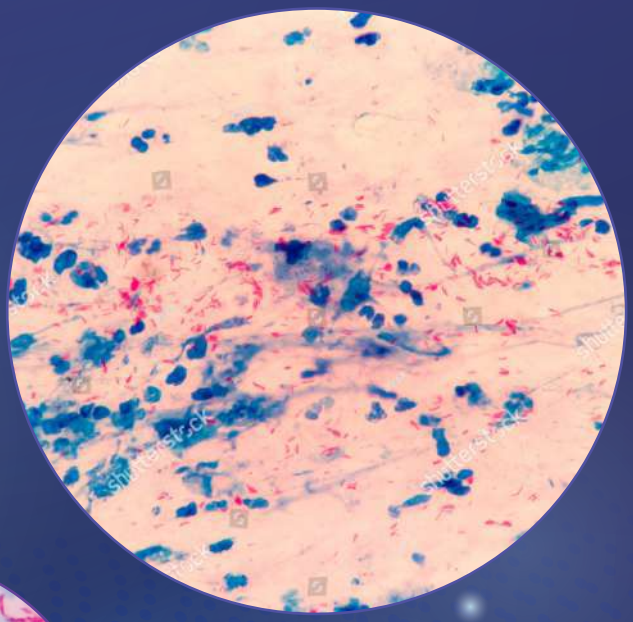
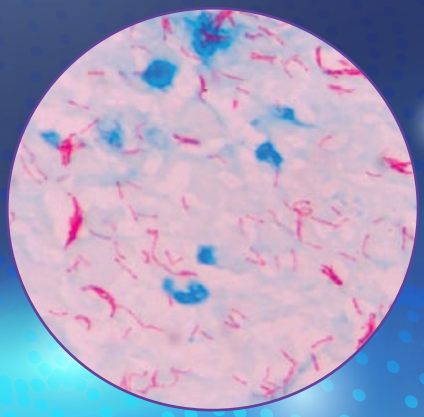
LABORATORY MANUAL FOR TUBERCULOSIS CONTROL

FIFTH EDITION, 2021





NATIONAL PROGRAMME FOR TUBERCULOSIS CONTROL AND CHEST DISEASES, MINISTRY OF HEALTH,SRI LANKA

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FIFTH EDITION, 2021

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FOREWORD

The National Programme for Tuberculosis Control and Chest Diseases (NPTCCD) is the national focal point for tuberculosis control and prevention activities in Sri Lanka. Successful outcomes of tuberculosis management, at individual as well as at public health level depends on early and accurate diagnosis. Therefore, it is essential that all laboratory diagnostic settings, methods, procedures and performances are in accordance with to the current international requirements and standards.

The laboratory network of the NPTCCD includes the microscopy centers, laboratories at district chest clinics and at hospitals, intermediate tuberculosis laboratories and the National Tuberculosis Reference Laboratory (NTRL) at Welisara. Performing standard techniques in TB microbiology is very essential for having accurate and reliable results in TB diagnosis and monitoring since some of the treatment options depend solely upon the lab diagnosis. In addition to provision of diagnostic services, the laboratories also support the national TB control by providing reports and returns to maintain the TB surveillance system in the country, which is essential in controlling TB burden in the country.

This island wide laboratory service network is coordinated, monitored and supervised by the NTRL team, headed by the Consultant Microbiologist.

The current Laboratory Manual by the NPTCCD was published in 2010. Hence, updating the manual to meet the current needs of TB laboratory diagnosis as well as to incorporate the latest evidence, including quality assurance of the laboratory activities is a timely necessity. Therefore, it is essential that all diagnostic settings, methods, procedure, and performance are not only complies to the current international requirements, but also to be standard, nationally.

I am thankful to the Sri Lanka College of Microbiologists for providing the technical assistance in formulating the revision of the Laboratory Manual for Tuberculosis Control (5th Edition). I would like to give my special thanks to all the Consultant Microbiologists who were in the editorial board namely Dr Jayanthi Elvitigala, Dr Dammika Vidanagama, Dr Dushani Jayawardena, and Dr Bhagya Piyasiri for their commitment and enthusiasm. In this service endeavor I am appreciating the hard work done by Dr Dushani Jayawardena, the Consultant Microbiologist of the NTRL, Welisara. Finally, my appreciation also extended to Dr E.M.D.N.S Ekanayake, Medical Officer, NTRL for her contribution in the revision of Lab Manual and Dr R.J.M. Amali.J Senanayake, Medical Officer of NPTCCD for coordinating this activity.

I sincerely hope that Laboratory Manual for Tuberculosis Control (5th Edition) will help considerably in our efforts aiming at elimination of Tuberculosis from Sri Lanka.

Dr H.D.B Herath

Director, NPTCCD

PREFACE

The fourth edition of laboratory manual for tuberculosis control by National Programme for Tuberculosis Control and Chest Diseases was published in 2010. It resulted in the revision and development of guidelines for laboratories with an aim to standardize the laboratory methods and practices throughout the country.

It has been just more than a decade since 4th edition was published. New knowledge and recent advances in technology now has led to the development of rapid, easy to perform and more sensitive tests in particular molecular diagnostics tools to detect tuberculosis cases.

In parallel with these advances, more sensitive newer diagnostic tests have been introduced in to the national tuberculosis control programme to improve case detection. It was highlighted that the recommendations for these newer tests be included in the TB laboratory manual in order to share new knowledge with laboratory personnel working in TB laboratories.

The aim of this manual is to give basic and fundamental knowledge on principles, methodologies and interpretation of test results of the new diagnostics to the laboratory personal working in the TB laboratories in the country while emphasizing the safe laboratory practices.

This manual contains the new diagnostics such as culture and drug susceptibility testing using liquid media, line probe assay and Xpert MTB/RIF, interferon gamma release assay and lateral flow urine lipoarabinomannan assay. In addition, the diagnostic tests in the 4th edition were detailed. Many current WHO recommendations, already published in guideline documents for TB diagnosis, are referred to in this guidance.

Each chapter of the manual has been written by one of the authors who have long experience in the field of TB diagnosis. All chapters were reviewed by the authors. Their tremendous hard work amid busy schedules especially during this period is highly appreciated.
Without their support, this would not have been a success.

Dr. Dushani Jayawardhana
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ABBREVIATIONS

ADA	**-** Adenosine Deaminase
AFB	- Acid Fast Bacilli
AST	- Antibiotic Susceptibility Testing
ATT	- Anti Tuberculosis Treatment
BAL	- Broncho Alveolar Lavage
BAP	- Blood Agar Plate
BCG	- Bacillus Calmette-Guérin
BSC	- Bio Safety Cabinet
BSL	- Bio Safety Level
CCC	- Central Chest Clinic
CSF	- Cerebro-spinal Fluid
CXR	- Chest X Ray
DCC	- District Chest Clinic
DR TB	- Drug Resistant Tuberculosis
DST	- Drug Susceptibility Test
DTCO	- District TB Control Officer
EPTB	- Extra Pulmonary Tuberculosis
EQA	- External Quality Assessment
FL	- First Line
FM	- Fluorescence Microscopy
HIV	- Human immunodeficiency Virus
IGRA	- Interferon Gamma Release Assay
INAH	- Isoniazid
IQC	- Internal Quality Control
ITL	- Intermediate Culture Laboratory
IU	- International Unit
LAM	- Lipoarabinomannan
LED	- Light Emitting Diode
LJ	- Lowenstein-Jensen
LPA	- Line Probe Assay
LTBI	- Latent Tuberculosis Infection
MC	- Microscopy Center
MGIT	- Mycobacterium Growth Indicator Tube
MLT	- Medical Laboratory Technologist
MOTT	- Mycobacteria Other Than Tuberculosis
MTB	- *Mycobacterium tuberculosis*
MTBC	- *Mycobacterium tuberculosis* Complex
mWRD	- molecular WHO recommended Rapid Diagnostics Test
NAAT	- Nucleic Acid Amplification Test
NEQA	- National External Quality Assurance
NPTCCD	- National Programme for Tuberculosis Control and Chest Disease
NTM	- Non Tuberculosis Mycobacteria

NTRL	- National Tuberculosis Reference Laboratory
PCC	- Probe Check Control
PCR	- Polymerase Chain Reaction
PHLT	- Public Health Laboratory Technician
PMDT	- Programmatic Management of Drug Resistant TB
PPE	- Personal Protective Equipment
PTB	- Pulmonary Tuberculosis
QC	- Quality Control
RBRC	- Random Blinded Re-Checking
RDHS	- Regional Director of Health Services
RIF	- Rifampicin
RR/MDR TB	- Rifampicin-Resistant or Multidrug TB
SL	- Second Line
SPC	- Specimen Processing Control
SR	- Sample Reagent
TB	- Tuberculosis
TH	- Teaching Hospital
TST	- Tuberculin Skin Test
VWS	- Ventilated Work Station
WHO	- World Health Organization
XDR-TB	- Extensively Drug Resistant Tuberculosis
ZN	- Ziehl Neelsen

DEFINITIONS

Presumptive Tuberculosis: A case of presumptive Tuberculosis (TB symptomatic) is a person who presents with symptoms or signs suggestive of tuberculosis (TB), particularly cough for two weeks or more.

Case of bacteriologically confirmed TB: A patient whose sputum or another biological specimen is positive for AFB by smear microscopy, culture or WHO approved Rapid molecular Diagnostics (mWRD) such as Xpert MTB/RIF.

Culture positive TB: A patient with or without sputum smear positive for AFB but sputum or any biological specimen culture testing positive by culture for *M. tuberculosis*.

mWRD positive TB: A patient with or without sputum smear positive for AFB but sputum or any biological specimen testing positive on Xpert MTB/RIF for *M. tuberculosis.*

Pulmonary Tuberculosis (PTB): Any bacteriologically confirmed or clinically diagnosed case of TB involving the lung parenchyma or the tracheo-bronchial tree with or without the involvement of any other organs in the body.

Extra pulmonary Tuberculosis (EPTB): Any bacteriologically confirmed or clinically diagnosed case of the involving organs other than the lung parenchyma or tracheo-bronchial tree, e.g. pleura, lymph nodes, abdomen, genitourinary tract, skin, bones and joint, meninges.

Rifampicin Resistance (RR): Resistance to rifampicin detected using phenotypic or genotypic methods, with or without resistance to other anti-TB drugs except isoniazid.

Multi-drug resistance (MDR): TB in a patient, whose infecting isolates are resistant in-vitro to both isoniazid and rifampicin with or without resistance to other first line drugs.

Pre-XDR-TB: TB caused by *Mycobacterium tuberculosis* (M. tuberculosis) strains that fulfil the definition of MDR/RR-TB and that are also resistant to any fluoroquinolone.

Extensively Drug Resistant TB (XDR-TB): TB caused by *Mycobacterium tuberculosis* (M. tuberculosis) strains that fulfil the definition of MDR/RR-TB and that are also resistant to any fluoroquinolone and at least one additional Group A drug.

01 INTRODUCTION

This manual is intended for laboratory staff serving in the National Tuberculosis Laboratory Network and other governmental and private sector laboratories involving in the diagnosis of tuberculosis (TB). It provides basic knowledge on commonly used methods in the diagnosis of TB.

1.1 Tuberculosis

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (MTB) complex. It occurs in both adults and children.

TB commonly affects the lungs but can also affect any other organ in the body. Most infections show no symptoms, in which case it is known as latent tuberculosis infection (LTBI). About 10% of latent infections progress to active disease if left untreated.

In pulmonary TB (PTB) where the lung parenchyma and the bronchial tree are affected by TB, the tubercle bacilli may be excreted in sputum and it is the most infectious form of TB maintaining the transmission in the community.

TB is one of the top 10 causes of death worldwide. It is also the leading killer of people living with HIV (Human Immunodeficiency Virus) and a major cause of deaths related to antimicrobial resistance.

In 2019, an estimated 10.0 million (range, 8.9 –11.0 million) people fell ill with TB worldwide while people living with HIV accounted for 8.2% of the total. According to the WHO (World Health Organization) global tuberculosis report 2020**,** around 1,400,000 people died of TB in 2019. A total of 206,030 people with multidrug or rifampicin resistant TB (RR/MDR TB) were detected and notified in 2019.

One third of the world's burden of TB or about 4.9 million prevalent cases are found in the South East Asia and 50 percent of the deaths due to TB occur in this region.

In Sri Lanka, the incidence of TB was estimated at 64/100,000, with 7258 notified cases in 2020, the second lowest in the Region. TB burden was as high as 740 per 100,000 population in 1964. This number was reduced to an estimated incidence of 66/100,000 by the year 2000. It has been more or less static throughout past decades (End term Programme Review Report 2021).

1.2 Organization of TB control in Sri Lanka

National TB control efforts are directed by the National Programme for Tuberculosis Control and Chest Diseases (NPTCCD), which is a directorate under the Ministry of Health, Sri Lanka. The Central Unit, National Tuberculosis Reference Laboratory (NTRL), Central Drug Stores of the NPTCCD, and Central Chest Clinic (CCC) Colombo and District Chest Clinics (DCC) Gampaha are under the direct administrative purview of the NPTCCD.

The NPTCCD implements its activities through a network of District Tuberculosis Control Officers (DTCOs) and DCCs which carry out TB control functions in the district level, as well as the follow up of other chest conditions. Except in Colombo and Gampaha where the CCC Colombo and DCC Gampaha come directly under the NPTCCD, the other DCCs report to the Regional Director of Health Services (RDHS).

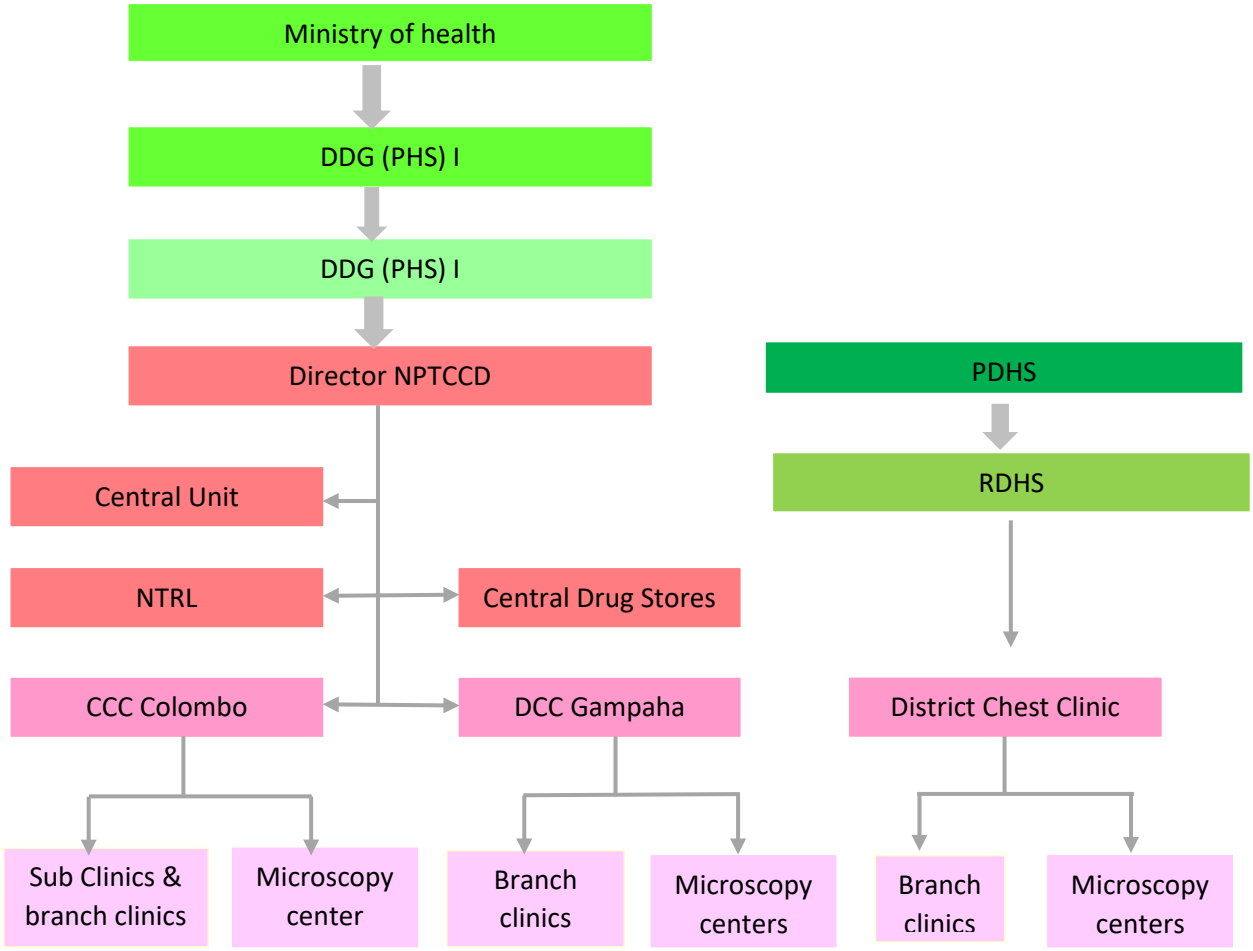


Figure 1.1 – Organizational Structure of National TB Control Programme at National and Provincial Level

1.3 Diagnosis of TB and laboratory Diagnostics

The diagnosis of TB is often the result of a combination of clinical, radiological, epidemiological evidence, microbiological and histological examination of the suspected clinical specimen.

Traditionally, TB diagnosis has been performed using sputum smear microscopy, a method developed more than 100 years ago, and remained the cornerstone of TB diagnosis in the world for many decades. For universal application in resource limited setting, microscopy of stained sputum smears is still the best choice among the TB diagnostic methods despite suboptimal sensitivity.

TB cultures can provide a definitive diagnosis of TB. Culture of MTB on liquid or solid media is a more sensitive method for TB diagnosis than smear microscopy and it permits testing for drug resistance. However, diagnosing TB with culture can take weeks because of the slow growth rate of mycobacteria.

Due to Low sensitivity of smear microscopy and the long turnaround time taken for TB cultures, rapid TB diagnostic tests with high sensitivity have been attempted for few decades to improve the case detection including smear-negative TB cases. Conventional Polymerase Chain Reaction (PCR) methods were developed but the test was cumbersome, needed additional infrastructure, trained staff and time consuming.

Molecular diagnostic test, Xpert MTB/RIF assay came in to light in 2010 and was promising against these challenges. The Xpert MTB/RIF assay was endorsed by WHO in 2011 and is considered an important breakthrough in the fight against TB. It is an automated real time PCR test utilizing the GeneXpert platform. Xpert MTB/RIF assay is a single test that can detect both MTB complex (MTBC) and rifampicin resistance simultaneously within a short period of time with minimal hands on technical time. For the first time, a molecular test was simple and rapid in diagnosing TB. Xpert MTB/ultra, an improvement of MTB/RIF assay with lower detection limit with higher sensitivity was introduced later by the same manufacturer. More recently, Xpert MTB/XDR was endorsed by the WHO for the detection of rifampicin, isoniazid and fluoro-quinolones for drug resistant patients.

In 2008, the WHO endorsed the use of the line probe assay (LPA), for the rapid detection of multidrug resistant TB (GenoType MTBDR*plus*). The version 2 of this assay was developed to improve the sensitivity of MTBC detection and to simultaneously detect resistance to rifampicin and isoniazid. Second line (SL) line probe assay (GenoType MTBDR*s*l*V*2) which detects the mutations associated with fluoro-quinolone and second-line injectable drug resistance became available in 2015.

1.4 Newer tests

New classes of technologies[2] recommended and associated products evaluated for TB diagnosis include,

- Abbott RealTime MTB and Abbott RealTime MTB RIF/INH (Abbott)
- BD MAX MDR-TB (Becton Dickinson),
- cobas MTB and cobas MTB-RIF/INH (Roche)
- FluoroType MTBDR and FluoroType MTB (Bruker/Hain Lifescience)
- Xpert MTB/XDR (Cepheid)
- Genoscholar PZA-TB II (Nipro)

1.5 Advancement in diagnosis of TB in Sri Lanka over the years

Over many decades, diagnosis of TB in Sri Lanka has been mainly based on the sputum smear microscopy and limited to a small number of TB cultures and DST (Drug Susceptibility Test) performed for first line (FL) drugs isoniazid, rifampicin, ethambutol and streptomycin using solid media (LJ - Lowenstein Jenson medium).

In 2010, LPA was introduced in to the national TB control programme by NTRL as the first rapid molecular diagnostic test to detect MTB and drug resistance to both rifampicin and isoniazid simultaneously. This reduced turnaround time to 48 hours compared to 8 weeks taken for solid DST to detect drug resistant TB (DR TB), thus resulting in early treatment for drug resistant TB without delay.

In 2012, rapid molecular diagnostic test, Xpert MTB/RIF assay which detect MTB and rifampicin resistance simultaneously was established at NTRL. The test was simple and easy to perform without much infrastructure facilities. Turnaround time was further reduced to 02 hours. Xpert MTB/RIF assay improved the case detection especially among smear negative cases. As a result, Xpert MTB/RIF assay was expanded to 09 districts at the end of 2018. By the end of 2019, there were 29 Xpert testing sites covering all the districts in the country.

TB culture and DST using liquid media were established at NTRL in 2008, 2020 respectively. Currently susceptibility testing to first line drugs including pyrazinamide is available using liquid DST. As turnaround time of liquid DST is short (10 to 14 days) compared with solid DST (08 weeks), NTRL is in the transition from solid DST to liquid DST.

1.6 Attempts to overcome challenges

Sri Lanka is a low burden country for TB and TB is still a major health problem in the country. Of the estimated 14,000 cases, 7,258 were notified in Sri Lanka with little more than the half of those confirmed microbiologically in 2020. The total number of cases continues to remain static for nearly two decades with small annual fluctuations. There is a gap of 4000 to 5000 missing TB cases each year over these years.

This highlights the need for improving case detection using more sensitive diagnostic tests. Optimum utilization of diagnostic tests is essential to detect suspected TB cases in a systematic way at all levels to close the diagnostic gap.

molecular WHO recommended rapid diagnostic tests (mWRD) were scaled up and rolled out to all 26 districts in the country by 2019. This was aimed at improving case detection with provision of universal drug susceptibility testing for microbiologically confirmed TB cases. The turnaround time for TB & RRTB diagnosis remarkably improved following the roll out of mWRD. Diagnostic algorithm (Annex 1, 2) was improved in 2018 to optimize the utilization of TB diagnostics including microscopy, chest X Ray (CXR), mWRD and culture.

1.7 TB laboratory services

TB diagnostic services are freely available and easily accessible to patients at all levels of health care institutions throughout the country. Laboratories in both government sector and private sector offer tests for the diagnosis of TB. Diagnostics services offered for TB are provided free of charge in the government health sector under the technical guidance of NTRL and function as a network of National TB Laboratories.

NTRL is the apex laboratory of the National TB Laboratory Network which consists of 04 intermediate TB culture laboratories (ITL), 29 GeneXpert testing sites, 26 district chest clinic laboratories and 150 microscopy centers (MC) across the country.

In addition to the laboratories in the ministry of health, TB laboratories in the department of prison and rehabilitation, medical faculties of universities and in the ministry of defense also contribute in the diagnosis of TB.

