do you have information on the advantage of the same-day diagnosis?

1. Reduce Turn around time (TAT) 3. Rediuse work load 4. I dont know
2. Reducce diagnos defolters 5. Reduce TAT,work load and diagnos defoulter

209 . Do you have information on the limitation of spot-morning-spot sputum sample for the diagnosis of Tb and which could be that.

1. Time of sample collection 2. hase high Result reporting time
3. Work load 4. Have high cost for health fasility and patients
5. All are limtation of spot-mornig-spot sample

210 . Which of the following can be the cause of diagnos defaulter?

1. Time of sample collection
2. Number of sample
3. Time of sample collection,work load, delay of result and number of sample
4. Work load
5. Quality of the test

Part-III: Attitude related questions

301 . Do you agree that the same-day diagnosis reduce turn around time (TAT) ?

1. I strongely agree 2. Agree 3. Neutral 4. Dis agree

5. I strongly dis agree

302 . Do you believe that the same-day diagnosis has equal to that of spot-morning-spot for the diagnosis of tubecrculosis ?

1. I strongely believe 2. I believe 3. Neutral 4. I don't believe

5. I don't strongly believe

303 . Do you agree that you can get acid fast staining bacilli (AFB) result of your patient in one day?

1. I strongely agree 2. I Agree 3. Neutral 4. Dis agree

5. I strongly dis agree

304 . Do you believe that the same-day diagnosis reduce the worke load?

1. I strongely believe 2. believe 3. Neutral 4. I don't believe

5. I don't strongly believe

305 . Do you agree that the same –day diagnosis has adevantage for DOT coverage?

1. I strongely agree 2. I Agree 3. Neutral 4. Dis agree

5. I strongly dis agree

306 . Do you believe that Sputum smear microscopy is the cheapest and quickest method to diagnose pulmonary tuberculosis in a tubrculosis suspect?

1. I strongely believe 2. I believe 3. Neutral 4. I don't believe

5 . I don't strongly believe

307. Do you believe that the same-day diagnosis reduces health facility cost and patient cost?

1. I strongely believe 2. I believe 3. Neutral 4. I don't believe

5. I don't strongly believe

308. When you think about TB diagnosis, do you seriously worry on time of sample collection?

1. Very serious 2. Somewhat serious 3. May or may not be serious

4. Not very serious 5. serious

309. Do your believe that diagnosis delay is a cause for TB diagnosis defaulter? .

1) I strongly believe 2. I believe 3. Neutral

4. I don't believe 5. I don't strongly believe

310. Do you agree that reducing number of sample and complete diagnosis in one day

Can reduce TB diagnosis of defaulter tubrculosis cases ?

1. I strongely agree 2. I Agree 3. Neutral 4. Dis agree

5. I strongly dis agree

Part-IV: Practice related questions

401. How often are you working in Tb clinic?

1) Always 2) usually 3) Some times

4) rarely 5) Never

402. Have you agree that receiving pre service /in service training on subjects related to TB improve TB diagnosis?

1) Strongly agree
2) Tend to agree
3) Neither agrees nor disagrees
4) Tend to disagree
5) Strongly disagree

403 . Do you perform sputum smear examination?

1) Always 2) Usually 3) Some times

4) Rarely 5) Never

404 . How frequent do you order AFB for your patient?

1) Always 2) Usually 3) Some times

4) Rarely 5) Never

405. Do you order spot,x-spot sample for AFB diagnosis?

1) Always 2) Usually 3) Some times

4) Rarely 5) Never

406. In the spot-morning-spot smear microscopy laboratory results are sent back to the OPD with in one day?

1) Always 2) Usually 3) Some times

4) rarely 5) Never

407. How frequently do you order flourecent microscopy for Tb diagnosis?

1) Always 2) Usually 3) Some times

4) Rarely 5) Never

408. How frequently do you order the conventional acid fast staining method (spot-morning-spot)

1) Always 2) Usually 3) Some times

4) Rarely 5) Never

409. How frequently Lab. requests are submitted with complete information?

1) Always 2) Usually 3) Some times

4) Rarely 5) Never

410. Where do patients produce sputa?

1. Always In the open air
2. Usually In the latrine
3. Sometimes In the waiting room
4. Rarely In the laboratory
5. I dont know

Section- II: KAPquestionnaires for laboratory personnel

[This section is preferably filled in by laboratory personnel working in TB laboratory]
N.B [please read the instruction and ask verbal consent to answer the question]
Dear Colleague.