Private sector laboratories especially in the major cities increasingly involved in the diagnosis of TB now.

1.7.1 National TB laboratory network

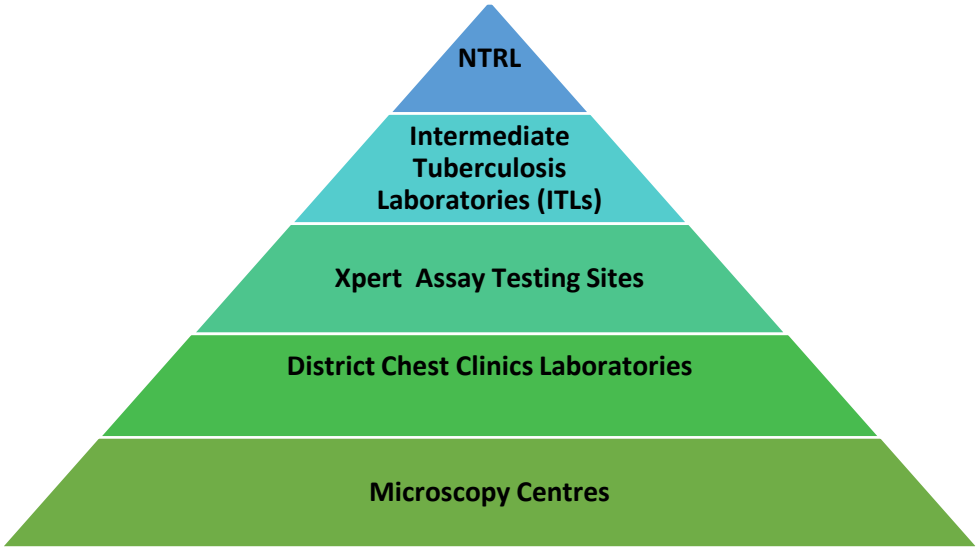


Figure 1.2 – National Tuberculosis Laboratory Network.

A. Microscopy centers

The MCs are located country wide mainly at hospitals in the periphery as the ground level facility for smear microscopy. These centers are linked to district laboratories. The district level laboratories are the next level of laboratories which provides TB smear microscopy services. Some of the hospital laboratories provide the services specially when there are no dedicated microscopy centers in the facility. These are also considered as microscopy centers, in functionality for External Quality Assessment (EQA) purposes.

Microscopy centers have basic facilities for sputum collection and smear microscopy. Smear microscopy is performed by the Public health laboratory technician (PHLT) under the supervision of the DTCO in the relevant district.

B. District chest clinic laboratories

DCC laboratories have facilities for sputum collection, smear microscopy for TB diagnosis. Other tests such as full blood count, liver function test required for monitoring of patients are also performed at DCC laboratories. Some of the DCC laboratories (DCC Anuradhapura, DCC Colombo) perform Xpert MTB/RIF assays using GeneXpert platform. Specimens collected at DCC laboratories and hospitals in the district for further TB diagnostics e.g. TB culture and MTB/RIF assays are dispatched to the NTRL or relevant GeneXpert sites and ITLs by the DCC laboratories.

DTCOs are responsible in carrying out EQA for smear microscopy for peripheral microscopy laboratories in the district.

Figure 1.3– Distribution of MC in Sri Lanka.

Figure 1.4 – Distribution of DCC Laboratories in Sri Lanka.

C. GeneXpert sites

GeneXpert platforms for Xpert MTB/RIF assays are mainly available in major hospitals in the districts, the list of sites given in the Annex 18. In addition, Xpert assay facilities have also been established at DCC Colombo, DCC Jaffna, DCC Kandy, DCC Anuradapura and Faculty of Medicine Colombo. The assay is performed by MLTs (Medical Laboratory Technologists) working in the microbiology laboratories where the GeneXpert machines are installed and under the supervision of respective Consultant Microbiologists.

Figure 1.5 – GeneXpert sites in Sri Lanka, 2021 (Annexure 18).

D. Intermediate TB culture laboratories (ITLs)

Currently, there are four ITLs in the country (Table 1.1). Two more ITLs have been proposed; one at Teaching Hospital (TH) Anuradhapura and Teaching Hospital Batticaloa.

ITLs provide TB diagnostic services including smear microscopy, TB culture, and Xpert MTB/RIF assay. Each ITL has a catchment area of its own for receiving clinical specimens for TB cultures and Xpert MTB/RIF assay. All cultures which showed a growth (positive cultures) are transported to NTRL for DST.

ITLs are under the supervision of Consultant Microbiologist. TB cultures and Xpert MTB/RIF assays are performed by MLTs.

Liquid culture facilities will be expanded to intermediate culture laboratories during the next three years to capture all the presumptive smear negative TB cases.

Table 1.1 – Intermediate Laboratories in Sri Lanka.

	ITLs	Catchment Area	Tests available	Supervised by
1	DCC Jaffna	Jaffna, Mullaitivu, Kilinochchi, Mannar	Smear Microscopy, Xpert assay, Solid culture	Consultant Microbiologist, TH Jaffna
2	DCC Kandy	Kandy, Matale, Nuwara Eliya	Smear Microscopy, Xpert assay, Solid culture	Consultant Microbiologist, National Hospital, Kandy
3	TH Karapitiya	Galle, Matara, Hambanthota	Xpert assay, Solid culture	Consultant Microbiologist, TH Karapitiya
4	TH Ratnapura	Ratnapura, Badulla, Monaragala	Xpert assay, Solid culture	Consultant Microbiologist, TH Ratnapura
5	TH Anuradhapura (Proposed)	Anuradhapura, Polonnaruwa, Vavuniya		
6	TH Batticaloa (Proposed)	Batticaloa, Ampara, Kalmunai, Trincomalee		

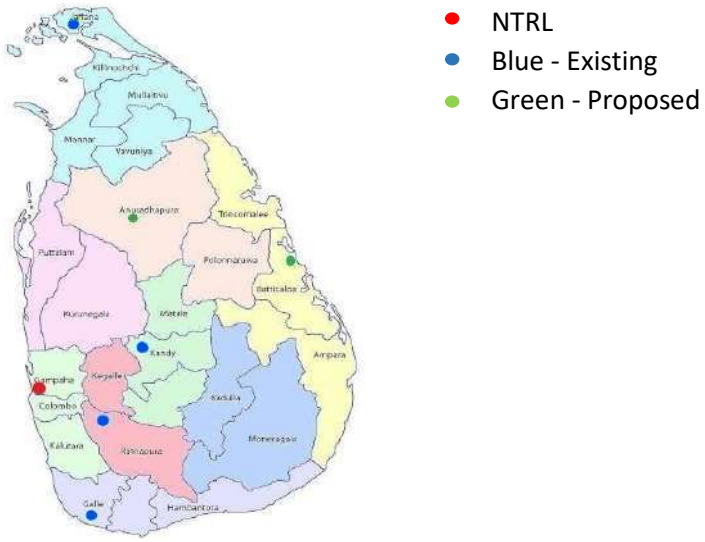


Figure 1.6 – Distribution of ITLs and NTRL in Sri Lanka.

E. National Tuberculosis Reference Laboratory (NTRL)

National Tuberculosis Reference Laboratory is the reference laboratory in the country for TB diagnosis. It is the apex laboratory of the national TB laboratory network. The BSL3 (Bio Safety Level) laboratory was planned in 2010, built during 2013-2014 and commissioned in 2015.

The NTRL comprises of fully functional microscopy, culture, DST, molecular testing sections and a media preparation section for solid culture media (LJ) to fulfill the need of the ITLs in the country.

NTRL participates in EQA programme conducted by supranational TB reference laboratories. NTRL is expected to obtain ISO 15189:2013 by 2021-2022.

Facilities

NTRL has a full battery of diagnostic facilities and is well equipped with bright field, LED (light emitting diode) fluorescent microscopes for smear microscopy and functions as the NEQAs (National External Quality Assurance) provider for the network of TB laboratories.

Fully automated TB culture system (BD BACTEC) for liquid culture, liquid DST and other necessary equipment for culture and DST using solid media are available with backup systems in the laboratory.

mWRD, GeneXpert which is an automated real time PCR is used for rapid diagnosis of TB and detection of rifampicin resistance. LPA technology (Hain LifeSciences) is also available as a molecular diagnostic test for diagnosis of TB and detection of resistance to first line drugs, rifampicin and isoniazid and to second line drugs fluro-quinolones and aminoglycosides.

Services provided by NTRL

- Diagnostic tests
    - Smear microscopy.
    - Solid culture and identification/DST.
    - Liquid Culture and identification/DST.
    - Xpert MTB/RIF assay.
    - LPA – For RR/MDR TB (1st line & 2nd line DST).

- Capacity building – NTRL organizes and deliver training as follows,
    - In course training - PHLTs, MLTs, Nurses, Medical officers, DTCOs, post graduate trainees in microbiology, respiratory medicine and venereology are given training in TB diagnosis and infection control.
    - Recruitment training.
    - Periodic in service refresher training for all categories of staff.

- Supervision & Monitoring
    - NTRL is responsible for supervision and monitoring of all the laboratories in TB laboratory network. It is the responsibility of the NTRL to ensure that all laboratories including private a sector which provide TB diagnostic services meet the WHO quality standards.
    - It's also the responsibility of NTRL to carry out supervision, on site evaluation and proficiency testing for DCC annually.

- National External Quality Assurance (NEQA) programme
    - Currently, NTRL carries out EQA for smear microscopy for DCC laboratories. Proficiency testing panels are provided to all microscopy centers in the network on annual basis.
    - NTRL is expected to establish EQA programme for Xpert assays and drug susceptibility test from 2022.

- Research
    - Develop and carry out operational research and make recommendation for introduction of new technology to the network laboratories where relevant.

- Laboratory data management
    - NTRL collects data of microscopy, Xpert assays and cultures performed on monthly basis from all the laboratories in the network. These data is used to monitor the laboratory performance, case detection and quality of the services provided by each laboratory.
    - Drug resistant data (Rifampicin & Isoniazid) generated at other TB diagnostic laboratories including private sector should be reported to NTRL.

- Laboratory Information Management System (LIMS).
    - NPTCCD has planned to establish LIMS at NTRL connecting MCs, GeneXpert sites and ITLs in 2022. This will enable easy monitoring of functions and performance of TB diagnostic laboratories.

1.7.2 Other laboratories providing TB diagnostic services

- Government sector laboratories not under the ministry of health

- Private sector laboratories

Most of the laboratories except very few provide smear microscopy services to detect TB. Currently only three laboratories provide TB culture diagnosis. All laboratories are expected to send monthly summary to NTRL and all drug resistant isolates should be sent to NTRL for confirmation and further testing.

References

1. WHO Consolidated Guidelines on; Module 3: Diagnosis - Rapid Diagnostics for Tuberculosis Detection.
2. WHO global tuberculosis report, 2020.

02 GENERAL INFORMATION ON TUBERCULOSIS

Tuberculosis is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. TB can affect any organ in the body. The most frequent site of involvement (80%) is the lungs and is termed pulmonary tuberculosis (PTB). When the disease occurs outside the lung it is termed extra-pulmonary tuberculosis (EPTB).

2.1 Natural history of tuberculosis

TB is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. TB can affect any organ in the body. The most frequent site of involvement (80%) is the lungs and is termed PTB. When the disease occurs outside the lung it is termed EPTB.

The main reservoir of infection is a patient with PTB. When such patient coughs or sneezes they produce aerosols containing large number of bacilli, which are infectious. When a previously healthy person inhales these infectious particles the smallest particles can penetrate into the pulmonary alveoli and cause infection.

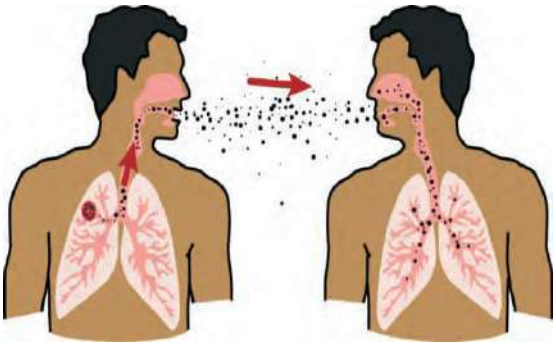


Figure 2.1 - Transmission of droplets.

Five percent of individuals progress from infection to disease at this initial exposure due to factors attributable to the host such as immunocompromised states or due to the large numbers and/ or virulence of the infecting mycobacteria. This progression from infection to disease is increased by 25% fold in HIV infected patients.

In the large majority of cases the immune system is able to contain bacilli at the primary focus. However, few bacilli can survive at these sites for months or years. These bacilli are known as “latent bacilli.” In 10%, these “latent bacilli” can get reactivated during an individual's life time. The risk is greatest during the first two years after initial exposure.

Bacilli from the primary infectious focus can get transported throughout the body by the lymphatic and blood systems. A limited number of bacilli may survive at these extrapulmonary sites and give rise to the various forms of EPTB months or years later.

In those with infectious PTB, 30% are spontaneously cured by the body's defense mechanisms. If untreated, 50% die within 5 years, 20% continue to excrete bacilli and remain sources of infection for many years before dying.

The highest priority on tuberculosis control is the identification and cure of infectious pulmonary cases.

Factors that increase the likelihood of becoming infected

The following increase the rate of transmission due to increase in the intensity and/ or duration of exposure,

- Household contacts of a sputum positive patient.
- HIV
- Underprivileged populations living in crowded improvised dwellings.
- Workers accommodated in crowded inadequately ventilated dormitories, boarding houses.
- Refugees.
- Prisoners.
- Those living in mental health institutions, nursing homes.
- Health care workers.
- Smokers, drug addicts, alcoholics.
- Malnutrition.
- Conditions leading to immunodeficiency such as HIV infection, poorly controlled diabetes, chronic renal failure, malignancies, those on immunosuppressive drugs such as, long term oral steroids.

A. Pulmonary tuberculosis (PTB)

Symptoms

- Cough of more than two weeks duration with sputum production.
- Chest pain.
- Breathlessness.
- Low grade fever.
- Loss of appetite, loss of weight.
- Malaise.
- Night sweats.
- Haemoptysis.

Over 90% of patients with PTB have cough.

Most acute respiratory tract infections resolve in three weeks. If a patient has cough and constitutional symptoms mentioned above for three or more weeks PTB should be strongly suspected.

B. Extra-pulmonary Tuberculosis:

The most frequent sites of extrapulmonary involvement are the lymph nodes (tuberculous lymphadenitis) and pleura (tuberculous pleurisy). Miliary tuberculosis is a disseminated form of TB characterized by the chest radiographic appearance of multiple small pin head-size lesions in both lung fields.

Neurological involvement can occur in the form of tuberculous meningitis, tuberculoma (manifesting as a space occupying lesion), Pott's disease of the spine, para-vertebral abscess (psoas abscess) etc. TB of the eye (tuberculous uveitis), abdominal tuberculosis (tuberculous peritonitis, appendicular and other bowel masses), genitorurinary and skin involvement has also been reported.

The clinical symptoms, signs, and investigations depend on the affected organ system. Diagnosis is confirmed by culture of a pathological specimen and/or by histopathological examination of relevant biopsy specimens of affected organs or tissue.

2.2 Definitions for drug resistant TB

Mycobacterium tuberculosis can develop resistance to the anti-microbial drugs used to treat tuberculosis.
Most people with TB are cured by a strictly followed drug regime provided to the patient with support and supervision. Inappropriate or incorrect use of antimicrobial agents or drugs or use of ineffective formulations such as use of single drugs, poor quality medicine or bad storage conditions and premature treatment interruptions can cause drug resistance which can then be transmitted by person to person.

Patterns of resistance observed in MTB strains:

- **Rifampicin-susceptible, isoniazid-resistant TB (Hr-TB):** caused by M. tuberculosis strains resistant to isoniazid and susceptible to rifampicin
- **Mono-resistance:** TB in a patient, whose infecting isolates of M. tuberculosis are resistant in vitro to one of the first line anti-tuberculosis drugs except rifampicin. Rifampicin mono resistance is categorised separately.
- **Poly-resistance:** TB in a patient, whose infecting isolates of M. tuberculosis are resistant in vitro to more than one first-line anti-tuberculosis drug, other than to both isoniazid and rifampicin.
- **Multi drug resistant TB (MDR-TB**): TB caused by Mycobacterium Tuberculosis (M. tuberculosis) strains that are resistant to at least both rifampicin and isoniazid with or without resistance to other first or second-line TB medicines
- **Pre-XDR-TB:** TB caused by Mycobacterium tuberculosis (M. tuberculosis) strains that fulfil the definition of MDR/RR-TB and that are also resistant to any of the Fluoroquinolone (Levofloxacin/Moxifloxacin) (1)
- **Extensively Drug Resistant TB(XDR-TB):** TB caused by Mycobacterium tuberculosis (M. tuberculosis) strains that fulfil the definition of MDR/RR-TB and that are also

resistant to any fluoroquinolone and at least one additional Group A drug(Bedaquiline , Linezolid)(1)

- **Rifampicin resistance (RR):** Resistance to Rifampicin detected using phenotypic or genotypic methods, with or without resistance to other anti TB drugs except Isoniazid.

Reference: (WHO 2021; Meeting report of the WHO expert consultation on the definition of extensively drug-resistant tuberculosis

2.3 Case definitions

A. Classification based on history of previous TB treatment (patient registration group)

In order to identify those patients at increased risk of acquired drug resistance and to prescribe appropriate treatment, a case should be defined according to whether or not the patient has previously received TB treatment. The registration group focuses only on history of previous treatment irrespective of bacteriological confirmation or site of disease. Accordingly, all patients can be categorized as 'New' patients or 'Previously treated' patients.

They are defined as follows:

New patients - A patient who has never taken treatment for TB OR a patient who has taken anti-tuberculosis drugs for less than one month.
New patients may have positive or negative bacteriology and may have disease at any anatomical site.
Previously treated patients - Those who have received 1 month or more of anti-TB drugs in the past are classified under this category. They may have positive or negative bacteriology and may have disease at any anatomical site. They are further classified by the outcome of their most recent course of treatment as 'relapse', 'treatment after failure' and 'treatment after loss to follow-up'. (Refer table 2.1)

Relapse - Patients who have previously been treated for TB, were declared cured or treatment completed at the end of their most recent course of treatment, and are now diagnosed with a recurrent episode of TB (either reactivation of dormant bacilli or a new episode of TB caused by reinfection).

Treatment after failure - Patients who have previously been treated for TB and whose treatment failed during or at the end of their most recent course of TB treatment.

Treatment after loss to follow-up - Patients who have previously been treated for TB and were declared lost to follow-up at the end of their most recent course of treatment. (These were previously known as treatment after default patients.)

Other previously treated patients - Patients who have previously been treated for TB but whose outcome after their most recent course of treatment is unknown or undocumented.

Patients with unknown previous TB treatment history
Patients who do not fit into any of the categories listed above.

Table 2.1 Classification based on history of previous TB treatment

Registration group (any site of disease)		**Outcome of most recent prior treatment**
New		
Previously treated	Relapse	Cured
		Treatment competed
	Treatment after failure	Treatment failed
	Treatment after loss to follow-up	Lost to follow-up
	Other previously treated patients	Patients who have previously been treated for TB but whose outcome after their most recent course of treatment is unknown or undocumented.
Patients with unknown previous TB treatment history		All cases that do not fit into above definitions

New and relapse cases of TB are considered incident TB cases.

B. Classification based on HIV status

HIV-positive TB patient refers to any bacteriologically confirmed or clinically diagnosed case of TB who has a positive result from HIV confirmatory test.

HIV-negative TB patient refers to any bacteriologically confirmed or clinically diagnosed case of TB who has a negative result from HIV testing conducted at the time of TB diagnosis. Any HIV-negative TB patient subsequently found to be HIV-positive should be reclassified accordingly.

HIV status unknown TB patient refers to any bacteriologically confirmed or clinically diagnosed case of TB who has no result of HIV testing. If the patient's HIV status is subsequently determined, he or she should be reclassified accordingly.

C. Standard treatment categories

Table 2.2 – First line treatment

TB treatment category	**Type of patient**	**Intensive phase**	**Continuous phase**
New	New smear positive or negative pulmonary/extra pulmonary	2 HREZ (FDC 4)*	4 HR (FDC 2)*
Re treatment	Previously treated	3 HREZ (FDC 4)*	5 HRE (FDC 3)*

*

H=isoniazid, R=Rifampicin E= Ethambutol P=Pyrazinamide FDC=fixed dose combination. HREZ (FDC4), HRE (FDC3), HR (FDC2) are now available as fixed dose combination (FDC) pills .This will improve compliance and prevent development of resistance.

D. Treatment outcome

Treatment failed - A patient whose treatment regimen needed to be terminated or permanently changed to a new regimen or treatment strategy.

Cured - A pulmonary TB patient with bacteriologically confirmed TB at the beginning of treatment who completed treatment as recommended by the national policy, with evidence of bacteriological response and no evidence of failure.

Treatment completed - A patient who completed treatment as recommended by the national policy, whose outcome does not meet the definition for cure or treatment failure.

Died - A patient who died before starting treatment or during the course of treatment.

Lost to follow-up - A patient who did not start treatment or whose treatment was interrupted for 2 consecutive months or more.

Not evaluated a patient for whom no treatment outcome was assigned.

Treatment success - The sum of cured and treatment completed.

References

1. National Manual for Tuberculosis Control – NPTCCD, 2016.

03 SPECIMEN COLLECTION AND TRANSPORT

A good quality specimen is the key for obtaining a satisfactory and quality assured test result. Therefore, one must pay attention to instruct and support the patient to produce a good quality specimen. Transporting in appropriate conditions and submitting the specimen to the laboratory is of equal importance. A specimen should always be accompanied by a request form.

The medical officer requesting the test should furnish an appropriate request form (TB 05 for smear microscopy, TB 06 for TB culture & molecular testing – Annex 4, 5).

3.1 Specimen collection for smear microscopy

Specimens are collected for,

A. Diagnosis
B. Follow up – To monitor response to treatment

A. For diagnosis

Sputum is the most commonly used specimen for the diagnosis of PTB. Early morning specimens have the highest yield of Acid Fast Bacilli (AFB). Three sputum specimens should be collected and processed for microscopy to ensure optimal recovery of TB bacilli from sputum to improve the sensitivity of smear microscopy.

Biological specimens other than sputum can be collected for smear microscopy. However, the sensitivity is low due to a smaller number of bacteria (paucibacilli) in those specimens.

It is not recommended to perform smear microscopy from blood or frankly blood stained specimens due to the low sensitivity of the procedure. It is also not recommended to routinely perform smear microscopy from urine specimens due to the frequent detection of saprophytic mycobacteria colonizing the urogenital tract.

B. For follow up – To monitor patients on treatment

Sputum specimens for microscopy should be collected from patients with PTB at intervals for monitoring response to treatment as given in Table 3.1

Table 3.1 – Schedule for follow up sputum examination

Follow up sputum examinations are done during the treatment for monitoring the effectiveness of treatment and to declare them as 'CURED' at the end of treatment.

Category	Time
New smear-positive PTB cases	• End of 2nd month • End of 3rd month if smear is positive at 2nd month • End of 5th month • End of treatment
New smear-negative PTB cases	• End of 2nd month • End of 6th month
Previously treated cases - Relapse - Failures - Return after lost to follow up	• End of 3rd month • End of 4th month if smear is positive at 3rd month • End of 5th month • End of treatment

3.1.1 Instructions for collection of sputum

Patients should receive clear instructions on the proper collection of sputum specimen for TB diagnosis. Sputum collection is a procedure generating infectious aerosols. Therefore, it is advised to collect sputum inside a coughing booth.

Coughing booth should be constructed allowing good sunlight and with hand washing facilities at a distant place away from the general traffic.

- When a coughing booth is not available, a designated open air space with good sunlight can be used and the collection area should be away from public areas.

When specimen is collected at home, it should be done in a well-ventilated area outside the house.

A. Steps in specimen collection

Step 1 - Instruction to the laboratory staff
Step 2 - Instructions to the patient.
Step 3 - Guide to obtain a good quality specimen.

Step 1 - Instructions to the laboratory staff

- Use a clean, wide mouthed container with a lid for microscopy (Figure 3.1).
- Give the patient a lab serial number, and write it on the body of the container.
- Provide the patient with information on collection of specimen (Refer step 2, 3).
- Direct the patient to the coughing booth to collect the specimen.

Step 2 - Instructions to the patient.

Explain clearly to the patient by medical officers/nursing officers/MLTs/PHLTs.

- Why sputum is needed.
- Three specimens are required.
- Spot-morning-spot (for diagnosis) or Morning-spot (for follow on).
- What a good specimen is (thick, purulent sputum, approximately 3–5ml in volume) and how to obtain it.
- The need for obtaining a specimen collection container from the laboratory (Figure 3.1).
- Opening and tight closing of containers.
- Not to soil the exterior of the container.
- Not to expose the specimens to sunlight.
- The need to return the specimen to the laboratory.

Step 3 - Guide to obtain a good quality sputum specimen (Figure 3.2)

- Gargle with water to rinse out mouth. Drinking a glass of warm water may help to bring out sputum.
- Inhale deeply 2-3 times through your mouth and cough up mucous from deep in your chest.
- Place the coughed-up sputum in to the container.
- Screw the lid tightly so it does not leak.
- Hand over the specimen to the laboratory.

Figure 3.1 - Sputum container for smear microscopy

Figure 3.2 – Guide to obtain a good quality sputum specimen

3.2 Specimen collection for culture and molecular diagnostic tests.

Instructions for collection of sputum and other specimens for culture and molecular diagnostic tests are similar to those given for specimen collection for microscopy. However, patient should be provided with a sterile, screw-capped container for culture and molecular diagnostic tests. If both, GeneXpert and culture tests are requested, patient should be provided with 2 specimen containers.

- Universal bottle for culture (Figure 3.3).
- Disposable, sterile, screw-capped bottle for GeneXpert (Figure 3.4).

Figure 3.3 - Container for TB culture.

Figure 3.4 – Sputum container for TB molecular tests.

3.3 Collection of other specimens for microscopy, culture & GeneXpert

Use the sterile, screw-capped container to collect the specimen.

Body fluids (cerebrospinal fluid, pleural fluid, pericardial fluid, synovial fluid, peritoneal fluid, blood, pus and bone marrow)
• Body fluids should be aseptically collected using aspiration techniques or surgical procedures.
Pleural effusion
• Pleural effusion is a suboptimal specimen: tubercle bacilli are mainly in the pleural wall and not in the fluid. The minimum volume for pleural effusion is 20–50ml. A pleural biopsy specimen is preferable to pleural fluid.
Aseptically collected tissues

- Tissues should be placed in sterile containers without fixatives or preservatives and transported quickly to the laboratory. For prolonged transportation, dehydration should be prevented by adding sterile saline (01ml) (to keep the moisture) and maintaining a temperature of 02-08°C.

Urine

- Urine is expected to be contaminated. To minimize excessive contamination of urine specimens, external genitalia should be washed before specimen collection. As excretion of tubercle bacilli is intermittent, three consecutive early-morning midstream specimens must be collected. Once received in the laboratory, a urine specimen must either be processed immediately or centrifuged and the pellet refrigerated.

Trans-bronchial and other biopsies

- Trans-bronchial and other biopsies taken under sterile conditions should be kept wet during transportation by adding 0.5–01ml sterile 0.9% saline.

Other respiratory specimens

- Other respiratory specimens that can be submitted to the laboratory for mycobacterial culture are bronchial wash (minimum volume 02–05ml) and bronchial alveolar lavage (minimum volume 20–50ml).

Gastric lavage from children

- In children who produce little, if any sputum, aspiration of the early morning gastric fluid can be used for TB diagnosis. The gastric aspirate should be transported immediately to the laboratory and neutralized by adding 100mg of sodium bicarbonate.

Table 3.2 – Requirement for specific specimens.

Specimen	Container	Volume	Storage
Sputum	For microscopy – Wide mouthed, clean, transparent.	2-3ml	2-8°C
	For culture – Sterile, screw capped, transparent		
Bronchial secretions (endotracheal secretions and bronchial wash)	For culture – Sterile, screw capped, transparent	2-5ml	2-8°C
Broncho- alveolar lavage (BAL)	For culture – Sterile, screw capped, transparent	20-50ml	2-8°C
Pleural Fluid	For culture – Sterile, screw capped, transparent	20-50ml	2-8°C
Urine	For culture – Sterile, screw capped, transparent	20-50ml	2-8°C

3.4 Specimen collection in children

The Xpert MTB/RIF assay has been recommended by WHO for children as the initial diagnostic test. Specimens tested with Xpert MTB/RIF assay from children are similar to that for adults.
If child can produce sputum, sputum should be the first and priority specimen to be tested by Xpert test. However, most young children will not be able to produce adequate sputum specimens upon request, instead gastric aspirate; nasopharyngeal aspirate can be collected to diagnose PTB.

Collecting specimens for pulmonary TB – If child cannot produce sputum.

- Sputum induction with nebulised hypertonic saline may facilitate collection of tracheo-bronchial secretions, especially in children who have a dry cough or no cough.
- Gastric lavage is the most common procedure for collecting specimens for Xpert MTB/RIF assay or culture and DST, especially early in the morning before the child has had anything to eat or drink.
- Fibre-optic bronchoscopy may be the best next step if gastric aspirates fail, in settings with appropriate facilities and technical expertise.

Collecting the respiratory specimen at optimal times is important to enhance the yield.

- Early morning expectorated sputum.
- Induced sputum after fasting 02-04 hours.
- Early morning fasting gastric aspirate, before mobilization.

Sputum (induced or expectorated) should be minimum 03ml, Gastric aspirate 05ml, gastric lavage 10ml, BAL 03ml, nasopharyngeal aspirate 02ml.

Collecting specimens for extra pulmonary TB

Other specimens tested with Xpert MTB/RIF include cerebrospinal fluid (CSF), lymph node aspirate and lymph node biopsy for extra pulmonary TB (EPTB). Serosal fluids include pleura, pericardium, peritoneum and synovium may also be helpful in diagnostics, but bacteriological yield is higher in tissues than fluids.

3.5 Induction of sputum

Sputum induction is a procedure used for patients who have trouble producing sputum spontaneously. It is simple and non-invasive. If successful, often precludes the need for bronchoscopy.

It is an aerosol generating procedure. Therefore, the infectious droplets, if present, will be expelled into the room air. Strict airborne respiratory precautions should be observed whenever sputum induction is performed.

Location of sputum induction rooms

Sputum induction rooms and local exhaust devices should be placed near patient care areas, where staff can monitor and assist patients as needed. The room should be located away from waiting rooms and other areas where patients or visitors are likely to enter and risk being exposure.

Infection control of sputum induction room

1. Induction of sputum should only be conducted in a single room with a ventilation system that allows for the total exhausting of air from the room to the external environment. The minimum requirement is a single room with door closed and air exhausted to the outside of the building without recirculation.
2. Staff performing this procedure should have a documented record of their tuberculin skin test (TST) or Interferon gamma release immunoassay (IGRA) status.
3. Staff must wear the recommended TB respiratory protection e.g. N95 masks while in the room and disposable gloves when handling sputum specimen.
4. The breathing circuit used for sputum induction should have a filter on the expiratory side to reduce environmental contamination.

Accredited staff

This procedure should only be performed by trained health care worker, physiotherapists or nurses.

Procedure

1. Use 20ml of 3% hypertonic saline solution for nebulization.
2. Instruct the patient to thoroughly clean the mouth by brushing with a toothbrush if this has not been done since a meal, or forceful rinsing and repeated gargling with tap water until the returned fluid is free from debris.
3. Bring the patient to the designated room for sputum induction. Seat them comfortably in an upright position.
4. Explain the procedure and possible side effects to the patient (e.g., coughing, dry mouth, chest tightness, nausea and excess salivation).
5. Instruct the patient to:
    - inhale and exhale through the mouth piece only
    - expectorate sputum coughed up into the container.
6. Shut all doors and windows and put the "Do Not Enter: Induced Sputum in Process" sign on the outside of the door. A view window in the door should be provided to monitor the patient from outside the room.
7. Turn the machine on (a fine mist should appear above the level of the hypertonic solutions).
8. Place the mouth piece into the patient's mouth, reemphasizing mouth breathing (the fine mist should not be seen through the clear T-piece in inspiration, and the patient should experience a salty taste in their mouth).
9. Allow the patient to inhale the hypertonic mist for approximately 5 minutes. Then instruct them to take several deep breaths off the nebulizer. If the patient does not initiate coughing spontaneously, ask them to attempt a forced cough.
10. The health care worker may use gentle chest physiotherapy e.g., vibration and percussion to produce sputum.
11. Patient must be observed during the procedure at all times carefully for signs of respiratory distress.
12. The procedure should be stopped when:
    - the patient has produced 1-2ml of sputum for each specimen.
    - 15 minutes of nebulisation is reached.
    - the patient complains of dyspnoea, chest tightness or wheeze.

13. Terminate the procedure if unsuccessful after 15 minutes, or if the patient is showing signs of respiratory distress or is light headed or feels nauseated.
14. Label the specimen container appropriately.
15. Assess the patient's condition post procedure, and take appropriate action if required.
16. Dispatch the specimen to the laboratory for relevant investigations.

3.6 Storage of specimens

Although TB bacilli can survive in sputum for one week in the absence of preservatives, the probability of successfully culturing the bacilli decreases with time and this is especially critical for paucibacillary specimens. If specimens cannot be transported to the laboratory within one hour, it is recommended to store them at 02-08°C. This does not apply to whole blood specimens, which should not to be refrigerated.
On arrival at the laboratory, specimens should again be refrigerated until they are processed. The delay between collection and inoculation should not exceed seven days.

3.7 Transportation of specimens

Specimens should be collected according to instructions and delivered as quickly as possible to the laboratory when culture and molecular tests are requested. Every effort must be made to organize and expedite specimen transportation and processing.

Specimens, including clinical specimens and culture isolates should be transported from one laboratory to the other in three-layer packaging at 02-08°C in cool boxes as mentioned below,

- Closed specimen bottle (primary receptacle) should be put into a zip lock polythene/plastic bag (secondary receptacle).
- Absorbent material should be kept in between primary and secondary receptacle.
- The secondary receptacle should be placed in a third receptacle, e.g. cool box.
- Specimen containers should be kept in upright position, need to be displayed outside of the 3rd receptacle.
- Request forms should not be kept inside the 3rd receptacle (cool box).

The laboratories receiving specimens should check and document the temperature at the receiving counter itself.
PPE is not necessary for people who transport specimens in the triple package.

Primary receptacle: A watertight, leak-proof receptacle containing the properly labeled specimen. The receptacle should be wrapped in adequate absorbent material to absorb all fluid in case of breakage.

Secondary receptacle: A durable, watertight, leak-proof receptacle to enclose and protect the primary receptacle. Sufficient absorbent material must be used to cushion multiple primary receptacles if few primary receptacles are placed in one secondary receptacle. E.g. A disposable zip lock polythene/plastic bag/plastic container.

Third receptacle (Outer package)**:** The outer most package which protects the package from physical damage and water. The secondary receptacle is placed inside this receptacle.

When transporting specimens (e.g. TB culture isolates) outside the country, the regulations specified by the International Air Transport Association (IATA) for international transfer of infectious substances, should be followed.

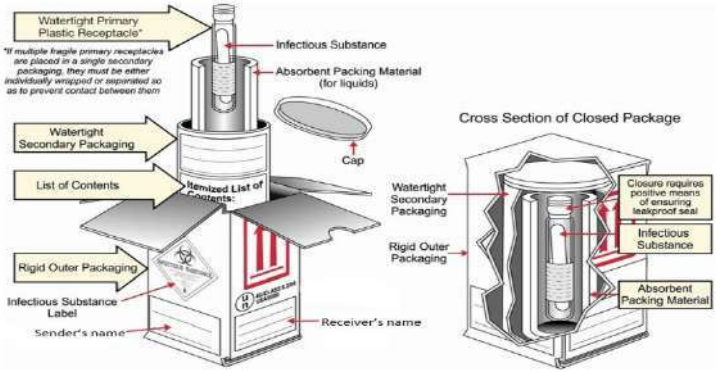


Figure 3.5 – Three Layer package

3.8 Registration of specimens at the laboratory

Laboratory personnel receiving the specimens should check the request form (TB 05 and TB 06) for completeness and check with the label/bar code on the specimen container for accuracy and patient identification.

Upon arrival in the laboratory,

- The quality of sputum specimens should be assessed. Sputum specimens should appear thick and mucoid or clear but with purulent material. The colour could vary from opaque white to green. Bloody specimens will appear reddish or brown.
- Before the patient leaves the laboratory, visually examine the sputum specimen for quality. If the specimen is not satisfactory, ask the patient to cough again to obtain a satisfactory specimen.
  Note: clear saliva or nasal discharge is not suitable as a specimen for microscopy.
- Volume and quality of specimen should be documented in the TB 05 (microscopy) or TB 06 form (culture and molecular diagnostic tests) as well as in TB register (TB 04) when the specimen is received for microscopy (Annex 4, 5, 3).
- Accept even a small quantity if the patient has difficulty producing sputum. Blood-streaked sputum is suitable, but pure blood should not be examined for microscopy.
- For optimum patient management, process the specimen as soon as possible (e.g. <24 hours).
- For microscopic examination, the interval between collection and staining is not critical. The specimen must be processed within three days of the specimen collection. Acceptable results can be obtained even if specimen delivery has been delayed.

3.9 Rejection criteria

1. All specimens should be processed, except for broken or leaking containers, unlabeled specimens which should be discarded.

2. Requesting clinician should be informed over the phone. Laboratory staff should document on the request form the name and designation of health staff informed and another specimen should be requested.
3. Requesting clinician should also be informed when specimens are mislabeled and patient details and the type of specimen written on the request form should be verified with that on the label of specimen container.
4. When possible, encourage the patient/ physician to submit a new specimen if patient has submitted a salivary specimen to the laboratory and this should be documented on the request form; however, even saliva may yield positive results.

Registration of specimens received for microscopy

Accurate entry of the patient data in the laboratory register is of high priority as this data is used to trace the patients.

- Register the patient in the TB Laboratory Register (TB 04) and assign a laboratory serial number to the patient (Refer Annex 03 for TB 04).
- The laboratory serial number begins with 1 on 1st of January each year and continues serial number with each patient until 31st of December of the same year.
- Assigning a serial number,

  When a patient comes for sputum examination,

  - For diagnosis - give one laboratory serial number for three specimens (E.g. 235A, 235B, 235C).
  - For follow up at 2 month - Give a new laboratory serial number (E.g. 253A, 253B).
  - For subsequent follow up - new laboratory serial number at each visit (E.g. 260A, 260B).
- Write the laboratory serial number on the laboratory request form and on the side of the sputum container, never only on the lid.

References

1. GLI Laboratory Diagnosis of Tuberculosis by sputum microscopy, The hand book, Global Edition, 2013.
2. Sputum induction guidelines - Tuberculosis (nsw.gov.au)

04 SMEAR MICROSCOPY

Smear microscopy for AFB with bright field microscopy is the primary diagnostic tool for the detection and control of TB mainly in pulmonary TB. It is a cost-effective method of diagnosing infectious cases of PTB in a resource poor setting.

This rapid and reliable method of identification of patients with PTB is dependent on the bacillary load in sputum. If the sputum has less than 5000 bacilli/ml, smear microscopy by ZN (Ziehl Nielsen) staining is unlikely to diagnose PTB. However, smear microscopy is non-specific in identifying MTB from NTM/MOTT (Non-Tuberculosis mycobacteria/Mycobacteria other than TB) as it appears the same. In areas of high TB prevalence, positive smears have a very high probability of being MTB.

The reliability of sputum microscopy depends on the quality of sputum specimen and hence on sputum collection. Sputum production in early morning often shows a higher concentration of the organism. Importantly, the reliability of sputum microscopy depends on the proper preparation and interpretation of slides. Therefore, laboratory technicians must be properly trained and the quality assurance procedures and quality assessment must be regularly carried out.

Sri Lanka has a network of laboratories for TB microscopy through which the services are provided in a decentralized fashion.

Functions of microscopy centers and DCC laboratories for microscopy

Microscopy centers

1. Perform sputum smear microscopy using ZN method.
2. Receive, process and report on sputum specimens.
3. Ensure 3 sputum specimens are examined for diagnosis and 2 during follow-up.
4. Participate in EQA.
5. Maintain the records and statistics.

District Chest Clinic Laboratory

1. Perform sputum smear microscopy.
2. Prepare and distribute reagents and other laboratory requirements to the microscopy centers.
3. Estimate the reagent requirement for the DCC and the MC for smear microscopy.
4. Quality assurance of the sputum smear microscopy.

4. 1 Smear microscopy

Staining for microscopy

The two most common methods of staining, which determine the acid-fast nature of mycobacteria are ZN staining and auramine staining. Two staining techniques are linked with different methods of microscopic examination,

1. Bright field microscopy – Staining by ZN method – Using light microscope.
2. Fluorescence microscopy – Staining with auramine – Using florescent microscope.

MTB may infect almost any organ in the body. Therefore, the laboratory may receive sputum as well as a variety of extra-pulmonary specimens e.g. body fluids, tissue, pus etc. The benefit of microscopy on these specimens is limited because of their paucibacillary nature. Therefore, extra pulmonary specimens should always be referred for further testing to confirm MOTT.

Main aims of sputum microscopy are to,

1. Diagnose patients with infectious PTB.
2. Monitor progress of TB patients who are on treatment.

Advantages of sputum microscopy are many,

1. Useful diagnostic tool for the diagnosis of infectious TB.
2. Simple to perform.
3. Easy to read.
4. Quick results.
5. Inexpensive.
6. Minimal infrastructure is required.

For diagnostic purposes collection of three sputum specimens are recommended as,

Spot-Early Morning-Spot

4.1.1 Bright Field Microscopy

Bright field microscopy requires simple laboratory facilities. Infrastructure wise, a BSL 2 facility is appropriate.

Finding AFB is more likely with examination of three specimens than that with one or two specimens.

A. Staining for microscopy by ZN method

ZN staining method uses a technique where the mycobacteria retain a primary stain after exposure to de-colorization with acid alcohol, hence the term “acid fast bacilli” (AFB). Therefore, the mycobacteria appear red (the color of the primary stain), slender rods with one or more granules giving rise to beaded appearance, against a blue background demonstrating the acid-fast nature.

Principle of acid-fast staining (in mycobacteria)

The mycobacteria have a cell wall rich in mycolic acids and it is waxy. In staining procedure heat often softens the wax in the cell wall and as the phenol is more soluble in lipids or waxes than in water or alcohol, the dye-phenol mixture (carbol fuchsin) enters the cell and the cell appears red.

When the smear is decolorized with decolorizing agent (3% HCl in 95% alcohol) the acid fast cells resist the decolorizing agent entering the cell due to the presence of large amount of lipoidal materials in their cell wall. The non-acid fast organisms lack the mycolic acid lipoidal material in their cell wall due to which they are easily decolorized, leaving the cells colorless. When the smear is stained with counter stain, methylene blue can be absorbed only by the decolorized cells. The color of the counter stain appears blue while acid-fast cell retain the recolor.

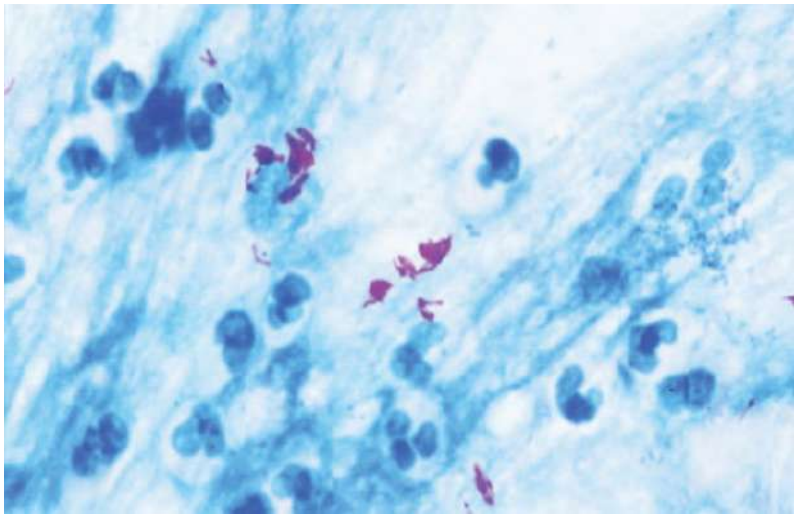


Figure 4.1 – Clump of AFB seen in a sputum smear (ZN stain, examined at ×1000).