My name is_____ I am here to conduct MSc research, entitled "Evaluation of the same-day diagnosis of TB in comparison to spot-morning spot method and assessment of knowledge, aptitude and practice on health personnel in same selected health institute in Addis Ababa" Your facility is one among randomly selected health facilities to participate in this study. We would appreciate your assistance in responding to this questionnaire. The information you provide me is confidential and will not be shared with any one else without your consent. No one including your supervisor will know what you tell me.
The information you provide me is extremely important and valuable, as it will help the NTP and the private health facilities in understanding areas and ways of collaboration for the rapid expansion of TB care. However, you have all the right not to respond to questions.

Name of the health facility_____ QRE Code no_____

Sub city _____ Kebele _____

Date_____/_____/_____

Part-I: General Questions

101.What is your educational status?

1.Diploma
2. BSc.
3. MSc
4. Certificate
5. Others

102. What is your the level of your profesion?

a) Junior technician
b) Senior technician
c) Technologist
d) Microbiologist
e) Others

103. How many years do you work in TB laboratory?

1. Less than or equal to one year
2. Two years
3. Three years
4. Four years
5. Five and more years.

Part-II: Knowledge level questions

201. How many sputum specimen(s) is/are required to diagnose pulmonary TB?

1. One
2. Two
3. Three
4. Four
5. I don't know

202 . Would you please tell me the sputum collection time (schedule) for the conventional method for the diagnosis of pulmonary tuberculosis at the time of the diagnosis?

1. Spot-morning-spot
2. Spot-X-Spot
3. Morning-spot-morning
4. Spot-morning-morning
5. Morning-spot

203 . Do you know the sample collection time of the new WHO policy (the same-day sampling method) on the diagnosis of Tb microscopy?

1. Spot-morning-spot 2. Spot and X-Spot 3. Morning-spot
4. Sputum smear not done 5. I don't know

.

204 . What familiar method of staining acid-fast bacilli do you use?

1. Gram stains 2. Ziehl-Nelson 3. Simple stain 4. Wright stain 5. I don't know

205 . What is the same-day diagnosis of Tb microscopy?

1. Doing spot-morning-spot sample
2. Doing two spot sample with in two days
3. Doing spot-X-spot sample and giving result in the next day
4. Doing spot-morning-spot with in one day
5. Doing spot-X-spot sample with in one day and gevining the resualt in the same day

206 . Do you have information on the advantage of the same-day diagnosis?

1. Reduce turn around time (TAT) 3. Reduce work load 4. I dont know

2. Reduce diagnosis of defaulters 5. Reduce Turn around time (TAT) ,work load and diagnosis of defailters

207 . What are the adevantage of light emitting diode – fluorescent microscopy (LED-FM) over the convantional fluorescent microscopy (FM) for the diagnosis of TB?

1. Less expensive 2. Require less power (run on batteries)
3. Very long half-life 4.Perform equally well without a darkroom
5. All are adevantage of light emitting diode – fluorescent microscopy (LED-FM)

208 . Do you have information on the limitation of spot-morning-spot sputum sample for the diagnosis of Tb.

1. Time of sample collection 2. Have high Result reporting time

3. Work load 4. Have high cost for health fasility and patients

5. All are limetation of spot-mornig-spot sample

209. Do you know how to dispose infected used materials like sputum containers?

1. By burning 2. By burning after boiling 3. By burning after disinfecting

4. By burial 5. By burying after disinfection

310. What are the main reason(s) that AFB examination is done (ordered)?

1. For diagnosis and follow up
2. Only for follow up
3. Only for diagnosis

4. For screening latent TB

5. Only for Tb surveillance

Part-III: Attitude related questions

301. Do you agree that you can give AFB resualt of your patient in one day?

1. I strongely agree 2. I Agree 3. Neutral 4. Dis agree

5. I strongly dis agree

302 . Do you believe that the same-day diagnosis reduce the worke load?

1. I strongely believe 2. believe 3. Neutral 4. I don't believe

5. I don't strongly believe

303 . Do you believe that Sputum smear microscopy is the cheapest and quickest method to diagnose pulmonary tuberculosis in a TB suspect?

1. I strongely believe 2. I believe 3. Neutral 4. I don't believe

5 . I don't strongly believe

304. Do you believe that light emitting diode - fluorescent microscopy (LED-FM) can replace convational fluorescent microscopy (FM) for TB diagnosis?