Steps in sputum microscopy

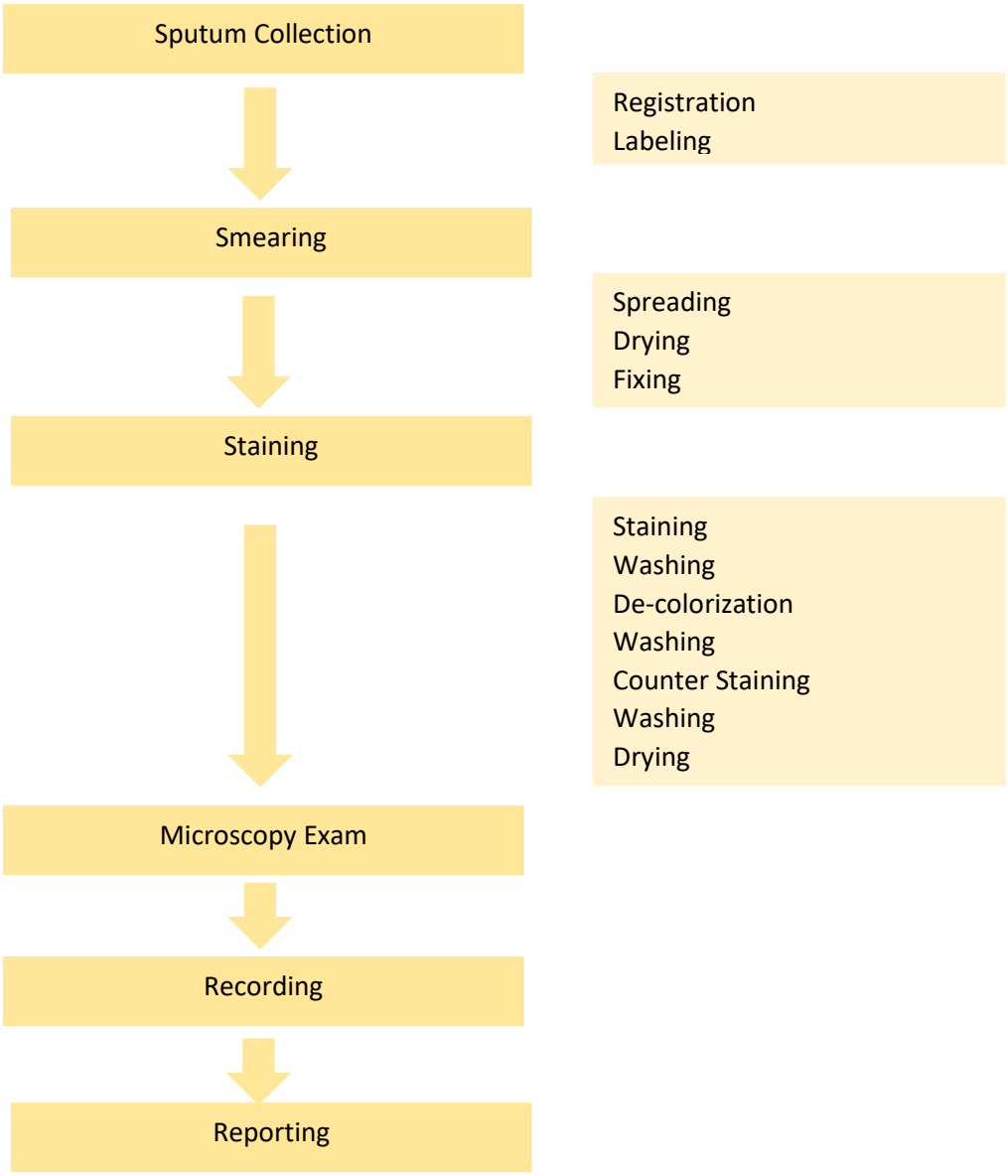


Figure 4.2 - Procedure of smear microscopy.

B. Sputum collection

A good specimen of sputum is necessary for obtaining good results in examination. Therefore, one should pay attention to collection of a proper specimen, storage and transport of the specimens with no deterioration of the quality. Refer chapter 3 – Sputum collection and transport.

The documentation in relation to sputum examination is of utmost importance. Therefore, the technicians should undergo training in this area very well.

Allocating a laboratory serial number

The laboratory serial number begins with 1 on 1st of January each year and continues serially with each patient until 31st of December of the same year, e.g. 1203/21 (No/Year)

When a patient comes for sputum collection,

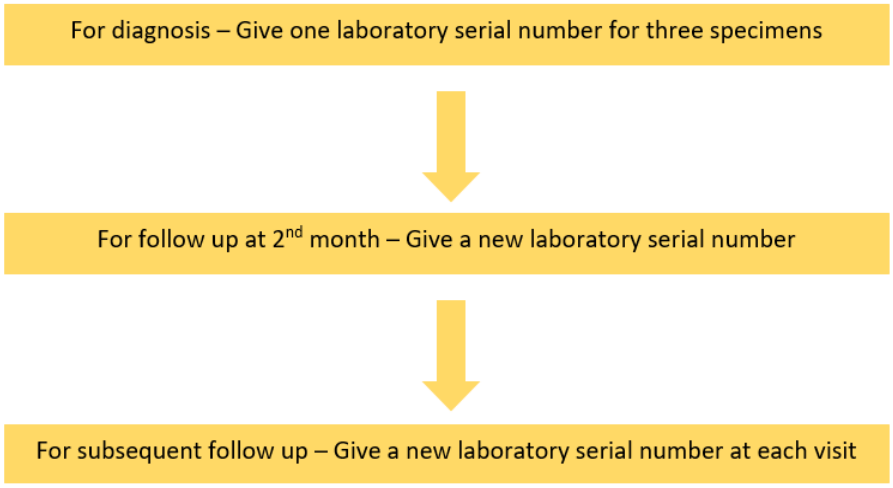


Figure 4.3 - Allocation of lab serial numbers.

Write the laboratory serial number on the laboratory request form and on the side of the sputum container using a permanent marker. Never label on the lid. This is because the lid from one container may be placed on another container causing incorrect labeling of specimen.

Quality of sputum specimen

Before preparing the smears one must assess and record the visual appearance of the specimen. The assessment should be entered on the laboratory request form by ticking the appropriate box.

Figure 4.4 – Specimen Quality

C. Preparation and staining of sputum smears

Make sure the laboratory serial number on the request form matches the laboratory serial number on the container.

- Arrange the specimen containers in serial order.

Steps in the preparation of smears

Step 1 - Labeling the slide

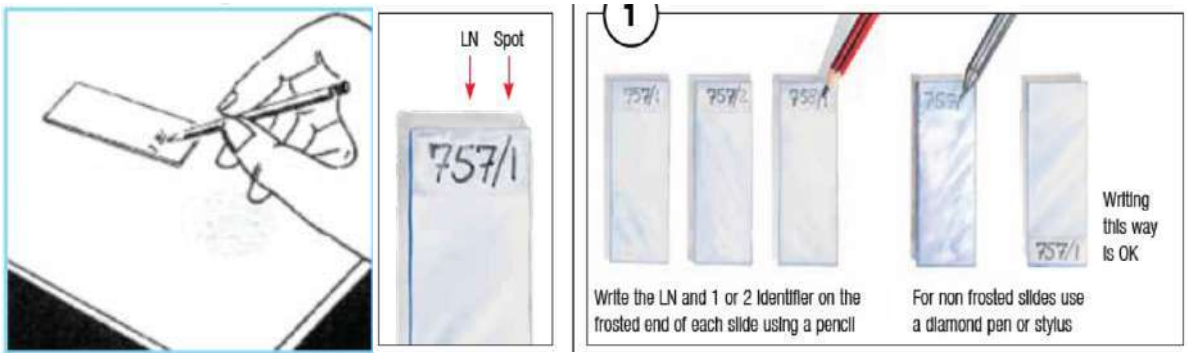


Figure 4.5 – Labeling the slide

- Select a new clean unscratched slide and write the laboratory serial number and the specimen number A, B or C using a diamond pencil on one end of the slide. (A, B and C indicate the 1st 2nd and the 3rd specimen). See figure 4.5.

Step 2 - Smearing

Figure 4.6 Breaking off of wooden sticks

- Break a wooden stick into two pieces with rough ends. See figure 4.6.
- A wire loop may be used. When a wire loop is used, it should be flamed until red-hot and allowed to cool.

Figure 4.7 – Smearing with a wire loop

- Ensure the number on the slide corresponds to the number on the sputum container.
- Using the jagged ends of the broken stick or wire loop, select and pick up a small portion of purulent particles, and transfer on to the slide. See figure 4.7.

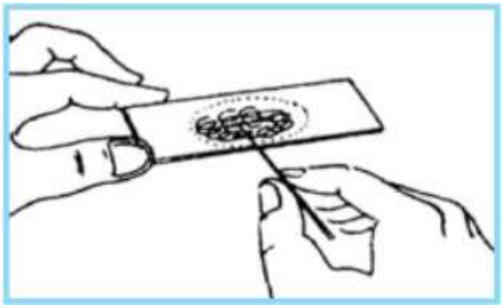


Figure 4.8 – Smearing with a wooden stick

- Use a separate stick for each slide.
- With the stick, spread the sputum evenly to cover the central portion of the slide, using a continuous movement. See figure 4.8.
- Place the used wooden sticks in a container with disinfectant (e.g. Phenolic disinfectant).

Figure 4.9 – Disinfectant sand bath.

- If a wire loop is used, sterilize the loop between successive specimens by first dipping it in a flask containing disinfectant and sand and then holding it to the Bunsen burner and flame until it is red hot. See figure 4.9.

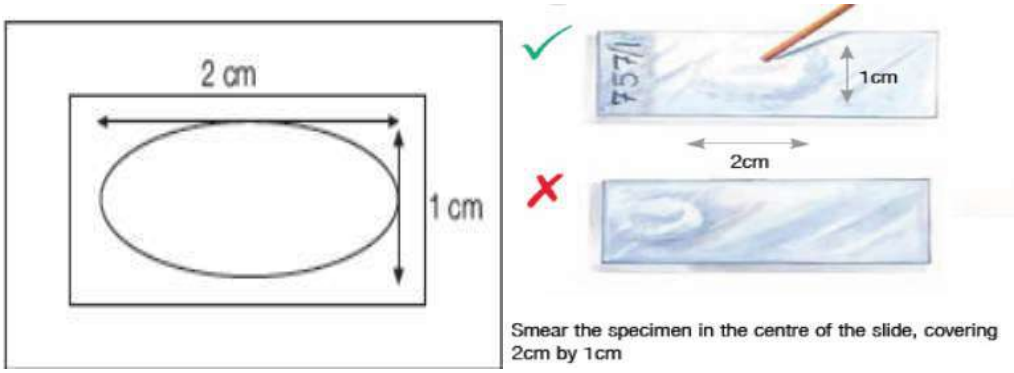


Figure 4.10 – Size of the smear.

- The size of the smear should be approximately 2 x 1cm. See figure 4.10.
- The smear should be spread evenly and not too thick nor too thin.
- It should be thin enough to read newsprint through.

Step 3 – Drying

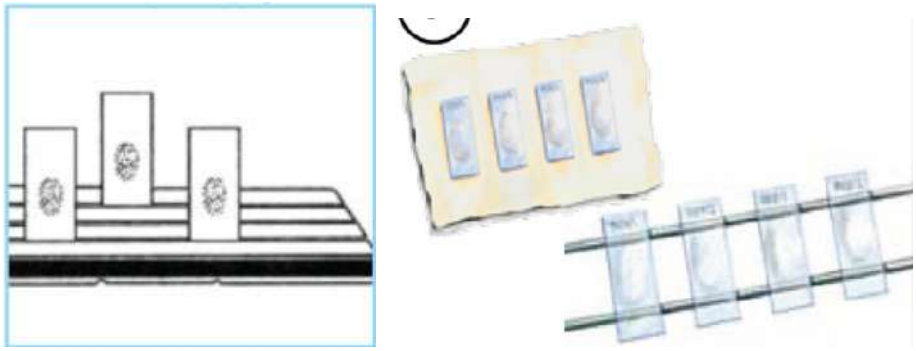


Figure 4.11 – Air drying of slides on the slide rack.

- Place the smeared slides on the drying rack. See figure 4.11.
- Let the slides dry in the air for about 15 – 30 minutes.
- Do not use the flame for drying. Drying has to be done in a drying rack or a paper if a drying rack is not available.
- Replace the lid of the sputum container, but do not dispose of the specimens until the smears have been examined and results recorded.

Step 4 – Fixation

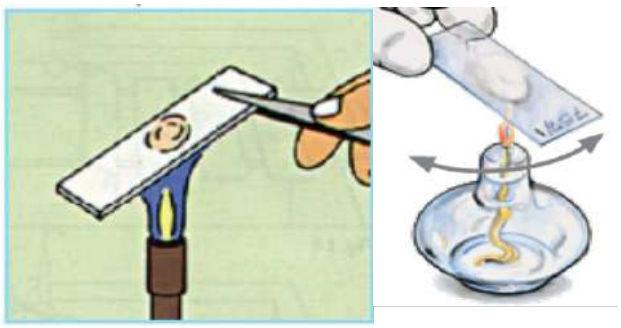


Figure 4.12 – Fixation of slide.

- Make sure the slide is completely dry.
- Hold the dry slide using forceps with the smeared side facing upwards.
- Pass the slide 2-3 times over the flame of the Bunsen burner for about 2-3 seconds each time. Fixation ensures that the sputum will stick to the glass slide. If not heated sufficiently, the AFB may be washed off during staining. See figure 4.12.
- Do not heat the slide for too long or keep it stationary over the flame. It could damage the bacilli.

Step 5 - Staining

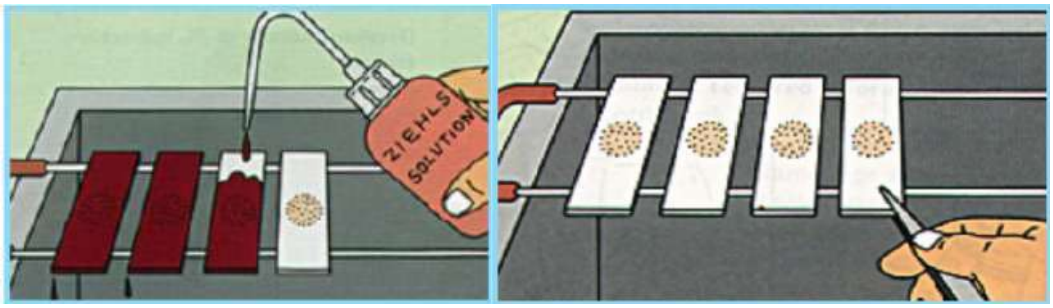


Figure 4.13 – Staining.

- Place the slides in serial order on the staining rack with the smeared side facing upwards. Leave space between the slides so that they do not touch each other. See figure 4.13.
- Never stain more than 12 slides at a time.
- Include positive and negative controls with each day's reading.
- Pour filtered 1% carbol fuchsin to cover the entire surface of the slide.

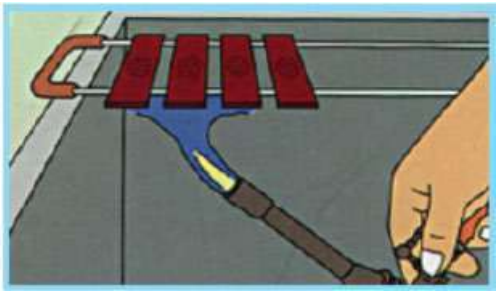


Figure 4.14 Gentle heating beneath the slide

- Heat the slide gently by-passing flame underneath the slide, until vapour rises. See figure 4.14.
- When the slide is heated, carbol fuchsin on the slide penetrates the wall of the TB bacilli to stain the bacilli red.
- Leave the carbol fuchsin on the slide for 5 minutes and maintain the heat by flaming intermittently.
- Do not allow the carbol fuchsin to boil or dry on the slide. Boiling will alter the shape of TB bacilli and could result in a false negative reading.

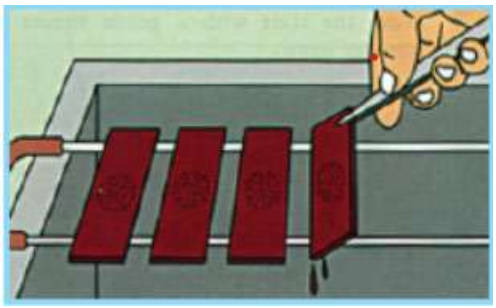


Figure 4.15 – Removal of excess stains.

- Tip off excess staining solution. See figure 4.15.

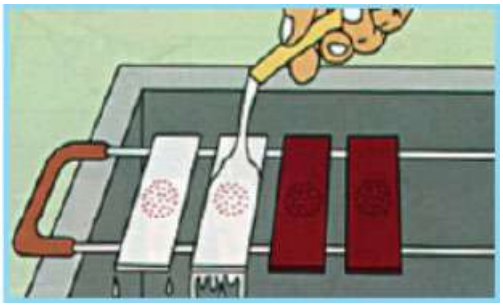


Figure 4.16 – Washing off of excess stain.

- Rinse the slides under a gentle stream of running water until all excess stain is washed off. See figure 4.16.

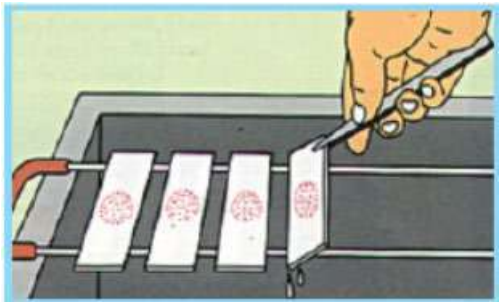


Figure 4.17 –Removal of excess water.

- Tilt the slides to drain off excess water. See figure 4.17.

Step 6 – De-colorization

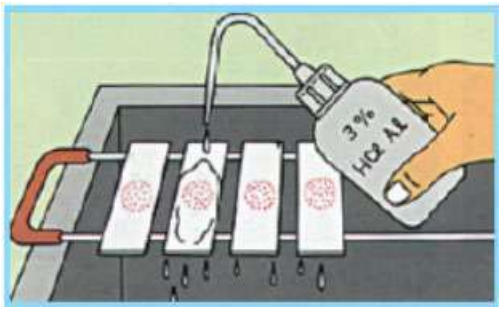


Figure 4.18 – De-colorization.

- Sputum smears appear red in colour.
- Replace slides on the staining rack.
- Pour 3% acid alcohol onto the slides (Figure 4.18).
- Let it stand for 2 minutes.

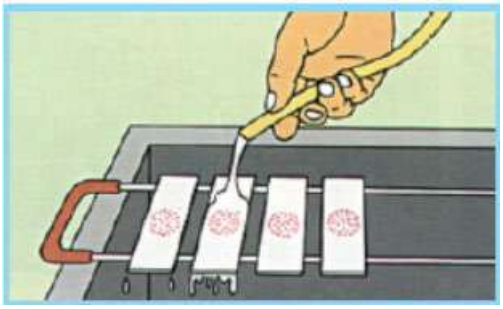


Figure 4.19 – Washing off of acid alcohol & stain.

- Gently rinse the slides with water to remove the acid alcohol and excess stain. (Fig 4.19).
- If the slides are still red repeat the process by adding more acid alcohol until the red color has disappeared, but do not over de-colorize.

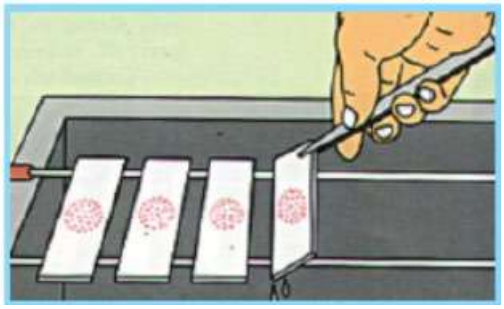


Figure 4.20 – Removal of excess water.

- Tilt the slides to drain off the water. See figure 4.20.

Step 7 – Counter staining

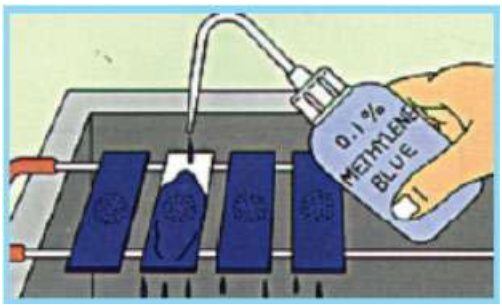


Figure 4.21 – Counter staining with methylene blue.

- Pour 0.1% methylene blue on the slide. See figure 4.21.
- Allow standing for 1 minute.

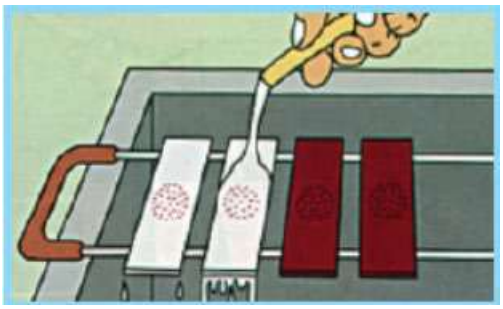


Figure 4.22 – Washing off with water.

- Gently rinse the slide with tap water. See figure 4.22.

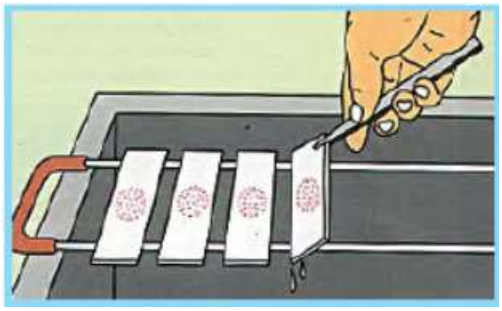


Figure 4.23 – Removal of excess water.

- Tilt the slide to drain off the water. See figure 4.23.
- Allow the slide to dry and then examine it under the microscope.

Staining Procedures should be posted above the staining area.

D. Microscopic examination of smears

Never examine the slide while it is wet. Examining a wet slide may damage the microscope. It will also make it difficult to focus and read it correctly.

Do not dry the slides in a blotting paper. Air dry.

Keep all the materials ready,

- Microscope
- Bottle of immersion oil
- Lens paper
- Laboratory request forms
- Slide box for examined slides
- Stained slides

Set up the microscope:

- Place the slide on the microscope stage.
- Use the 10X objective to focus to find a suitable area of the slide to examine.
- Put one drop of immersion oil on the stained smear. Allow the drop of oil to fall freely on to the slide.

Never touch the slide with the oil applicator. This could carry AFB from one slide to the next slide resulting in false positive results.

- Turn the nosepiece and bring the 100X objective into place and focus using the fine adjustment knob.

Never let the lens touch the slide. This can damage the lens and may break the slide.

i. Examination procedure

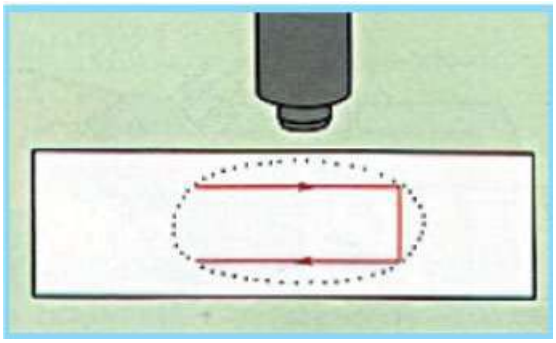


Figure 4.24 – Direction of smear examination.

1. Examine at least 100 microscopic fields.
2. For an experienced laboratory technician this will take at least 5 minutes.
3. Examination must be systematic and standardized. Begin examining the slide at the left end of the smear. See figure 4.24.
4. Systematically examine each field thoroughly beginning at the periphery of the field and ending at the center.
5. After examining one field, move the slide longitudinally so that the next field to the right can be examined. In this manner all the microscopic fields along the length of the smear can be examined.
6. Move the slide a small distance vertically so that a second length can be read from right to left.
7. Search for and identify the tubercle bacilli, which appear as fine red beaded rods against a blue background. They may be slightly curved and occur singly, in pairs or in clumps.
8. Count the number of AFB seen per field and record the results in the laboratory request form.

ii. Grading of the sputum smear microscopy results

The number of bacilli found in the smear is very important, because it relates to the degree of the infectivity of the patient and to the clinical severity of the disease.

Table 4.1- Grading of smear microscopy results

Number of bacilli seen	Result	Grading	No of fields to be examined
No AFB seen per 100 fields	Negative		100
1-9 AFB per 100 fields	Positive (Scanty)	Record the exact No. seen	100
10-99 AFB per 100 fields	Positive	1 +	100
1-10 AFB per field	Positive	2 +	100
More than 10 AFB per field	Positive	3 +	100

1. After completing the examination, remove the slide carefully from the microscope stage.

2. Clean the slides to remove the immersion oil.
3. Before examining the next slide, wipe the immersion oil on the lens with a piece of lens paper.

Grading improves the laboratory technician's attention and facilitates supervision. It helps to assess the load of disease and response to treatment during follow up.

E. Reagent preparation

General Guidelines

- It is recommended to wear Personal Protective Equipment (PPE), e.g. laboratory coats, gloves, etc. When the stains are prepared.
- Always consider the workload when preparing stains. This is to make sure that the prepared reagents are used within 6 months and to avoid wastage.
- All the reagents should be properly labeled and stored appropriately after preparation.
- Maintain proper records with regard to stain preparation.
- Always quality control the batch of stain prepared.

When stain solutions are kept for several months and sediment is observed/stain particles are seen in the smears, the stains should be filtered and be quality controlled.

i. Quality control of stains (IQC)

- All containers of stains and reagents should show the name, concentration, volume, the date manufactured, the date received, the date first opened, and the date of expiry.

- Each new batch of staining solutions must be checked by staining a known positive slide and a known negative slide as controls before being used or sent out. (Make positive control smears with 1+ sputum.)
    - Check whether particles have started to form in the carbol fuchsin.
    - It is recommended to use reagents prepared within six months.

ii. Preparation of reagents

1. 1% Carbol Fuchsin

    Basic fuchsin 10g
    Absolute alcohol 100ml
    Phenol 50g
    Distilled water 900ml

Label:

1% Carbol Fuchsin 100ml
Prepared: 2021-01-01 Date of expiry: 2021-06-30

a) Dissolve Basic fuchsin in Absolute alcohol in a flask. (If phenol crystals are used heat the crystals to melt and add the melted phenol to the above solution). Then add distilled water to make up the final volume.
b) Filter the solution and store in an amber bottle. Label the bottle with the name of the reagent and the dates of preparation and expiry.

2. 3% Acid alcohol solution

Alcohol 95% 970ml
Concentrated hydrochloric acid 30ml
Label:

3% Acid alcohol solution 100ml
Prepared: 2021-01-01 Date of expiry: 2021-06-30

a) Carefully add concentrated hydrochloric acid to 95% alcohol. Always add acid slowly to alcohol and not vice versa.
b) Store in an amber-coloured bottle. Label the bottle with the name of the reagent and dates of preparation and expiry.

3. 0.1% Methylene blue

Methylene blue 0.5g
Distilled water 500ml
Label:

0.1% Methylene blue 100ml
Prepared: 2021-01-01 Date of expiry: 2021-06-30

a) Dissolve the methylene blue in distilled water.
b) Store in an amber-coloured bottle. Label the bottle with the name of the reagent and dates of preparation and expiry.

F. Clean and store the slides

- Clean the slides to remove the immersion oil. Dry them and store them in slide boxes till reviewed by the supervisor.
- All slides should be stored according to the serial numbers month wise.

G. Recording and reporting of results

i. Reporting the results

- Reporting in laboratory request form (TB 05).
- Verify that the laboratory serial number on the slide is the same as that on the laboratory request form and record the results with the grading on the laboratory request form.

Results should be recorded:
• Negative as “Neg”. • Positive as “POS”, and tick off 1+, 2+ or 3+ in the appropriate column.

- All positive results should be written in RED ink.
- Write the date of the report and sign the form.
- Send the completed laboratory request form back to the treating medical officer promptly, preferably within 24 hours of receipt of specimen.
- If the patient was referred from another health unit, the patient should be given a copy of the completed laboratory request form and the original sent to the referring health unit. If the result is positive for AFB, the patient should be asked to go to the DCC with the report.

ii. Recording the Results

- Recording in TB Laboratory Register – TB 04 (Annex 03).
- Verify that all information for each patient is entered completely and accurately in the appropriate columns in the TB Laboratory Register.
- It should include the following:
    - Laboratory serial no
    - Date received
    - Patients name, sex, age and address
    - Name of health institution
    - Reason for examination (For diagnosis of follow-up)
    - District TB No for follow-up case.
- Record the results with the grading from the laboratory request form in the TB laboratory register.
- Results should be recorded as follows:
    - Negative as 'NEG'.
    - Positive as 'POS' 1+, 2+, or 3+.
    - All positive results should be written in RED ink.
- All information requested in the laboratory register must be entered.
- A blank space is NOT a negative result, but a MISSING record.

Causes of false positive results

- Presence of food particles, fibers, and pollen.
- Saprophytic acid-fast bacilli.
- Scratch marks on the slides.
- Precipitated stains (using unfiltered carbol fuchsin).
- Inadequate de-colorization.
- Re-use of containers or positive slides.
- Contamination from another positive smear,
    - Not using a separate wooden stick/applicator for each specimen.
    - allowing the oil applicator to touch the smear.
    - allowing the oil immersion lens to touch the smear.
    - Not cleaning the immersion lens after each examination.
    - Not keeping space between slides when staining.
- Errors in handling specimens.
- Labeling the sputum containers and slides with errors.

Causes of false negative results

- Poor sputum specimen - in quantity and quality.
- Incorrect storage of sputum specimens.
- Poor selection of sputum for smear preparation.
- Incorrect preparation of smears and staining of slides.
- Over de-colorization.
- Not examining at least 100 microscopic fields.
- Errors in labeling sputum containers, slides and laboratory request forms.
- Errors in recording and reporting.

Consequences of false positive results

- Patients are started on treatment unnecessarily.
- In the case of follow up examinations, Intensive Phase of treatment is continued longer than necessary.
- Tuberculosis medication is wasted.
- Patients may lose confidence in the NPTCCD.

Consequences of false negatives results

- Patients with TB are not treated, resulting in suffering, spread of TB, and death.
- Intensive Phase of treatment is not extended for the required duration, resulting in inadequate treatment and sometimes development of resistance.
- Patients may lose confidence in the NPTCCD.

4.2 Florescence microscopy

In 2011 WHO released a new policy for TB diagnosis based on LED based fluorescent microscopy (FM). The technique was introduced to TB laboratory network of Sri Lanka in 2017 replacing the conventional fluorescent microscopy which was limited only to the central level. LED microscopes are now in many district chest clinics with decentralization of the technology.

LED FM offers considerable advantages over conventional fluorescent microscopy. It is less expensive than the conventional method. The life span of the LED is longer than the mercury bulbs used in conventional FM. However main disadvantage is the high initial cost of the fluorescent microscope and the running expenditure and the need for uninterrupted power supply.

For a laboratory with a high work load LED FM is more suitable than light microscopy for screening the patients as it is more sensitive than the bright field microscopy. The important advantage of the fluorescence technique is allowance of examination of a much larger area per unit of time as those are examined in a lower magnification.

FM requires trained, experienced technicians and a fluorescent microscope. LED modules that can be adapted to ordinary microscope or new LED microscopes are simpler, cheaper and safer alternatives to traditional mercury vapor lamp microscopes and do not require dark rooms.

Fluorescence staining is performed in a similar fashion to ZN staining, but carbol fuchsin is replaced by a fluorescent dye (auramine-O, rhodamine, auramine-rhodamine, acridine orange etc.), the acid for de-colorization is milder and the counter stain, though not essential, is useful to quench background fluorescence.

When the fluorescent dye (auramine O) is used and when examined under FM the organism appear bright yellow against a dark background and the smear can be scanned in a much shorter time than by the ZN method.

The same area that needs examination for 10 minutes in bright field microscope can be examined in 2 minutes in fluorescence microscopy.

Principle

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.

With auramine staining, the bacilli appear as slender bright yellow green luminous rods, standing out clearly against a dark background. The identification of the mycobacteria with auramine O is due to the affinity of the mycolic acid in the cell walls for the fluorochromes.

The advantage of fluorescence microscopy is that a low magnification objective is used to scan the smear allowing a much larger area of the smear to be seen. Therefore a smear can be scanned in a much shorter time than by the ZN technique and more sensitive. If the work load is high it may be more cost effective to use fluorescence microscopy.

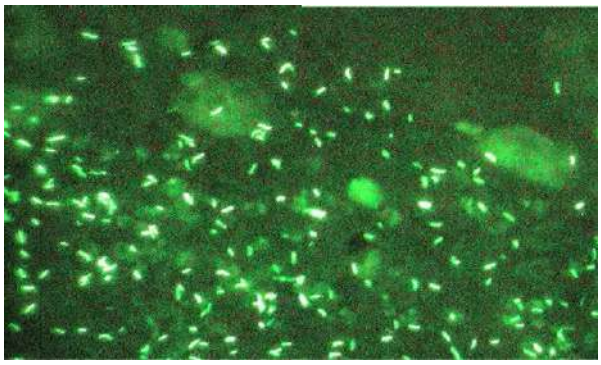


Figure 4.25 - TB Bacilli seen with Auramine O stain.

Materials required for staining,

1. Auramine-Phenol solution
2. 1% Acid alcohol
3. Methylene blue

A. Staining procedure

1. Place the slides on a staining rack, with the smeared side facing up, the slides not touching each other. See figure 4.26.

Figure 4.26 – Placement of slides on the rack

2. Flood the slides with freshly filtered auramine - phenol. Let stand for 20 minutes. See figure 4.27.

Figure 4.27 – Slides in auramine-phenol stain.

3. Wash well with running water, taking care to control the flow of water so as to prevent washing away the smear. See figure 4.28.

Figure 4.28 – Washing off with water.

4. Decolorize by covering completely with acid-alcohol for 2 minutes, twice. Figure 4.29.

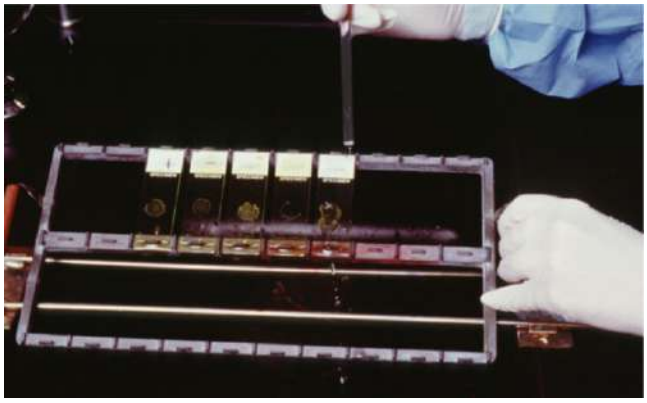


Figure 4.29 – De-colorization with acid alcohol.

5. Wash well with running water, as before to wash away the acid alcohol. See figure 4.30.

Figure 4.30 – Washing off with water.

6. Cover the slide with methylene blue for 1 minute. See figure 4.31.

Figure 4.31 – Counter staining with methylene blue.

7. Wash as before with water and slope the slides to air dry. See figure 4.32.

Figure 4.32 – Air drying of slides.

- Keep the stained smears in the dark in a slide box or a folder.
- Exposure to light fades away the fluorescence.

B. Preparation of stains and reagents (Auramine technique)

Prepare the stains in a well-ventilated area wearing PPE. Clean the spills immediately. Avoid contact with skin and mucous membranes.

i. Solution A - 1% Auramine in ethanol

1. Add 100ml ethanol/methanol to a glass flask.
2. Add 10g of auramine powder.
3. Mix gently until dissolved.
4. Store in a dark bottle and label.
5. Expires in 12 months.

ii. Solution B - 3% phenolic solution for auramine.

1. Dissolve 30g of phenol in 900g of water.

iii. 0.1% Auramine-Phenol solution:

1. Add 50ml of solution A to a dark brown bottle.
2. Add 450ml of solution B.
3. Mix well.
4. The mixture expires in 02 months.

iv. 0.5% acid alcohol

1. Fuming hydrochloric acid (HCl) 5ml.
2. Ethanol/ methanol 1000ml.
3. Carefully add HCl to alcohol.
4. Store in a dark bottle.

v. Counter stain - 0.3% methylene blue

1. Metheylene blue 3g.
2. Distilled water 1000ml.

C. Examination procedure and reporting of results

Read the smears on the same day they were stained.

First examine a known positive slide to ensure that the microscope is correctly set up.

Smear needs to be observed in “linear pattern”. For a trained and experienced lab PHLT, each smear would take approximately a minimum of 2 minutes for 100 fields or three horizontal sweeps.

In the fluorescent staining, smears are examined at much lower magnifications than used for ZN stained smears. Use the 20X objective to scan the smear and the 40X objective for confirming suspicious objects.

Each field examined under fluorescence microscopy, therefore, has a larger area than that seen with bright field microscopy. Thus, a report based on a fluorochrome-stained smear examined may contain much larger numbers of bacilli than a similar report from the same specimen stained with ZN.

- With auramine staining, the bacilli appear as slender bright yellow fluorescent rods, standing out clearly against a dark background. Rule out any artifacts. Refer figure 4.33.

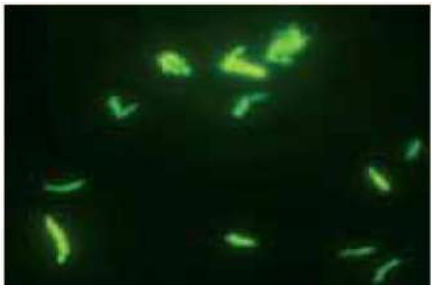


Figure 4.33 – Correct Staining.

- At least one length of the smear should be examined before declaring it negative. See figure 4.34.

Figure 4.34 – Direction of smear examination.

- Do not re-stain the scanty smears with ZN staining.
- Confirmation required by another technician or prepares another smear, stain and read.

Grading of the smears for reporting

4.2 - Grading chart for fluorescence staining method result reporting

What you see (x200)	What you see (x400)	How to report
No AFB in one length	No AFB in one length	No AFB observed
1-4 in one length	1-2 one length	Confirmation required*
5-49in one length	3-24 one length	Scanty
3-24 in one field	1-6 one field	1+
25-250 in one field	7- 60 one field	2+
>250 in one field	>60 one field	3+

Positive smears can be graded into four degrees of positivity.

Trouble shooting

Table 4.3 – Trouble shooting 1: staining with too much fluorescence

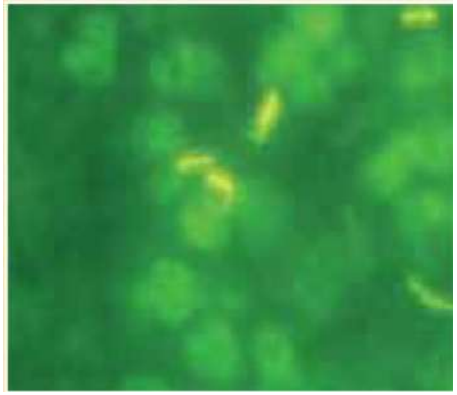
Problem	Cause	Remedy
Too much fluorescence 	Insufficient de-colorization	Check de-colorization time.
	Counter stain too weak or no alcohol	Prepare new reagent.
	Auramine has dried on the smear.	Check smears are level over sink.
		Add sufficient stain.
	Auramine not filtered.	Filter auramine at time of use.
	Smear too thick.	Prepare new smear.
	Do not heat during staining.	

Table 4.4 – Trouble shooting 2: pale acid fast bacilli

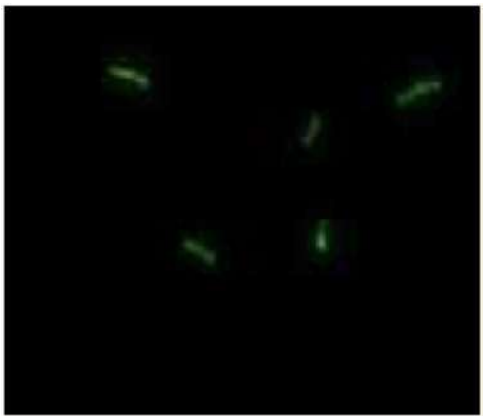
Problem	Cause	Remedy
Pale acid fast bacilli 	Auramine has expired or stored in direct sunlight	Replace reagent.
	Auramine<0.1%	Recheck stain preparation and QC results.
	Staining time <20 minutes	Stain for at least 20 minutes.
	Smear overheated during fixation step.	Pass smear through the flame 3 times. 1-2 seconds each time.
	Over colorized	Do not exceed the maximum time (1-2 minutes only)
	Stained smears exposed to day light.	Keep slides in the dark using slide box or similar.
		Read smears as soon as possible.
	Smear too thick.	Prepare new smear.

Table 4.5 – Trouble shooting 3: background too dark

Problem	Cause	Remedy
Background too dark 	Counter stained too long or decolorized too long	Do not exceed 1 minute. Do not exceed 2 minutes.
	Inadequate washing step after counter staining.	Extend washing step
		Ensure washing step is complete.
	Counter stain concentration too strong	Recheck stain preparation and QC results.
	Smear too thick.	Prepare new smear.

4.3 Quality assurance of sputum smear microscopy

Sputum smear microscopy is the cornerstone for both, the diagnosis and follow-up of TB patients. Reliable laboratory results are essential for proper categorization of the patients, to start the continuation phase of treatment and to declare the patient as 'cured'. To improve the efficiency and reliability of smear microscopy services, a quality assurance programme is needed.

Therefore quality assurance of sputum smear microscopy is an essential component of effective TB control.

The components of quality assurance program are:

- Internal Quality Control (IQC)
- External Quality Assessment (EQA)
- Quality improvement

Quality assurance ensures that the information generated by the laboratory is accurate, reliable and reproducible.

Internal Quality Control

IQC of microscopy is a process of effective and systematic internal monitoring of the performance of bench work in the microscopy laboratory.

Quality improvement

Quality improvement is a process by which the components of TB laboratory services are analyzed continuously to improve the reliability, efficiency and utilization. Data collection, data analysis and problem solving are the key components of this process. It involves continuous monitoring, identification of defects, followed by remedial action to prevent recurrence of problems. Often problem solving can only be done efficiently during on-site supervisory visits. This is the quickest and

most effective form of quality improvement because of personal contact and permits on the spot corrective action.

External quality assessment

EQA is designed with onsite evaluations and with programmes to compare results from different laboratories by means of various methods organized by an external agency such as a reference laboratory.

A. Internal quality control

- IQC activities should be part of everyday laboratory workload.
- It is the responsibility of every Laboratory Personnel.
- Demonstrates that the results generated by a laboratory are likely to be reliable and accurate.
- Results of IQC activities must be documented.

Quality control measures, which must be in place in all tuberculosis laboratories include,

1. Laboratory arrangement and administration

- Work areas, equipment and supplies should be arranged for efficient work flow.
- Benches should be cleaned daily with a disinfectant (e.g. 5% phenol).
- All laboratory procedures should be written out and kept for easy reference.
- All records should be retained for two years.

2. Laboratory equipment

- Equipment should meet the manufacturer's claims and specifications.
- Written operating and cleaning instructions must be kept in a file for all equipment.
- Dated service records and log books must be kept for all equipment.

3. Supervision of sputum collection, smear preparation, smear staining and microscopy reading

- Adhere to standard operating procedures.
- Stain slides in batches with a maximum of 12 sides per batch. Do not stain more than twelve slides at a time.
- Include positive and negative controls with each day's reading.
- Maintain the internal quality control register.

4. Reagents and stains

- All bottles with the reagents should be labeled with the name and concentrate of the reagent, date of preparation, the date of expiry, batch no and the volume.
- Stocks should be limited to six months supply and ensure that bottles with short expiry are used first.

5. Recording and Reporting

- Microscopy results should be sent out as soon as possible, preferably within 24 hours of receipt of specimen.
- All positive results should be written in RED ink.
- All microscopy results must be recorded in laboratory register.
- Analyze microscopy results on a monthly basis to detect changes which may indicate a problem.
- All records should be retained for at least two years.

B. External quality assessment.

EQA is to help laboratories, identify errors and improve practices for better performance. TB laboratory network guided and supervised by a hierarchial laboratory system with the National Reference laboratory at the apex is essential for EQA.

EQA neither identifies individual slide errors nor validates individual patient diagnoses. EQA activity provides the technicians with an opportunity to strengthen skills. Good performance in EQA activities reassures lab technicians that their results are contributing to TB diagnosis and control in a useful manner.

Components of EQA

1. Random Blinded Rechecking (RBRC)
2. Onsite Evaluation
3. Proficiency testing or panel testing

1. Random Blinded Rechecking

Random blinded rechecking is a process of rereading a sample of slides from a laboratory to assess whether that laboratory has an acceptable level of performance.

The instructions for RBRC are provided to the DCC laboratories and to the microscopy centers by the NTRL at the beginning of each year.

a) Method of RBRC

- A statistical sampling method called Lot Quality Assurance Sampling (LQAS) is used for RBRC of slides. The sample size is based on the annual negative slide volume and the positivity rate. (Sampling 10% negatives & 100% positives is no longer recommended).
- From the sample obtained decisions are made on the overall quality of the lot.

Lot Quality Assurance Sampling (LQAS) method for RBRC is the standard sampling method.

To determine the sample size in LQAS, following information is needed,

- No of slides performed per year by each laboratory.
- Number of positives detected by the laboratory.
- Slide positivity rate.

Slide Positivity Rate (SPR)

$$SPR = \frac{\text{Number of positive smear per year}}{\text{Total number of slides examined}} \times 100 \text{ Annual}$$

According to the positivity rate and the number of negative slides processed by the laboratory, the sample size for RBRC (annual sample size) is decided using specially prepared tables to identify the appropriate sample size. (E.g. is shown below.)

A table for determining annual sample size (80% sensitivity, 100% specificity and '0' acceptance number, CI= 95%)

Table 4.6 - Determining annual sample size.

Number of negative slides per year	% Slide positivity rate			
	5	10	15	20
200 - 350	107 (9)	72(6)	54(5)	43(4)
351 - 750	154(13)	89(9)	62(6)	48(4)
751 - 3000	180(15)	96(8)	66(6)	49(4)
3001 - 5000	208(18)	103(9)	69(6)	50(5)
5000 – 50,000	216(18)	104(9)	69(6)	51(5)

Monthly sample size is in (n)

b) Storage of slides

- Laboratory must store the slides in a way that allows retrieval of every slide identified for the re-checking.
- Slides are labeled as in lab register to ensure that the correct slide is matched to the result.
- Slides are stored in slide boxes in the same manner as they are listed in the lab register.
- There is no need to store +ves and –ves separately.
- Prior to placing slides in storage boxes excess oil should be allowed to drain off the slides.
- Store slides in boxes not touching each other.
- Always store slides in closed boxes away from direct sunlight.

c) Selection of slides

A person other than the laboratory personnel who performed the slides should select the slides for RBRC. DTCO or the supervisory officer from the DCC laboratory can be given this responsibility. They can do this during the monthly visit.

First step in selection is the marking of selection in the laboratory register. Slides should be collected from the slide boxes next. E.g. 10 slides required to be collected.