1. I strongely believe 2. I believe 3. Neutral 4. I don't believe

5. I don't strongly believe

305. Do you agree that reducing number of sample and complete diagnosis of pulmonary tuberculosis in one day?

1. I strongely agree 2. I Agree 3. Neutral 4. Dis agree

5. I strongly dis agree

306. Do you believe that on job or off job training improve skill of lab personals?

1. I strongely believe 2. I believe 3. Neutral 4. I don't believe

5 . I don't strongly believe

307. Do you agree that participating in external quality assurance (EQA) improve TB laboratory quality?

1. I strongely agree 2. I Agree 3. Neutral 4. Dis agree

5. I strongly dis agree

308. Do you believe that implementing the same-day diagnosis in external quality assurance (EQA) participating TB laboratory is more important?

1. I strongely believe 2. I believe 3. Neutral 4. I don't believe

5 . I don't strongly believe

309. Do you agree that implementing external quality assurance (EQA) for fluorescent stained smear microscopy is difficult?

1. I strongely agree 2. I Agree 3. Neutral 4. Dis agree

5. I strongly dis agree

310. Do you believe that the same-day diagnosis increase smear detection rate?

1. I strongely believe 2. I believe 3. Neutral 4. I don't believe

5 . I don't strongly believe

Part-IV: Practice related questions

401. How often are you working in Tb laboratory?

1) Always 2) Usually 3) Some times

4) Rarely 5) Never

402. Have you received pre service /in service training on subjects related to TB diagnosis in the past three years?

1) Yes, in service training 3) Both pre & in service training

2) Yes, pre service training 4) No 5) I dont know

403 . Do you perform sputum smear examination?

1) Always 2) Usually 3) Some times

4) Rarely 5) Never

404. In the spot-morning-spot smear microscopy at what time laboratory results are sent back to the OPD?

1) Always within two working days 3) Some times within one working days

2) Usually within three working days 4) Rarely reported within two working days

5) Never reported within tow working days

405. How frequently Do you do flourecent microscopy for Tb diagnosis?

1) Always 2) Usually 3) Some times

4) Rarely 5) Never

406. How frequently do you do the conventional one(spot-morning-spot)

1) Always 2) Usually 3) Some times

4) Rarely 5) Never

407. What type of Microscope slide used for AFB examination in the facility?

1. Always new
2. Usually reused
3. Some times new sometimes reused.
4. Most of the time new some times reused
5. Most of the time reused, sometimes new

408. Where do patients produce sputa?

1. Always In the open air
2. Usually In the latrine
3. Sometimes In the waiting room
4. Rarely In the laboratory
5. I dont know

409. When smearing specimen near by the window, what do you think it should be the position of the technician?

1. Always standing by the side
2. Usually facing the window
3. Sometimes Standing opposite to the window
4. Rarely back to the window
5. I dont know

410. Do you use the standard grading system for reporting AFB results?

1) Always 3) Some times
2) Usually 4) Rarely 5. Never

Section II. Patient Identification and demographic questions

Date___/___/_____

Patient Name ----------

Sex ----------

Age----------

Code No_____

Card No. ____

Hospital/ health centres No.____

Occupation

1. Farmer

2. Merchant

3. Governmental worker

4. Other (specify) ----------

Laboratory data

Date of sample collection _____ (dd/mm/yyyy)

Time of sample collection .1st _____ 2nd _____ 3rd _____4th--------

Total no of sample received _____

Result:

a) Completed_____ b) Incomplete _____c) excluded_____

Action taken for the incomplete data_____ (please use additional blank paper if the space is not enough)

Gross appearance of sputum

Haemoptysis____ Purulent _____ Mucopurulent_____ Saliva _____

AFB Result

By principal investigator:

ZN convantional 1+_____2+_____3+_____

LED- FM Direct positive 1+_____2+_____3+_____

ZN same day-smear 1+_____2+_____3+_____

Date and signature of laboratory technician _____

Comment:

_____

_____

_____

_____

Annex VI: Laboratory procedure

Sputum collection *Procedure*

Give the patient confidence by explaining to him/her the reason for sputum collection

Instruct the patient to rinse his/her mouth with water before producing the specimen. This will help to remove food and any contaminating bacteria in the mouth

Instruct the patient to take two deep breaths, holding the breath for a few seconds after each inhalation and then exhaling slowly. Ask him/her to breathe in a third time and then forcefully blow the air out. Ask him/her to breathe in again and then cough. This should produce a specimen from deep in the lungs. Ask the patient to hold the sputum container close to the lips and to spit into it gently after a productive cough. Sputum is frequently thick and mucoid, but it may be fluid, with chunks of dead tissue from a lesion in the lung. The color may be a dull white or a dull light green. Bloody specimens will be red or brown. Thin, clear saliva or nasopharyngeal discharge is not sputum and is of little diagnostic value for tuberculosis.