If the supervisor observes that the laboratory processed 80 slides since the last monthly visit every eighth (80/10 = 8th) slide should be collected to obtain the required 10 slides. They may begin with any number between 1 to 8. If no 5 is selected 5th, 13th, 21st ….etc. should be collected to obtain 10 slides required for that month as shown below in the example of the laboratory register.

d) Method of selection of slides for Random Blinded Rechecking from TB laboratory register

Lab Serial No	Date	Name	Sex M/F	Address	Reason for Examination		Result			Signature	Remarks
					Diagnosis	Follow-up	1	2	3		
							Neg				
							Neg	Neg	Neg		
							Neg	Neg			
							1+	2+	8 AFB		
							Neg	Neg	Neg		
							Neg	Neg	Neg		
							Neg	Neg			
							Neg	Neg	Neg		
							Neg	Neg	Neg		
							Neg	Neg	Neg		
							6 AFB	1+	2+		
							Neg	Neg	Neg		
							Neg	Neg	Neg		
							1+	3+			
							Neg	Neg	Neg		
							Neg	Neg	Neg		
							Neg	Neg	Neg		
							Neg	Neg	Neg		
							Neg	Neg	Neg		
							2+	1+	3+		
							Neg	Neg	Neg		
							Neg	Neg	Neg		

e) Blinded Rechecking Process

- Selected slides are sent to the NTRL/DCC laboratory at the end of the every month (Annex 7 for microscopy centers & Annex 9 for DCC laboratories).
- A separate book is maintained for distribution of slides for blinded re-checking (Annex 8).
- All identification features are removed from the slide bundle and the result sheet is kept with the MO NTRL/DTCO.
- Slide bundle is allocated to a technician (1st controller) for blinded re checking.
- Smears are evaluated for the results as well as specimen quality (sputum vs. saliva), appropriate size and thickness, and quality of staining using Form A.
- When the results are reported by the 1st controller results are compared with the original result sheet.
- If a discrepancy is found those slides are given blindly to another technician (2nd controller) to recheck.
- Second controller's decision is taken as the final answer to decide on the discrepancies.

f) Feedback

Regular and timely feedback report to the DCC laboratory/MC is issued by the supervising laboratory on a monthly basis using the format in annex 10.

Feedback will include the return of slides with discordant results to be re-read by the original laboratory personnel of the respective laboratory.

g) Classification of Errors of RBRC

Correct : No errors
Minor error : QE - Quantification error
LFN - Low False Negative
LFP - Low False Positive
Major error : HFN - High False Negative
HFP - High False Positive

Table 4.7 - Classification of Errors of RBRC

Peripheral results by LT/MC	**Results of controller**				
	Negative	**1-9 AFB/100 fields**	**1+**	**2+**	**3+**
Negative	**Correct**	LFN	HFN	HFN	HFN
1-9 AFB/100 fields	LFP	**Correct**	**Correct**	QE	QE
1+	HFP	**Correct**	**Correct**	**Correct**	QE
2+	HFP	QE	**Correct**	**Correct**	**Correct**
3+	HFP	QE	QE	**Correct**	**Correct**

2. **Proficiency testing / panel testing (PT)**

Panel testing is one method of EQA that can be used to determine whether a laboratory technician can adequately perform AFB smear microscopy. A panel consists of a batch of stained and/or unstained smears that are sent out by the reference laboratory to the peripheral laboratories for processing, reading, and reporting of results. This method tests individual performance of technicians.

3. **On site evaluation**

Technical supervisory visits offer the best opportunity to assess the conditions and skills practiced in the laboratory. This is carried out by the trained staff from the NTRL for district chest clinic laboratories once a year and by district labs for the microscopy centers quarterly.

Aspects to be monitored during on site evaluation,

- Infrastructure and availability of standard operating procedures.
- Adequacy of supplies and reagents.
- Availability of a properly functioning microscope and other equipment.
- Work load and slide positivity rates.
- Safety and infection control practices e.g. disposal of contaminated material.
- Practice of satisfactory turnaround time in reporting.
- Adherence to correct procedures in sputum collection, smearing and staining.
- Practice of internal Quality control (IQC).
- Record keeping and documentation as per requirements of the NTRL and storage of slides.
- Participation in EQA activities.
- Training and re-training received.

Tools required for monitoring during onsite evaluation

Checklists - The checklist for on-site evaluation to be used at DCC laboratories and MC is provided in Annex 14.

During the supervisory visit, reviewing results of RBRC and providing suggestions for corrective action or implementing corrective action as needed, can be carried out in relevant laboratories.

Quarterly report on supervisory visits to microscopy centers should be sent to the NTRL by the DCC laboratories.

4.4 Use of microscope

For TB diagnosis with smear microscopy, use of binocular microscope is recommended. Proper maintenance of the microscopes is very important in microscopy. Therefore, basic facts about the microscope and the general maintenance are addressed in this section.

The main parts of a microscope are:

• Eye- pieces
• Microscope tube
• Nose piece
• Objective
• Mechanical stage

• Condenser
• Coarse and fine focusing knobs
• Light source

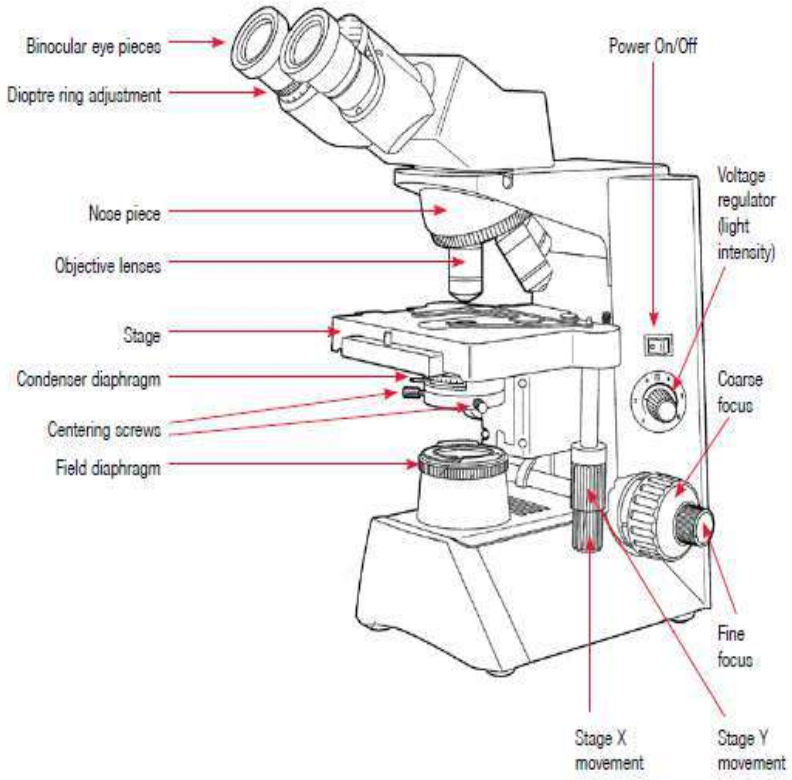


Figure 4.35 – Parts of the microscope.

A. Setting up the microscope

- Adjust the inter-pupillary distance until the two images right and left are merged. Refer figure 4.36.

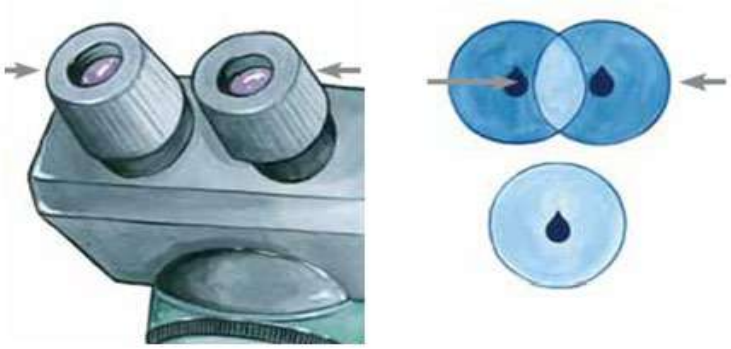


Figure 4.36 – Adjustment of the inter-pupillary distance.

- Focus the image with the fine focus knobs with right eye piece.
- Focus the image with left eye by adjusting diopter ring.
- Open the condenser iris diaphragm to light each field equally.
- Place one drop of immersion oil on smear and move the 100X lens on to it.

B. Care and maintenance of the microscope

In proper handling and maintenance of the microscope the following points should be observed.

- Always carry the microscope with both hands, one hand the base and the other firmly grasping the arm.
- Never carry the microscope with only one hand.
- Place and store the microscope in a dry, dust free and vibration free environment and away from chemicals.
- Place the microscope on a sturdy vibration free surface. Never keep it on a surface where a centrifuge is placed.
- Keep it away from refrigerators and air conditioners.
- Avoid exposing the microscope to direct sunlight.
- Avoid exposing the microscope to moisture.
  Humidity may allow fungus to grow on the lens and cause rusting of metal parts. To reduce moisture, keep dry blue silica gel or any other drying agent in the box, where the microscope is kept. Dry silica absorbs the moisture and when it is unable to absorb any more moisture, it changes colour from blue to pink. As soon as it changes colour the silica gel should be replaced or may be heated and reused.
- When the microscope is not in use, it should be kept in the box or covered with a plastic or polythene cover so as to keep it free from dust.
- If the microscope is used every day, do not remove it from the table and keep it covered with a plastic or polythene cover.
- During examination never let the immersion lens touch the slide. This can damage the lens and may break the slide.
- Use only the fine focusing knob while using the immersion lens.
- Keep the microscope and the lens clean.

After daily use

- Adjust the variable voltage regulator setting to the minimum before turning off the light.
- Turn off the microscope light source.
- Rotate the nose piece to bring the lowest power objective into position before removing the slide.
- Gently wipe the immersion oil from the objective, condenser and mechanical stage with lens paper.
- Replace the microscope cover.

Monthly

- Use an air brush to blow away dust.
- Clean the objectives, eye- pieces, and condenser with lens paper.
- Remove the slide holder from the stage and clean.

- Wipe the dust off the body of the microscope and the window of the illuminator in the base of the unit with a tissue.

Yearly

- Have the microscope inspected, cleaned, and lubricated by professional service personnel.

C. Microscope storage

Microscopes should ideally be stored in boxes with no moisture. Warming boxes can be used to serve this purpose.

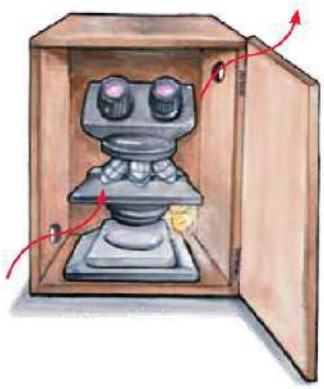


Figure 4.37– Storing the microscope in a warming box.

Table 4.8 – Troubleshooting with microscope

Problem	Possible causes	Solution
Dim Field.	• Condenser may be too low. • Condenser iris may be closed.	• Raise the condenser. • Open the diaphragm.
Dark shadows in the field which move when eye piece is moved.	• Eye piece may be dirty. • Eye Piece or objective may be contaminated with fungus. • Surface of eyepiece may be scratched.	• Clean the eye piece. • Eye piece may need repair. • A new eye piece may be needed.
Image is not clear.	• The smear may not be facing upwards. • May be an air-bubble in the oil. • There may be dirt on the objective. • Oil may be too thick	• Turn the slide over. • Move the 100x lens from side to side. • Clean the lens. • Use only good quality immersion oil.
The image through low power is not clear.	• There may be oil on the lens. • There may be dust on the upper surface of the lens. • The lens may be broken.	• Clean the lens. • New lens will be needed if it is cracked.

4.5 Designing microscopy areas/ centers

The microscopy centers/areas should be designed away from the busy pedestrian areas. A cough area should also be identified when a sputum microscopy area is designed. The area for microscopy should tally with the number of technicians working there and the work load.

Basic requirements for a sputum laboratory,

1. Good ventilation

    Directional air flow: The air flow should be directed out from in. It should never be the other way round. The wind should not be blowing from out to in, through the smear preparation bench to the technician.

2. Smear preparation area

    A bench to prepare the smears: Preferably placed near the window and away from the entrance.

3. Staining area with a sink to stain smears.

4. Examination area – a separate bench to examine the smears with microscope.

5. Recording area – separate bench for documentation.

6. Hand wash basin.

7. Good lighting.

8. Non slip, washable flooring.

9. Reception counter.

Refer figure 4.38 for the layout of the sputum smear microscopy area/center.

- A storage refrigerator is also necessary if the specimens cannot be examined on the same day and has to be stored for examination.
- The microscopy area should be furnished with a storage cupboard and a gown rack.
- It is better if a partition is introduced between the smear preparation area and the rest of the laboratory.

Ventilated work Station (VWS)

- A ventilated work station is a partially enclosed workspace. Air is drawn inward, away from the technician and exhausted outside the laboratory, VWS are inexpensive to build and require little maintenance. VWS do not replace careful attention to risk minimizing laboratory methods.

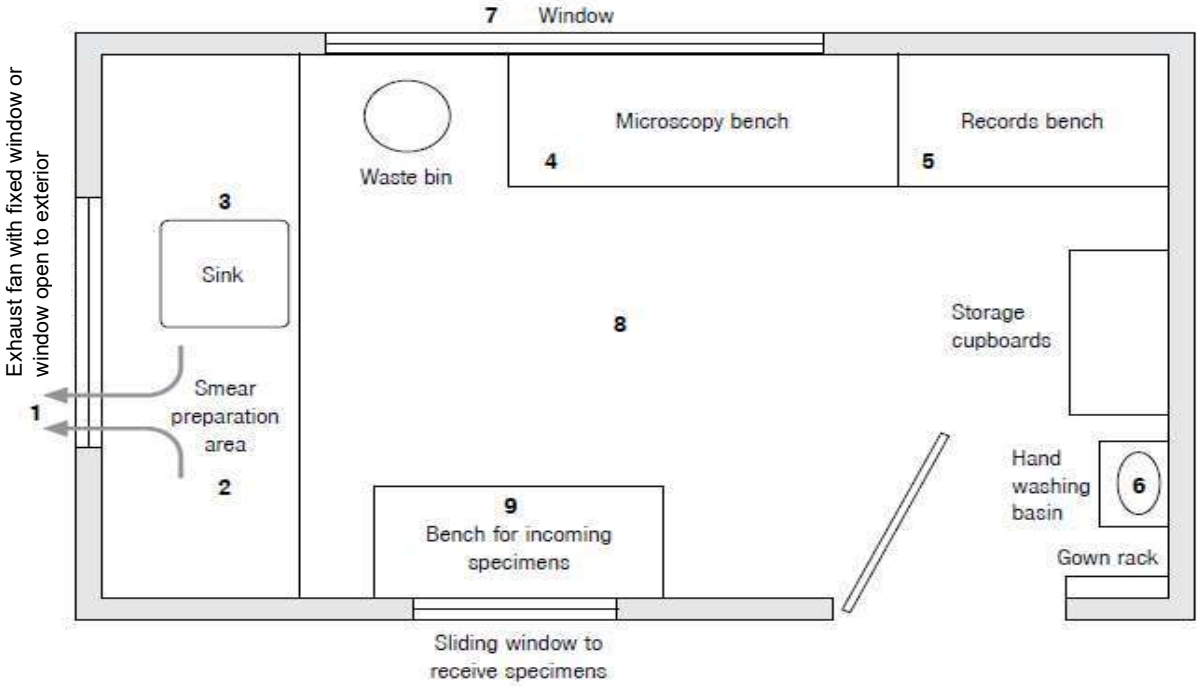


Figure 4.38 – Layout of a of a sputum smear microscopy area/ center

References

1. GLI Laboratory Diagnosis of Tuberculosis by sputum microscopy, The hand book, Global Edition, 2013.
2. Practical guide for Tuberculosis.

05 TB CULTURE

Laboratory diagnosis of TB relies mostly on direct microscopic examination of sputum specimens in most TB prevalent countries. In addition to low sensitivity, microscopy cannot distinguish viable from non-viable organisms and cannot identify drug resistant strains. Therefore, TB culture is highly recommended for bacteriological confirmation of TB.

There are two types of culture methods,

1. Solid culture method
2. Liquid culture method

5.1 Culture on solid media for the diagnosis of tuberculosis

Egg-based media (such as Lowenstein Jensen - LJ or Ogawa) or agar-based media (such as Middlebrook 7H10/7H11) are used for solid culture.

Solid culture on LJ medium is cost effective and is the most widely available culture method worldwide. Culture laboratories in Sri Lanka, NTRL and ITLs also use LJ medium for TB culture.

Indications for the TB solid cultures are defined as follows,

Indication for TB solid cultures

- Pre-treatment cultures patients who have a high risk of drug resistance.
  E.g. HIV positives, contacts of known drug resistant TB patients, prisoners, healthcare workers and drug addicts.
- From patients with RR detected by Xpert MTB/RIF assay.
- Pre-treatment cultures in sputum smear negative PTB patients.
- Patients of whom the sputum remains positive after three months of anti TB treatment (ATT).

ITLs are expected to perform TB cultures for the relevant catchment areas (Refer table 1.1) and send the culture isolates to the NTRL for confirmation and DST. Similar to sputum microscopy services, these laboratories too work in network fashion with the technical guidance of the NTRL.

Advantages of culture:

- Detects fewer bacilli (even 10) and increase the number of case detection.
- Provides definitive diagnosis.
- Allows identification of the organism.
- Provides material for DST.

Disadvantages of culture:

- MTB proliferates extremely slowly (generation time is 18-24hrs). Therefore, results take a long time (06-08 weeks) in solid cultures.
- Needs high degree of training.
- Needs special laboratory facilities.

5.1.1 Specimen collection & transport

Refer Chapter 3 – Specimen collection and transport.

5.1.2 Preparation of LJ medium

Potassium dihydrogen phosphate anhydrous (KH_2PO_4) - 2.40g
Magnesium sulphate- anhydrous - 0.24g
Magnesium citrate - 0.6g
Asparagine - 3.6g
Glycerol (reagent grade) - 12.0ml

↓ **Autoclave 121°C – 30 minutes cool**

+

Malachite green solution 20ml
(2% aqueous solution freshly prepared)

+

Homoginized whole eggs 1000ml

↓

Dispense 6-8ml to each sterile universal container

↓

Inspissation in slant position at 85°C for 50 minutes

↓

Sterility test at 37°C, 48hours

↓

Store in cool condition

5.1.3 TB culture on LJ medium

A. Procedure

(a) Materials

1. LJ medium.
2. Sterile, transfer pipettes with graduations marking volume (individually packed).
3. Tuberculocidal disinfectants.
4. Discard bags with bio-hazard bag insert, containing appropriate disinfectant.
5. Sterile loop or disposable applicator sticks.
6. ZN stain.
7. Parafilm.
8. Microscope slides (new and clean).
9. Paper towels soaked in disinfectant.
10. Sterile distilled water.
11. Permanent marker.
12. Pencil for labeling slide.
13. Study labels.
14. Mortar or blender (for biopsy).
15. Sterile 4% NaOH solution.
16. Shaker/Vortex mixer.
17. Wire racks for holding universal containers.

General facts:

- Specimens are to be checked against the accompanying request forms.
- Evaluate the quality of sputum specimens and make a note on the request form if a specimen resembles saliva (Refer chapter 3 – Specimen collection & transport).
- If the volume of specimen submitted is inadequate, record on the request form as volume is insufficient.
- Generate work sheet with specimen type and laboratory serial number.
- Transfer specimens to laboratory.
- Process sputum and other specimens in batches according to centrifuge capacity.
- Each culture media bottle should be labeled with the TB culture laboratory register number of the specimen before inoculation, with 2 slopes for each specimen.
- In the case of solid culture, the seeding surface of the slant should always be kept clear of any writing or stickers in order to allow an easy reading of the cultures.

(b) Specimen processing

It is important to adhere to the laboratory safety protocols.
For more information on safety, refer to chapter 9 - Laboratory safety.

Sputum and respiratory specimens - Using modified Petroff's method.

1. Open the caps of the specimen containers slowly, avoiding vigorous shaking of the specimen to minimize the aerosol production.
2. Always digest/decontaminate the whole specimen. Add equal volume of sterile 4% NaOH solution, aseptically.
3. Tighten the caps of the universal bottles and mix well.
4. Invert each bottle to ensure that NaOH solution contacts all the sides and inner portion of caps.
5. Place them in shaker & keep for 15 minutes at room temperature with shaking (Total contact time of 4% NaOH 20 minutes).
6. At the end of 15 minutes, universal bottles are to be removed from the shaker.
7. Add sterile distilled water up to the rim of universal bottles.
8. Centrifuge at 3000g for 15 minutes (use refrigerated centrifuge - aerosol containment centrifuge with safety shield, with at least 3000g, to be operated at 20-35°C).
9. Decant supernatant fluid in to discard jar with disinfectant.
10. Add distilled water up to neck and re-suspend the sediment.
11. Centrifuge at 3000g for 15 minutes.
12. Decant the supernatant in to discard bottle.
13. Inoculate the sediment on to 2 slopes of LJ medium.

Tissue biopsy

1. Homogenize the specimen in a sterile porcelain mortar or tissue grinder, using 2-5ml sterile saline.
2. Inoculate the suspension onto LJ medium.

CSF and other sterile fluid specimens

1. Aseptically collected CSF, centrifuge and inoculate sediment directly onto LJ medium, preferably in liquid medium.

Laryngeal swabs

1. Swabs are to be cultured as soon as possible.
2. Use sterile forceps to transfer the swab to a sterile universal bottle.
3. Add 2ml of sterile distilled water.
4. Decontaminate according to the Petroff's method.
5. Using sterile forceps, remove the swab from the tube.
6. Precede the rest of the steps as sputum.

Urine

1. Centrifuge and discard the supernatant.
2. Decontaminate the sediment according to the Petroff's method.

(c) Inoculation and incubation

1. The deposit is inoculated on to two slopes of LJ medium.
2. Use one loop-full (5mm wire loop) of sediment for each inoculation, spread inoculum evenly over entire surface of the medium.
3. Prepare a smear on a slide with the laboratory serial number from the deposit of each specimen.
4. Replace the cap of the bottle.
5. Incubate bottles immediately in an upright position with cap loose for the 1st week of incubation. Then tighten the cap securely and incubate at 37°C for up to 08 weeks.

(d) Culture examination

1. All cultures should be examined 48-72 hours after inoculation, to detect gross contaminants.
2. If contaminated cultures are found during examination,
    - Those where the surface has been completely contaminated or where medium has been liquefied or discoloured, they should be discarded.
    - If contamination is only present on a small surface area of the slant continue to incubate to allow MTB to grow.
3. Cultures can be read on the bench as long as the caps are NOT loosened. To observe fine growth, a strong direct light from a lamp must be shown on to the slant surface.
4. Cultures are examined weekly, up to 08 weeks.
5. Assess time for growth to appear on the medium.
    - After one week of incubation: detect rapidly growing mycobacteria which may be mistaken for MTB.
    - After three to four weeks of incubation: detect positive cultures of MTB as well as other slow-growing mycobacteria.
    - After eight weeks of incubation: may detect MTB and very slow-growing mycobacteria.

(e) Identification of cultures

- Typical colonies of MTB are dry, rough, crumble, buff coloured and slow- growers.
- All culture isolates should be tested for acid-fast bacilli using ZN or Auramine staining and for purity.
- Identification of MTBC can be confirmed with MPT-64 test (rapid immunochromatographic strip test).
    - MPT-64 negative isolates to be identified with further testing at NTRL and **should not** be reported as MOTT.
- Culture reports should be qualitative (e.g. positive or negative) as well as quantitative (e.g. number of colonies isolated).
- The following grading scheme should be followed,

Table 5.1 – Grading of positive cultures

Reading	Report
No growth	Negative
1-19 colonies	Positive (Number of colonies)
20-100 colonies	Positive (1+)
>100 discreet colonies	Positive (2+)
Confluent growth	Positive (3+)
Contaminated	Contaminated

5.2 Liquid culture – mycobacteria growth indicator tube (MGIT)

Mycobacterial culture is more sensitive, but growth of TB bacilli on conventional solid medium requires up to eight weeks, which would delay appropriate treatment in the absence of a confirmed diagnosis.

Liquid media (e.g. Middlebrook 7H9) offer a considerable time advantage over solid media: 07–14 days for culture positivity in liquid medium, compared with 21–42 days in LJ medium. Culture on liquid media also has higher sensitivity than solid cultures.

In general, liquid media contain modified Middlebrook 7H9 broth plus a mixture of antimicrobial agents. Several automated systems have been commercially developed for rapid detection of mycobacteria in liquid medium. One of the most widely used automated systems for rapid detection of mycobacteria in liquid medium is the BD BACTEC MGIT 960 system.

Specimens currently performed in liquid culture

- BAL, bronchial wash.
- Lung biopsy.
- Sputum specimens in which rifampicin resistance was detected by Xpert MTB/RIF assay.
- All extra-pulmonary specimens including pleural fluid, pleural biopsy, any other tissue specimens, pus, tissue or fluid aspirates.

5.2.1 Automated BD BACTEC MGIT 960 TB culture system

Principle

The MGIT (Mycobacteria Growth Indicator Tube) consists of liquid broth medium that is known to yield better recovery and faster growth of mycobacteria. The MGIT contains 7.0 ml of modified Middlebrook 7H9 broth base. MGIT growth supplement OADC (Oleic acid, Albumin, Dextrose and Catalase) is added to each MGIT tube to provide substances essential for the rapid growth of

mycobacteria. MGIT PANTA (Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim and Azlocillin) is an antibiotic mixture added to the medium to suppress contamination.

In addition to Middlebrook 7H9 liquid media, the MGIT tube contains an oxygen quenched fluorochrome embedded at the bottom of the tube. During bacterial growth within the tube, the free oxygen is utilized and depleted in the tube. The intensity of fluorescence is directly proportional to the extent of oxygen depletion and analysis of the fluorescence is used to determine if the tube is positive for growth.

MGIT Instrument

BD BACTEC MGIT instruments an automated system which continuously monitors the bacterial growth every 60 minutes. The machine signals when there is a growth. The instrument declares a tube negative if it remains negative for minimum of 42 days (up to 56 days).

A. Materials

1. Disposable, sterile, screw-capped, conical 50 ml plastic tubes.
2. N-Acetyl L-Cysteine (NALC) Powder.
3. NaOH pellets.
4. Sterile transfer pipettes with graduations.
5. Micro Pipettes and Tips.
6. Timer.
7. Vortex mixer, shaker.
8. Racks.
9. 5CC syringes.
10. Tuberculocidal disinfectant.
11. Discard bags with bio-hazard bag insert, containing appropriate disinfectant.
12. Sturdy labels.
13. Permanent markers.

B. Reagents

1. Machine specific reagents

- BD BBL MGIT Mycobacteria growth indicator tube
- BACTEC MGIT Growth supplement
- BBL MGIT PANTA

Reagents should be stored as per the manufacturer's instructions.

The following reagents are needed for processing of specimens.

A. NaOH-NALC reagent.
B. Phosphate buffer (pH 6.8, 0.067 M).

5.2.2 Specimen collection and transport

Refer chapter 3 – Specimen collection and transport.

Note: Pulmonary and extra-pulmonary (except for urine & blood) specimens can be processed using BD BACTEC™ MGIT™ 960 System. (E.g. Pus and other mucopurulent specimens, gastric aspirates, bronchial washings, laryngeal swabs, tissue biopsies, other body fluids such as CSF, synovial fluid and pleural fluid are accepted with this system)

5.2.3 Safety

Refer chapter 9 – Laboratory safety.

5.2.4 Procedure

All the procedures described here should be carried out in bio safety cabinet (BSC).

A. Digestion, Decontamination and Concentration
B. Preparation of reagents
C. Inoculation of MGIT medium
D. Preparation for smears for AFB

A. Digestion, Decontamination and Concentration

It is extremely important to follow the standard procedure for decontamination recommended for MGIT in order to obtain optimal results. A general outline of processing procedure for different types of clinical specimens is given below,

1. Record all the specimens processed in a batch using the lab processing worksheet. Also, record the name of the technologist's processing each batch.
2. Allow refrigerated specimens and the reagents to come to room temperature before processing.
3. Homogenize the tissue biopsies with a tissue grinder and transfer into a 50ml sterile centrifuge tube. If necessary, tissue may also be placed in a sterile Petri dish and be torn apart with the help of two sterile needles before grinding.
4. When processing a swab specimen, transfer the swab into a 50ml sterile, screw-capped centrifuge tube and add 2ml sterile water. If necessary, break off the swab stick.
5. If the volume of a liquid specimen is over 10 ml, first concentrate by centrifuging at 3000g for 20 minutes. If the specimen is a thick fluid, liquefy the specimen by adding a small quantity of NALC only (50mg powder) and mixing well. After the concentration step, suspend the sediment in 5ml sterile water.
6. Due to the low pH, gastric aspirates should be processed as soon as possible. Concentrate gastric aspirates by centrifuging at 3000g for 20 minutes. Re-suspend the sediment in about 5ml of sterile water.
7. Add NaOH-NALC-sodium citrate solution in a volume equal to the volume of specimen. Tighten the cap. Start the timer.
8. Vortex lightly or hand mix for about 15-30 seconds. Invert the tube so the whole tube is exposed to the NaOH-NALC solution.

9. Wait 15 minutes after adding the NaOH-NALC solution. Hand mix/invert every 5-10 minutes.
10. Make sure the specimen is completely liquefied. If still mucoid, add a small quantity of NALC powder directly to the specimen tube. Mix well.
11. At the end of 15 minutes, add phosphate buffer (pH 6.8) up to the top ring on the centrifuge tube. Mix well by inverting several times.
12. Centrifuge the specimen at 3000g for 20 minutes in a refrigerated centrifuge.
13. After centrifuging, allow the tubes to stand for 5 minutes to allow aerosols to settle.
14. Carefully decant the supernatant into discard jar with 5% phenolic disinfectant. Make sure the sediment is not lost during decanting of the supernatant fluid.
15. Add a small quantity (1-2ml) phosphate buffer (pH 6.8) and vortex the sediment to re-suspend.
16. Use the re-suspended pellet for making smears and for inoculation of MGIT tubes ± LJ media.

B. Preparation of reagents

Reconstitute MGIT PANTA with 15ml of MGIT growth supplement.

C. Inoculation of MGIT medium.

1. Label MGIT tubes with specimen number.
2. Unscrew the cap and aseptically add 0.8ml of MGIT growth supplement/PANTA to each MGIT tube. Use of adjustable pipettes is recommended.
3. Using a sterile pipette or a transfer pipette, add up to 0.5ml of a well-mixed processed/concentrated specimen to the appropriately labeled MGIT tube. Use separate pipette or pipette tip for each specimen.
4. Immediately recap the tube tightly and mix by inverting the tube several times.
5. Wipe tubes and caps with a disinfectant and leave inoculated tubes at room temperature for 30 minutes.

Inoculation of additional media

It is customary to use two different types of media for maximum recovery of mycobacteria. With the MGIT system, maximum recovery of mycobacteria may be achieved by using an additional solid medium, most commonly an egg-based medium such as LJ is used.

D. Smears for Acid-Fast Bacteria

Smear preparation - Prepare smears from the deposit of all processed specimens before inoculation into medium.

5.2.5 Entering the tubes into the BACTEC MGIT 960 instrument

All inoculated MGIT (7ml) tubes should be entered in to the BACTEC MGIT 960 instrument as soon as possible after scanning each tube (refer the BACTEC MGIT 960 instrument manual for details).

5.2.6 Incubation of MGIT tubes

MGIT tubes should be incubated until the instrument flags them positive.

After a maximum of six (06) weeks, the instrument flags the tubes negative if there is no growth. If an instrument positive tube is determined to be smear negative for either mycobacteria or contaminant, the tube may be re-entered to the instrument but within 05 hours.

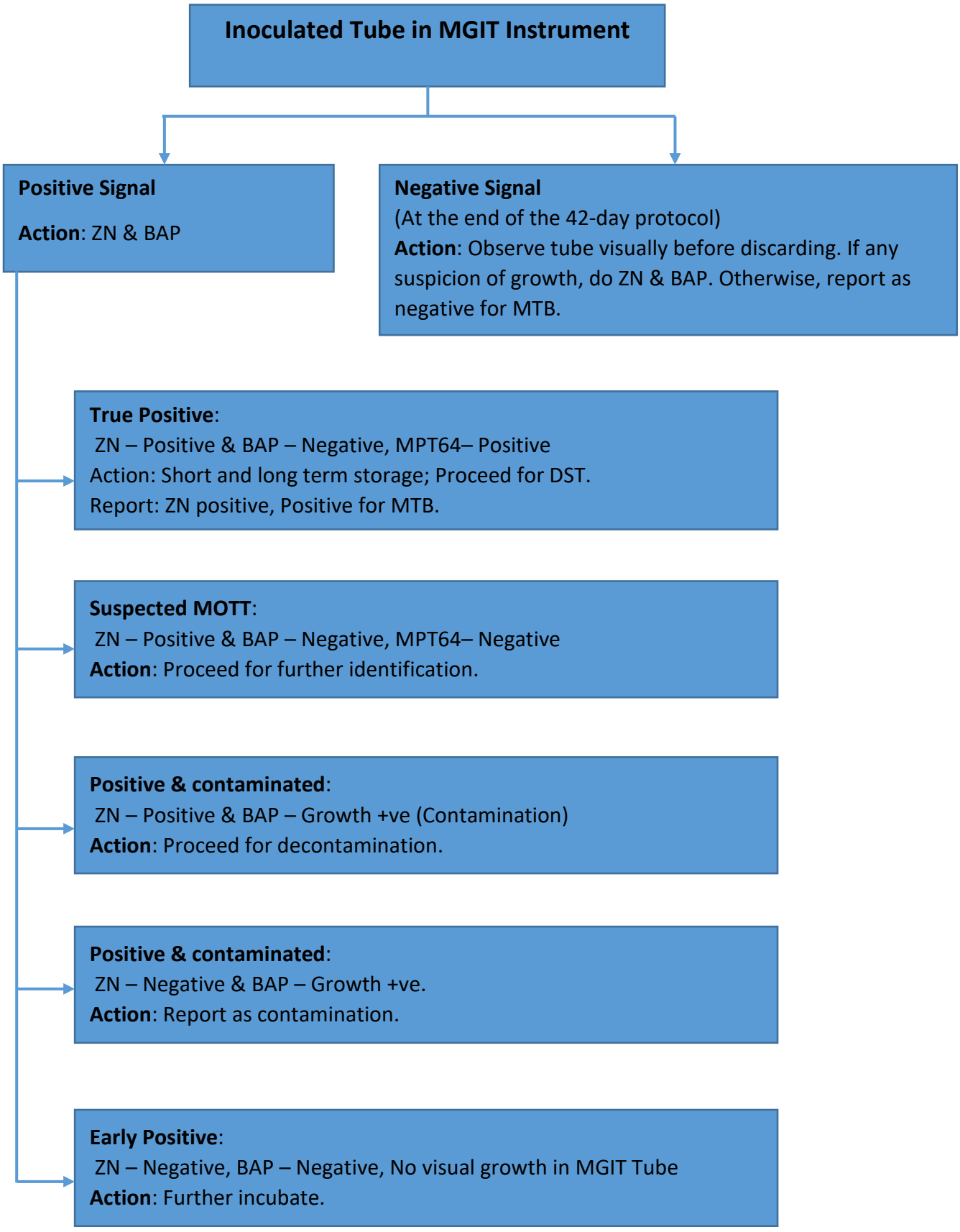
5.2.7 Detection of positive growth

When the instrument signals a tube positive for growth, the tube should be removed from the instrument. The tube should be observed visually. Mycobacterial growth appears granular and not very turbid while contaminating bacterial growth appears very turbid. Growth, especially of MTBC, settles at the bottom of the tube.

Identification of positive tubes

1. Work inside the BSC.
2. Prepare a smear on a glass slide from all positive tubes for ZN/ Auramine staining.
3. All positive tubes should also be sub-cultured on blood agar plates (BAP).
4. Identification of MTBC is confirmed by MPT 64 test.
5. DST should be performed on all MTBC isolates.

Figure 5.1 - General Algorithm MGIT 960 Cultures.



5.3 Recording and reporting

All positive culture identified as *M. tuberculosis* should be informed to the relevant clinicians and the DTCOs over the phone or by email. A hard copy of the report should be sent to the relevant clinician by hand/post (Refer Annex 16).

5.4 Quality control of media

A. Quality control of solid media

Record of batch numbers, dates of preparations, expiration dates and quality control results should be recorded when the solid media is put into use.

B. Quality control of MGIT media

The media are quality controlled for sterility, growth and selectivity.

Commercially prepared media need not be quality controlled, provided that documents of the quality control by manufacturer are available. The document should include information on the preparation date, lot number, and expiration date, the organisms used for quality control, the date of testing and the result.

Record lot number, expiry dates, etc. for new MGIT medium and supplement/ PANTA on the reagent/ media quality control form.

C. Quality control and monitoring of MGIT instrument

Perform daily functional and temperature checks of the MGIT instrument and record on the MGIT maintenance log. Each month, run the MGIT quality control report (a report generated by the MGIT 960 instrument), which lists the status of all the detectors in the instrument, along with the date and time of their last verification. The report also lists all manually blocked stations. Print out the report and maintain in the laboratory files.

References

1. European Centre for Disease Prevention and Control, 2016; Handbook on TB laboratory diagnostic methods for the European Union.
2. Global Laboratory Initiative advancing TB diagnosis; Mycobacteriology Laboratory Manual; First Edition, April 2014.
3. MGIT Manufactures' Manual.

06 ANTI TB DRUG SUSCEPTIBILITY TESTING

Mycobacterium tuberculosis develops resistance to the anti-TB agents used in the treatment (Anti TB Treatment - ATT). A combination of antibiotics is used to treat TB disease due to the possibility of emergence of resistance conferring mutations in MTB strains. The success of treatment depends on the susceptibility of the infecting strain to the antibiotic regime used.

Drug susceptibility testing (DST) detects drug resistance or confirms the emergence of drug resistance, ensures the correct choice of ATT regime providing the best chance of cure, and offers insight into appropriate prophylactic treatment of immediate contacts. DST is also used to estimate the prevalence of primary and acquired drug resistance in a community.

Laboratories use different methods to detect resistance of MTB to antibiotics used in ATT regimes. Some NAATs (Nucleic Acid Amplification Testing) based rapid methods (e.g. Xpert MTB/RIF assay) detect the presence of drug resistant MTB strains directly on specimens. Indirect DST methods are used to perform DST on MTB isolates grown from specimens.

Most phenotypic DST methods are based on detection of growth of a MTB strain on culture media in the presence of ATT agents. Three methods which have been traditionally used to detect the presence of resistance are absolute concentration method, resistance ratio method and proportion method.

Growth based indirect DST is commonly performed using the following two culture methods,

1. DST using solid culture media with manual processing and naked eye observation of growth.
2. DST using liquid media and automated culture systems for incubation and growth detection.

LJ medium is the commonly used solid culture medium for DST. LJ growth based phenotypic methods of DST have been well established at NTRL, Sri Lanka for a long time before the new molecular diagnostic methods was introduced.

Conventional DST methods using solid media take up to 03 to 08 weeks to observe a result. However, DST method using liquid media such as MGIT has reduced the turnaround time compared to that of solid media and takes an average of 15 days to obtain results.

NTRL provides the DST for all positive MTBC cultures isolated from patient specimens processed for culture at NTRL and the ITLs in the TB laboratory network of Sri Lanka. Currently, both solid and liquid media-based methods are used for DST. NTRL is in the process of transition from solid DST to MGIT based liquid DST (WHO recommended) method.

Susceptibility to all first-line drugs (isoniazid, rifampicin, ethambutol and pyrazinamide) and streptomycin are tested initially. Second-line DST is performed on isolates showing RR/MDR TB pattern on initial testing.

Indications for DST

The first line ATT drugs – RIF, INH (2 concentrations), EMB & PZA should be performed on,

1. On all the positive initial culture isolates.
2. On culture isolates grown from a different specimen source from the same patient.
3. On positive culture isolates grown at 03 months if a patient remains culture positive after treatment.
4. Isolates from patients who are clinically failing treatment.

Second-line drug susceptibility tests should be performed on:

1. All RR/MDRTB isolates (e.g. isolates demonstrating isoniazid and/or rifampicin resistance).
2. All isolates demonstrating resistance to ≥ 02 first line drugs.
3. Isolates from patients experiencing severe adverse reactions to first-line agents.

6.1 DST on LJ medium

LJ medium based DST is performed at NTRL using the proportion method recommended by international TB laboratory networks.

6.1.1 Laboratory safety

Since solid DST involves preparation of suspensions of MTB cultures it is considered as a high risk procedure which should be performed inside a BSC (class II A2) within a BSL 3 level laboratory under strict monitoring of safety parameters. Only technical staffs with special training are allowed to perform this procedure.

Refer chapter 9 – Laboratory safety.

6.1.2 Principle

DST is performed on MTB isolates obtained through culturing of patient specimens. It uses antibiotic solutions prepared using commercially available antibiotic powders for laboratory use. Critical concentrations of anti-TB agents decided by international convention are used for the test.

Antibiotics are incorporated into LJ media which are inoculated with a series of dilutions of the MTB strain to be tested. Antibiotic free LJ media are also inoculated with the dilutions of prepared MTB suspension as controls.

The inoculated media are incubated at 37±1°C and examined for contamination after 01 week of incubation. Growths are observed after 04 weeks of incubation for provisional results and after 06 weeks for definitive DST interpretation.

Any growth of MTB exceeding a critical proportion (usually > 01%) on an anti-TB drug containing medium is used for the interpretation of clinically significant resistance to the drug used in the medium.

6.1.3 Internal quality control

Each stage of DST procedure including equipment calibration, drug media preparation and inoculum preparation should be performed under strict quality control. Every new batch of drug containing media prepared for DST must be quality controlled. For each drug, a slant of the critical concentration should be tested as well as the specially prepared media with lower drug concentrations. A suspension of a freshly sub cultured *M. tuberculosis* H37Rv strain should be used with other reference MTB strains.

6.1.4 External quality assessment

EQA should be performed according to recommendations of the international TB laboratory network.

NTRL has established links with the supranational laboratories for annual EQA.

6.2 DST on liquid culture using BACTEC MGIT 960

There are different types of liquid media systems e.g. MGIT 320 or 960, VersaTREK which have been authorized for drug susceptibility testing for TB.

BACTEC MGIT 960 system is an automated system recommended by WHO for performing DST for anti TB drugs.

Susceptibility testing in the BACTEC MGIT 960 system is based on the same principle as isolation from sputum (detection of growth).

Positive MTBC cultures grown in MGIT (direct method) must be pure as contaminating bacteria can potentially cause false-resistant results. Broths should be sub-cultured on to LJ media or 7H10/7H11 and blood agar to assess purity and colony morphology. If a culture is mixed with MOTT or other bacteria, laboratories should attempt to re-isolate the MTBC.

Positive cultures grown on LJ media (indirect method) can also be used after confirmation of the identification as MTBC.

DST is performed using an AST (antibiotic susceptibility testing) set, which consists of a Growth Control tube and one tube for each drug, as well as a bar coded tube carrier that holds the set. A known concentration of drug is added to a MGIT tube, along with the isolate, and growth is compared with a drug free control of the same isolate.

The MGIT 960 system monitors these growth patterns and can automatically interpret results as susceptible or resistant. An isolate is defined as resistant if 1% or more of the test population grows in the presence of the critical concentration of the drug.

All first-line drugs (streptomycin, isoniazid, rifampicin, ethambutol (SIRE); and pyrazinamide (PZA)) can be tested in the MGIT 960 system. DST of second line drugs are well established with the MGIT system and results are reliable and reproducible. Therefore, the MGIT system is recommended for testing second line drugs.

6.2.1 Turnaround time

Laboratories should aim to report MTBC DST results within an average of 15–30 days from the time of the original specimen reception. The DSTs themselves can generally be completed within 07–14 days of obtaining the initial MTBC isolate from the primary cultures if DST is performed using liquid media.

6.2.2 Internal quality control

IQC using a pan-susceptible strain, such as H37Rv which is sensitive to all of the test drugs, must be performed;

• For each new batch of reagents (MGIT drug kits, other drugs, media, etc).

• Weekly - in a DST run when patient tests are run weekly.

• With each batch of patient isolates - when DST is performed less frequently.

6.2.3 External quality assessment

All laboratories performing DST for anti TB drugs should participate for EQA programme the proficiency panel received at laboratory should be tested and the results should be reported within the stipulated time period.

All drug resistant TB isolates should be sent to NTRL for confirmation, further testing and monitoring purposes.

6.3 Recording and reporting of DST results

RR/MDR and isoniazid resistant results are informed to coordinator for Programmatic Management of Drug Resistant TB (PMDT) and the relevant clinician on the same day.

All DST results should be reviewed by the consultant microbiologist before authorizing the release of reports (Annex 17). When critical patterns of resistance such as RR TB/MDR TB/pre-XDR TB/XDR TB/mono-resistance to isoniazid is detected, it should be immediately informed to the consultant microbiologist who in turn alerts the treating clinician and the PMDT coordinator of NPTCCD to review the patient, optimize ATT regime and implement infection control precautions.

All other laboratories performing DST, should send monthly DST results to NTRL for monitoring purposes.

References

1. Global Laboratory Initiative advancing TB diagnosis; Mycobacteriology Laboratory Manual; First Edition, April 2014.

07 MOLECULAR DIAGNOSTICS

Effective management of TB relies on the rapid diagnosis of TB, rapid detection of drug resistance and prompt initiation of an effective treatment regimen and infection preventive measures. Thus, there is a need for fast and accurate detection tests and rapid and accurate drug susceptibility testing for all TB patients. To meet these requirements, an increasing number of novel molecular diagnostic tools have been developed over the last two decades. These novel molecular TB diagnostics are rapid, accurate and easy to perform tests than conventional diagnostic tests.