If the sputum is insufficient encourage the patient to cough again until a satisfactory specimen is obtained. Remember that many patients cannot produce sputum from deep in the respiratory track in a few minutes. Give him/her sufficient time to produce an expectoration, which s/he feels, is produced by a deep cough.

If there is no expectoration, consider the container used and dispose of it in the appropriate manner.

Check that the container is securely closed and label the *container* (not the lid) clearly

Wash hands with soap and water.

Give the patient a new sputum container and make sure that s/he understands that a specimen must be produced as soon as s/he wakes up in the morning.

Demonstrate to the patient how the container should be securely closed.

Instruct the patient to bring the specimen back to the health center or laboratory

Ziehl Neelsen methods: Procedure

Prepare smear as describes; allow air to dry.

Heat fix smear either on an electrics slide warmer at 65 to 75 ^{o}c for at least 2 hours or pass slide through Bunsen burner flame as for other bacteriological smear. Don't over heat.

Cover smear with a 2x 3 cm piece of filter paper to holds the stain on the slide and to minimize precipitation of dye crystal on to the smear.

Flood the paper strip with carbool fuchsine.

Heat the slides to steaming with Bunsen burner or an staining electric racks, let stands 5 minutes .if the smear dries, add more stain add more stain but don't re heat.

Use forceps to remove paper strips from slides and to place them in discard containers wash slides with water (use tap water or water from reservoir bottles)

Flood smear with acid alcohol, allow to distain for 2 minutes

Wash smear with again with water and drain

Floods slides with methylene blue and counter stain for 1 to 2 minutes

Rinse with water, drain and air dry .do not blot

Examine smears under oil immersion objectives lens of the microscope

Examination of smears

The smear should be searched in an orderly manner by making a series of three parallel or nine sweeps the length of smears or nine parallel sweeps the width of the smears.

Reporting of smear results

Smear result should be quantified to be meaningful.

N^{o} of AFB seen report

None…….. No AFB seen

1-2/300 fields……..±

1-9/100 fields……. …….1^{+}

1-9 /10 field's……2^{+}

1-9 /fields……..3^{+}

>9 /fields……..4^{+}

STAINING WITH AURAMINE O SOLUTION

Principle of acid fastness

The nucleic acids of the cell become stained by Auramine O, which later does not lose its

Color despite the action of acids or alcohol.

Auramine O staining procedure:

1. Arrange slides in serial order on staining bridge, with smear side up, at a distance of at least 1cm between every slide.

2. Flood slides with filtered 0.1% Auramine O solution.

3. Do not heat.

4. Keep the staining reagent for at least 20 minutes. Make sure that the smear area is continuously covered with Auramine solution by adding more if needed.

5. Rinse with water and drain.

6. Apply decolorizing solution, 0.5% Acid alcohol, for 3 minutes.

7. Gently rinse with water until the macroscopically visible stain has been washed away and drained.

8. Flood smear with 0.5% Potassium permanganate solution for 1 minute. Time is critical because counterstaining for a longer time may quench the acid-fast bacilli fluorescence.

9. Gently rinse with water and drain.

10. Air dry on a slide rack.

Annex VI: WHO Grading of Sputum Microscopy Results

Table. 1 IUATLD/WHO recommended grading of sputum microscopy results

IUATLD/WHO scale (1000x field = HPF) **Result**	**MICROSCOPY SYSTEM USED**		
	Bright field (1000x magnification 1 length = 2 cm = 100 HPF	Conventional fluorescence (200-250x magnification 1 length=30 fields = 300 HPF	iLED fluorescence (400xmagnification; 1 length=40 fields = 200 HPF
Negative	Zero AFB/1 length	Zero AFB/1 length	Zero AFB/1 length
Scanty(actual count)	1-9 AFB/1 length or100 HPF	1-29 AFB/1 length	1-19 AFB/1 length
1+	10-99 AFB/1 length or100 HPF(1-9 AFB/10field	30-299 AFB/1 length	20-199 AFB/1 length
2+	1-10 AFB/1 HPF on average	10-100 AFB/1 Field on average	5-50 AFB/1 Field on average
3+	≥10AFB/1 Field on average	≥100 AFB/1 Field on average	>50AFB/1 Field on average

2). **N-acetyl L-cysteine- Sodium hydro oxide method**

NaOH is toxic, both for contaminants and also for tubercle bacilli; therefore, strict adherence to the indicated timings is required.

Reagents: NALC-NaOH: 4% and Phosphate buffer 0.067M, pH 6.8

Procedure:

Step1-Weight 4g in 100 ml distill water

Step2- Weight 2.97 g in 100 ml distill water

Step 3- Mix step 1 &2

Step 4- Add 0.5g NALC

Sputum processing

Step 1- transfer the sputum (at least 2 ml ,not more than 5 ml) in to a centrifuge

Step 2- add equal volumes of NALC-NaOH solution

Step 3- tighten cap of container and vortex slowly

Step 4-shake intermittently to aid homogenization and decontamination

Step 5-invert each bottle to ensure that NaOH solution contacts all the sides and inner portions of caps

Step 6-keep at 20 ^{o}c -25^{o}c for 15 min for decontamination

Step 7-fill the tube with phosphate buffer up to 50 ml mark on the tube

Step 8- vortex

Step 9- centrifuge at 3000g for 15 min

Step 10-carfully pours off the supernatant in to a discarded can containing 5 % phenol or other germicide

Step 11-inoculate deposit on to two slopes of L-J medium labeled with the ID number

Step12-use a pipette inoculate each slope with 3 to 4 drops

Step 13-smear on a slide with the ID number for microscope examination

3. Preparation of egg-based LJ media

LJ medium containing glycerol favors the growth of M. tuberculosis while LJ medium without glycerol but containing pyruvate encourages the growth of M. bovis. Both should be used in countries or regions where patients may be infected with either organism. And LJ medium prepared according to EHNRI standards.

Ingredients:

A) **Mineral salt solution**:

-Potassium dihyderogen phosphate anhydrous (KH2PO4) ---2.4g

-Magnesium sulphate (MgSO47H2O) 0.2g

-Magnesium citrate 0.6g

-Asparagines …….3.6g

Glycerol (reagent grade)……...12ml

-Distilled water……..600 ml

NB: Dissolve the ingredients in the distilled water by heating, autoclave at 121^{oc} for 30 minutes to sterilize. Cool to room temperature. This solution keeps indefinitely and may be stored in suitable amounts in the refrigerator.

B) **Malachite green solution:**

-Malachite green dye……2.0g

-Sterile distilled water …….100ml

NB: Using aseptic techniques dissolve the dye in sterile distilled water by placing the solution in the incubator for 1-2 hours. This solution will not store indefinitely and may precipitate or change to a less-deeply colored solution. In either case discard and prepare a fresh solution.

c). Homogenized whole eggs

Scrubbing thoroughly with a hand brush in warm water and a plain alkaline soap cleans fresh hens' eggs, not more than seven days old. Let the eggs soak for 30 minutes in the soap solution. Rinse eggs thoroughly in running water and soak them in 70% ethanol for 15 minutes. Before handling the clean dry eggs scrub the hands and wash them. Crack the eggs with a sterile knife into a sterile flask and beat them with a sterile egg whisk or in a sterile blender.

d). Preparation of complete medium

The following ingredients are aseptically pooled in a large, sterile flask and mixed well:

Mineral salt solution…….600 ml

Malachite green solution …….20 ml

Homogenized eggs (20-25 eggs, depending on size….1000ml

Finally the complete egg medium is distributed in 6-8ml volumes in sterile 14ml or 28ml McCartney bottles or in 20ml volumes in 20 x 150mm screw-capped test tubes, and the tops are securely fastened.

e). **Coagulation of the medium**

Before loading, heat the inspissator to 80^{oc} to quicken the build-up of the temperature. Place the bottles in a slanted position in the inspissator and coagulate the medium for 45 minutes at 80 ^{oc}-85^{oc} (since the medium has been prepared with sterile precautions this heating is to solidify the medium, not to sterilize it). Heating for a second or third time has a detrimental effect on the quality of the medium.

The quality of egg media deteriorates when coagulation is done at too high a temperature or for too long. Discoloration of the coagulated medium may be due to excessive temperature. The appearance of little holes or bubbles on the surface of the medium also indicates faulty coagulation procedures. Poor quality media should be discarded

f). Sterility cheek: After inspissations, the whole media batch or a representative sample of culture bottles should be incubated at 35^{oc}-37^{oc} for 24 hours as a check of sterility.

j).Storage: the LJ medium should be dated and stored in the refrigerator and can keep for several weeks if the caps are tightly closed to prevent drying out of the medium. For optimal isolation from specimens, LJ medium should not be older than 4 weeks.

3.1. **Quality Control**

3.1. **Sensitivity of plain egg based medium.**

Serious problems affecting the sensitivity of culture medium, i.e. its capacity to sustain consistent growth of tubercle bacilli, can be detected by seeding a 1/10.000 dilution of a suspension of Mycobacterium tuberculosis calibrated to McFarland No 1. (Equivalent to a bacterial suspension containing 1 mg/ml of tubercle bacilli)

- Prepare a McFarland No 1 suspension with a M. tuberculosis reference strain.
- Dilute the suspension with 10-fold dilutions to the 10^{-4} dilution.
- Five tubes of a previous batch of medium and 5 tubes of the new batch of medium are inoculated with 0.2 ml of the 10^{-4} diluted suspension.
- Incubate at 36°C+/- 1°C

- ❖ If the number of colonies obtained on the recently prepared or purchased batch is significantly lower than on reference batch of medium, the sensitivity of the new medium, whether prepared or purchased, is not adequate.

This register allows the identification and the elimination of deficient media batches. In the case of egg-based media, 20 days of incubation are usually enough to determine whether the sensitivity of the batch is satisfactory. If it is not, negative culture results obtained with tubes inoculated with the deficient medium will be invalidated and these cultures will be repeated. Media batches that are not homogeneous or contaminated, those that were exposed to high temperatures of inspissations as well as those showing low sensitivity, should never be used and should be discarded without delay.

3.2. **Reading: Solid media**:

- ➢ Make sure that cultures are checked at regular intervals:
- ➢ At 3 days of incubation to detect and to register early contamination
- ➢ Weekly to detect growth as early as possible.
- ➢ Confirm that new specimens have been requested in those cases when the smear positive specimens turn out to be culture negative or when all inoculated tubes/vials are contaminated.

3.3. Determination of the contamination rate: The contamination rate is a valuable indicator of the efficiency of procedures used for specimen processing. It is calculated as the percentage of contaminated tubes among all inoculated tubes or vials and not as the percentage of patients.

It should be within the range 2-4% and not exceed 5%, if the Petroff decontamination method is used. When available, computer databases should be preferred to hard copies forms to register and monitor results of positive patients and culture quality indicators

4. Main biochemical tests to identify M. tuberculosis

A). **Niacin accumulation test.** Nicotinic acid or niacin is produced by all mycobacteria, but some species, such as M. tuberculosis, Mycobacterium simiae and M. bovis BCG excrete it due to a blockade in their scavenging pathway. The excreted niacin accumulates in the culture

medium and is evidenced in the presence of cyanogen halide with a primary amine. Niacin-negative M. tuberculosis strains are extremely rare.

B). **Growth in the presence of p-nitro benzoic acid.** This compound inhibits the growth of several species in the M. tuberculosis complex: M. tuberculosis, M. bovis, M.africanum, and M. microti (Tsukamura 1984, Leão 2004).

C). **Nitrate reduction test.** This test is particularly useful for differentiating M. tuberculosis, which gives a positive reaction, from M. bovis, which is negative (Tsukamura 1984, Vincent 2003).

D). **Catalase test.** Catalase is an intracellular enzyme that transforms hydrogen Peroxide to oxygen and water. The 68°C catalase is a heat-tolerance test measuring the catalase activity at high temperature. Characteristically, M. tuberculosis gives negative results, as do other species in the M. tuberculosis complex. (Vincent 2003)..

5. **Waste management and other safety precautions-**

Used pipettes are collected inside the BSC in appropriate containers, metal or thermo resistant plastic bins, containing disinfectant (see SOP # 23). Test tubes with bacterial suspensions, if screw-capped tightly, can be sprayed with disinfectant and later be autoclaved as well as the pipettes. More or less open test tubes with suspensions in racks need to be tightly boxed before transfer to the autoclave. When tubes of solid cultures are discarded in solid containers (instead of autoclavable plastic bags), water with disinfectant should be added to the bottom of containers before autoclave. Otherwise steam may not be reach cultures and tubercle bacilli may be alive after a standard autoclave cycle. Gloves and other waste may be collected in an autoclavable plastic bag, which has to be closed and autoclaved