7.1 Xpert MTB/RIF assay for detection of *Mycobacterium tuberculosis* and rifampicin resistance

- Detection of MTB specific nucleic acid with molecular diagnostic techniques is a sensitive and specific method for bacteriological confirmation of TB.
- Xpert MTB/RIF assay is an automated, cartridge based NAAT for the diagnosis of TB using Xpert MTB/RIF cartridges and a GeneXpert® platform.
- GeneXpert® PCR platform is a real-time PCR system with a rapid DNA detection technology. Xpert MTB/RIF cartridges detect the presence of MTB and rifampicin resistance in respiratory or extra-pulmonary specimens obtained from patients.
- It is recommended by the WHO as a rapid diagnostic technique to diagnose pulmonary & extra-pulmonary TB and to detect resistance to rifampicin.

7.1.1 Indications for Xpert MTB/RIF Assay

A. Xpert MTB/RIF assay as the initial diagnostic test
(culture ± microscopy for AFB, can be done as additional tests)

1. Presumptive TB meningitis.
2. Critically ill patients in whom TB is suspected (PTB/EPTB).
3. Presumptive TB in paediatric patients (age ≤14 years).
4. TB testing on people living with HIV.
5. Presumptive PTB during pregnancy and peri-partum period.
6. Presumptive PTB in patients with evidence of immune-suppression.
7. Presumptive EPTB patients in whom tissue or fluid aspiration from affected site is possible.
8. All presumptive MDR TB patients.

B. Xpert MTB/RIF assay as a follow–on test

As per the National TB diagnostic algorithm (Annex 1, 2), microscopy for AFB and CXR is the initial diagnostic test in patients with presumptive PTB.
Xpert MTB/RIF assay is offered for patients when the sputum smear is negative for AFB and having clinical features suggestive of PTB or changes on CXR or positive Mantoux testing (smear negative PTB).

7.1.2 Principle

- Xpert MTB/RIF assay detects the rifampicin resistance determining region (RRDR) of the *rpoB* gene in the wild type of MTB**.** Mutations in RRDR are the most common mechanisms responsible for rifampicin resistance in MTB.
- Cartridges used are self-contained unit with specimen processing, amplification and detection integrated in to a single self-enclosed test unit.

7.1.3 Safety measures

Refer chapter 9 - Laboratory Safety.

7.1.4 Specimens

Refer chapter 3 – Specimen collection and transport.

Types of specimens:

1. Expectorated sputum specimen.
2. Induced sputum.
3. Broncho-alveolar lavage.
4. Aspirates from extra-pulmonary sites.
5. Tissue/Biopsy specimens (need special processing with tissue grinders before adding to the cartridge).

Suboptimal specimens (Poor sensitivity/low grade evidence according to WHO references)

1. Pleural fluid, blood/bloodstained specimens, urine, feces.
2. Blood-stained and xanthochromic CSF specimens may cause false-negative results from Xpert MTB/RIF assay.
3. Low volume specimens.

7.1.5 Specimen registration.

1. When a separate specimen is received for Xpert MTB/RIF assay, the same container can be used for specimen processing.
2. If both TB culture and Xpert MTB/RIF assay test is requested from the same specimen, a portion (0.5ml to 05ml depending on the total volume) should be carefully transferred into a separate sterile, screw capped container and sent to NTRL/ITL for TB culture.
3. Assign unique GeneXpert number.
4. Document in the GeneXpert register (Annex 19).

7.1.6 Procedure

Material

1. Commercially available test kit (CGXMTB/RIF kit).
   Contents of test kit,
   i. Xpert (MTB/RIF) cartridges

ii. 2ml sterile disposable pipettes
iii. Sample reagent bottles

2. Labeling material, marker pens.
3. Discard jar, phenolic/ other suitable disinfectant, yellow bags, waste bins.

Method

1. Specimen preparation is different for different types of specimens.
   A. Respiratory specimens, purulent or thick aspirates.
   B. CSF and other sterile/clear fluids.
   C. Tissue specimens.

Respiratory specimens/ thick fluids

1. Mark the level on container to assess the volume of specimen.
2. Transfer 02 volumes of SR to one volume of specimen in the container using a fresh, sterile, single-use transfer pipette.
3. Close the container tightly.
4. Vigorously shake with 10 to 20 back & forth movements or vortex for at least 10 seconds.
5. Incubate for 10 minutes at room temperature.
6. Shake/vortex again.
7. Incubate at room temperature for an additional 05 minutes.
8. Specimen should be perfectly liquefied with no visible clumps before transferring it in to the cartridge.
9. If still viscous, wait for 05-10 further minutes.

CSF & clear sterile fluids

1. Mark the fluid level on container to assess the volume of specimen.
2. Add an equal volume of sample reagent.
3. If specimen volume is <01ml, add SR to bring the total volume up to 02ml.
4. If there is less than 0.1 ml, it is an insufficient specimen for testing using the Xpert MTB/RIF assay.
5. Close the container tightly.
6. Vigorously shake with 10 to 20 back & forth movements or vortex for at least 10 seconds.
7. Incubate for 10 minutes at room temperature, and shake/vortex the specimen again.
8. Incubate the specimen at room temperature for an additional 05 minutes.

Tissue specimens

1. Using sterile pair of forceps and scissors cut the tissue specimen into small pieces in a sterile mortar (or homogenizer or tissue grinder).
2. Add approximately 2ml of sterile phosphate buffer (PBS).
3. Grind the solution of tissue and PBS using a mortar and pestle (or homogenizer or tissue grinder) until a homogeneous suspension has been obtained.
4. Use a transfer pipette to transfer approximately 0.7ml of the homogenized tissue specimen to a sterile, conical screw-capped tube. Avoid transferring any clumps of tissue that have not been properly homogenized.

5. Use a transfer pipette to add a double volume of the Xpert MTB/RIF SR (1.4ml) to 0.7ml of homogenized tissue.
6. Follow the next steps in the same manner as other specimens.

Transferring Prepared Specimen

1. Open the cartridge lid.
2. Transfer 2ml of the processed specimen using a fresh, sterile, single-use transfer pipette.
3. Close the cartridge lid properly.

Loading cartridges to the instrument

1. Load the cartridge into the GeneXpert instrument within 30 minutes of transferring processed specimen into the cartridge.
2. Follow manufacturer's instructions for cartridge loading and starting test.

Internal Quality Control (IQC) and Interpretation of results.

1. Internal quality control procedure is in cooperated in the cartridge as Probe Check Control (PCC) and Specimen Processing Control (SPC).
2. Interpretation step is automated. The results are interpreted by the GeneXpert Dx system.
3. Read the results displayed on the computer at the end of testing process.
4. Possible outcomes of the test and the next steps are listed in Table 7.1.
5. Record the observed result on the worksheet and proceed accordingly.
6. Enter results in the register (Annex 19).

Table 7.1 – Results of the test and relevant action

Result	Action
a) *M. tuberculosis* detected. • Very low/low /medium/high • Rifampicin resistance not detected,	Validate result and prepare report in standard format (Annex 23). Inform the medical officer.
b) *M. tuberculosis* detected. • Very low/low /medium/high • Rifampicin resistance detected OR indeterminate.	**Inform Consultant Microbiologist for advice regarding further testing, reporting and notification.**
c) *M. tuberculosis* not detected.	Validate result and prepare report in standard format (Annex 22) with comments on further testing (if any).
d) Test Invalid	Needs repeat testing, if specimen volume is adequate. Request a fresh specimen if specimen volume is inadequate.

e) Error	Document module & type of error and repeat test if specimen volume is adequate. Request a fresh specimen, if specimen volume is inadequate

7.1.7 Report validation and communication of results

- **Critical results**
  - CSF positives or rifampicin resistant/indeterminate results are considered as critical.
  - Inform consultant microbiologist immediately when critical results are noted.
- **Other MTB positives** should be informed to medical officers to inform the requesting clinician over the phone.
- Report should be printed appropriately for results a, b &c. (Annex 23, 24, 25 & 22).
- MLT performing test should validate results & sign with date on the worksheet and on the report.
- Report should be signed by the consultant microbiologist or a designated medical officer.

7.1.8 Documentation

- Details of specimens and results should be entered in the GeneXpert register (Annex 19) maintained in the testing site.
- Monthly performance summary report should be prepared and shared according to the instructions (Annex 21) given by NTRL.
- Documents on cartridge stock management and maintenance of GeneXpert machine should be maintained in separate files.

7.1.9 Disposal of used cartridges, disposable pipettes & containers.

Refer chapter 10 – Laboratory waste disposal.

- Place used cartridges and closed containers in a separate waste disposal container.
- Transfer to the washing room for autoclaving in yellow bags.
- Autoclave yellow bags at 121^{o}C for 15 minutes.
- Arrange safe transport of labeled autoclaved yellow bags to the clinical waste disposal area.

7.1.10 Maintenance and calibration of GeneXpert machine

Refer instructions given by NTRL & manufacturer.

7.1.11 Xpert® MTB/RIF Ultra Assay.

- Ultra is the next generation assay developed by Cepheid.
- It is performed using the same GeneXpert® platform.
- Ultra has a higher sensitivity than Xpert MTB/RIF particularly in smear negative culture positive specimens and in specimens from HIV infected patients with at least as good accuracy for rifampicin resistance detection.

- The Xpert® MTB/RIF Ultra assay can detect very small numbers of non-viable or non-replicating bacilli particularly in patients with a history of ATT.
- The increased sensitivity and loss of specificity are primarily related to the Xpert® MTB/RIF Ultra assay "trace" call. Trace results provide no information on rifampicin resistance.

7.1.12 Xpert MTB/XDR

Xpert MTB/XDR has been recommended by WHO to detect DR TB. Xpert MTB/XDR will be available at NTRL from 2022.

7.2 Line probe assay

Molecular diagnostic tools for the diagnosis of MDR TB effectively address the issue of the long turnaround time associated with culture and sensitivity testing. With the introduction of LPA for the rapid diagnosis of DR TB, there has been a significant reduction in time to initiation of treatment for MDR suspect cases. LPAs are WHO endorsed tests for rapid detection of MTB and drug resistance to first- and second-line agents.

LPA can be used to detect MTB and identification of different species of MTBC and MOTT.

All these assays are now commercially available. The Foundation for Innovative New Diagnostics (FIND) supported Hain Life science, Germany to develop this tool in the initial stages of developing new TB diagnostics.

7.2.1 Principle of Line Probe Assay

LPA detects DNA of MTBC from the products of specimens or isolates subjected to PCR. Initial steps of the assay are similar to those of a conventional PCR.

It is a strip-based test. The nitrocellulose strip has specific probes in the form of immobilized bands. The bands are for MTBC strain and for resistant genes. These bands with hybridization probes react with amplicons and develop a coloured band pattern visible to naked eye, after completion of the test (Figure 7.1).

There are two types of LPA for detection of drug resistance targeting the most common mutations causing resistance.

A. First line (FL- LPA) detects resistance to rifampicin and isoniazid.
B. Second line (SL- LPA) detects resistance to fluoroquinolones and aminoglycosides- amikacin, kanamycin and capreomycin.

7.2.2 Indications

The NTRL has identified the following indications for LPA,

Direct testing on specimens

1. FL-LPA – For smear microscopy positive specimens with rifampicin resistance detected by Xpert MTB/RIF.
2. SL-LPA - Smear positive specimens with rifampicin and/or rifampicin and isoniazid resistance detected by FL-LPA.

Indirect testing on culture isolates

1. FL-LPA - culture isolates with rifampicin resistance detected by Xpert MTB/RIF.
2. SL-LPA – culture isolate with rifampicin and/or rifampicin and isoniazid resistance detected by FL-LPA.

In addition to the equipment needed for PCR, twincubator or GT Blot 48 is used depending on method (manual or automated) for hybridization step.

The GenoType MTBDR*plus* and GenoType MTBDR*sl* are the two types of test kits used for the detection of resistance to first and second line drugs.

7.2.3 Procedure

- DNA extraction step
- Amplification step
- Hybridization step - Labeled PCR products are hybridized with specific oligonucleotide immobilized on a strip. Specific probes attached as parallel lines on nitrocellulose strip.

7.2.4 Interpretation of the test result

- Check whether the TUB line is present on the strip. Absence of this line indicates invalid result and further reading should not be done.
- If all wild types are developed with no mutation, it indicates that there is no resistance.
- Detection of rifampicin resistance is through the mutations of *rpoB* gene.
- Isoniazid resistance can be detected through the mutation of *kat* G gene and *inhA* gene.
- For second-line drugs, detection of
    - fluoroquinolone resistance is through the mutation of *gyrA and gyrB* genes
    - aminoglycoside resistanceis through *rrs* and *eis* genes.
- Mutation/s is/are detected by lack of binding to wild type probes or appearance of one or more MUT probes.

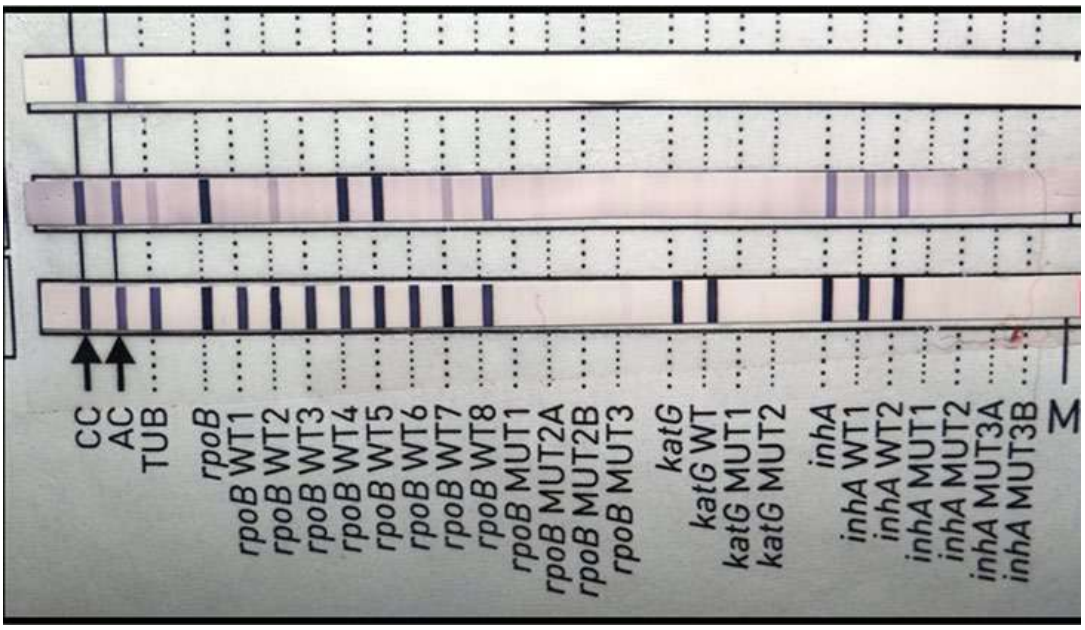


Figure 7.1 - Different types of resistance for rifampicin and isoniazid.

Table 7.2 – Interpretation of band patterns

Patterns	WT reaction zones	MUT reaction zones	Interpretation
1	All WT probes are developed.	All MUT probes are not developed	Resistance not detected
2	One or more WT probes are not developed.	One or more MUT probes in the corresponding region are developed	Depending on the specific drug: – Resistance detected (Rif, SLI drugs); – Mutations associated with high-level increase in MIC detected (H and Mfx); – Mutations associated with at least low-level increase in MIC detected (H and Mfx).
3	One or more WT probes are not developed	No MUT probes developed	Depending on the specific drug: – Resistance inferred (Rif, SLI drugs); – Mutations associated with high-level increase in MIC inferred (H and Mfx); – Mutations associated with at least low-level increase in MIC inferred (H and Mfx).

4	All WT probes are developed	One MUT probe developed	Resistance detected (Due to hetero resistance); Interpret according to case 2.

7.2.5 Reporting and recording

If resistance was detected by LPA, the coordinator for the PMDT should be informed about the resistant results on the same day to ensure initiation of appropriate treatment and infection control measures.

Report will be released to the relevant clinician after technical and clinical validation.

Conventional DST should be performed on the isolate to confirm rapid assay results.

7.2.6 Quality assurance

IQC - The assay controls supplied with the test kit must be included with each run, regardless of the number of specimens tested or strips used.

EQA – A panel of MTB strains sent by a supranational laboratory for proficiency testing should be tested annually to monitor the quality of the drug susceptibility by LPA.

References

1. GLI Line probe assay for drug resistant tuberculosis detection interpretation and reporting guide for laboratory staff and clinicians.
2. Xpert MTB/RIF implementation manual: technical and operational ‘how-to’; practical considerations. ISBN: 978 92 4 150670 0; World Health Organization 2014.
3. WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTF/RIF Ultra compared to Xpert MTB/RIF. Geneva: World Health Organization; 2017 (WHO/HTM/TB/2017.04).

8.1 Interferon gamma release assay (IGRA)

A person exposed to a patient with TB or MTB from other sources can develop active infection within weeks to months, but most infected individuals remain well. Latent tuberculosis infection is a state of persistent immune response to stimulation of MTB antigens without evidence of clinically manifested active TB.

The main purpose of diagnosing LTBI is to consider ATT agents for prevention TB disease. Until recently, the tuberculin skin test (TST) was the only available method for diagnosing LTBI. Cutaneous sensitivity to tuberculin develops from 2 to 10 weeks after infection.

However, some infected individuals, including those with a wide range of conditions including immunosuppression, but also others without these conditions, do not respond to tuberculin. Conversely, some individuals who are unlikely to have MTB infection exhibit sensitivity to tuberculin and have positive TST results after vaccination with Bacille Calmette-Guérin (BCG) or infection with mycobacteria other than MTBC, or due to unknown factors.

IGRAs can be used in place of TST in all situations that recommend TST as an aid in diagnosing MTB infection. IGRAs do not differentiate LTBI from active TB disease.

IGRAs are based on the amount of IFN-γ (Interferon) that is released by T lymphocytes or on the number of cells that release IFN-γ when exposed to the antigens of MTB.

Two types of IGRAs are available for the diagnosis of TB infection.

- QuantiFERON®-TB Gold In-Tube test (QFT-GIT)
- T-SPOT®*TB* test (T-Spot)

8.1.1 IGRA performance and reproducibility

Performance characteristics (sensitivity and specificity) of the two IGRAs currently available in the market (QFT-G and the T-SPOT) have been extensively reviewed.

Specificity of both assays (>95%) is said to be unaffected by BCG vaccination and is similar to that of the TST in non BCG-vaccinated individuals; in populations where BCG vaccination is administered, specificity of TST is significantly lower (60%).

IGRA requires a single patient visit to conduct the test. Results of IGRA can be available within 24 hours. IGRA does not boost responses measured by subsequent tests. Prior BCG vaccination does not cause a false positive IGRA test result.

Refer to guidelines for programmatic management of LTBI in Sri Lanka for indications.

8.1.2 General principles of QuantiFERON-TB Gold

Test is performed on blood specimens collected from suspected patients with LTBI after excluding the active TB disease.

The QFT-GIT system uses blood collection tubes that contain antigens representing specific MTB proteins or controls.

After blood collection (nil control, two antigen tubes, and a mitogen tube), the contents of the tubes should be thoroughly mixed with the blood. Incubation at 37°C±01°C should begin as soon as possible. Tubes should be incubated for 16 to 24 hours.

When incubation is complete, the tubes are centrifuged, plasma is harvested and the amount of IFN-γ produced is measured by immunoassays.

Each QFT-GIT result and its interpretation should be considered in conjunction with other epidemiological, historical, physical, and diagnostic findings. Therefore, test validation at country level should be done using blood specimens from different groups of people before using as a diagnostic tool for LTBI.

Actual test data should not be reported. It should be interpreted as per the manufacturer's instructions. Results for test specimens are reported in International Units (IU) relative to a standard curve prepared by testing dilutions of the secondary standard supplied by the manufacturer.

Any test which is not performed as per the manufacturer's instructions should not be accepted for making clinical decisions.

The magnitude of the measured IFN-γ level cannot be correlated to stage or degree of infection, level of immune responsiveness, or likelihood for progression to active disease

8.1.3 Safety

Care should be taken when handling materials of human origin. All blood specimens should be considered potentially infectious. Normal laboratory protective clothing should be worn. Correct laboratory procedures should be adhered to at all times.

8.2 Lateral flow urine lipoarabinomannan assay (LAM)

Lateral flow urine LAM assay tests based on the detection of the lipoarabinomannan (LAM) antigen in urine. LAM is MTB specific antigen excreted urine. It is used as potential point-of-care tests for TB in a few selected groups of patients infected with HIV.

Currently available urinary LAM assays have suboptimal sensitivity, and are therefore not suitable as general diagnostic tests for TB.

However, unlike traditional diagnostic methods, they demonstrate improved sensitivity for the diagnosis of TB among individuals co-infected with HIV. The estimated sensitivity is even greater in patients with low CD4 cell counts.

8.2.1 The lateral flow urine LAM assay (LF-LAM) strip-test

The Alere Determine TB LAM Ag (USA), hereafter referred to as AlereLAM, is currently the only commercially available urinary LAM test that potentially could be used as a rule-in test for TB in patients with advanced HIV-induced immunosuppression, and facilitate the early initiation of ATT.

AlereLAM is an immunocapture assay that detects LAM antigen in urine, LAM being a lipopolysaccharide present in mycobacterial cell walls released from metabolically active or degenerating bacterial cells during TB disease. The test should be performed the test as per the manufacturer's instructions and only for indications recommended by WHO.

All patients with signs and symptoms of pulmonary TB who are capable of producing sputum should submit at least one sputum specimen for Xpert MTB/RIF assay, as their initial diagnostic test. This also includes children and adolescents living with HIV who are able to provide a sputum specimen.

8.2.2 WHO recommendation for the use of LF LAM assay on urine

In inpatient settings to assist in the diagnosis of active TB in HIV positive adults, adolescents and children:

1. with signs and symptoms of TB (PTB and/or EPTB).
2. with advanced HIV disease or who are seriously ill.
3. irrespective of signs and symptoms of TB and with a CD4 cell count< 200 cells/mm^3.

In outpatient settings to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children:

1. With signs and symptoms of TB (PTB and/or EPTB) or seriously ill.
2. Irrespective of signs and symptoms of TB and with a CD4cell count < 100 cells/mm^3.

In outpatient settings, WHO recommends **against** using LF-LAM to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children

1. Without assessing TB symptoms.
2. Without TB symptoms and unknown CD4 cell count or without TB symptoms and CD4 cell count ≥ 200 cells/mm^3.
3. Without TB symptoms and with a CD4 cell count of 100–200 cells/mm^3.

LF-LAM should be used as an add-on to clinical judgment in combination with other tests; it should not be used as a replacement or triage test.

8.3 Adenosine deaminase (ADA)

In recent years, there has been a great demand for finding new microbiological, genetic, immunological, and biomedical diagnostic methods to diagnosis TB quickly and accurately. However, in some patients, the diagnosis of tuberculosis still cannot be confirmed using even in the best diagnostic methods.

ADA is an enzyme which contributes in purin metabolism. ADA is essential for proliferation and differentiation of lymphoid cells, especially T cells, and helps in the maturation of monocytes to macrophages. It seems ADA is an index for cellular immunity and studies have proved its value in TB diagnosis, even for assessing TB effusions and TB meningitis.

Measurement of ADA is a biomedical method. It has been widely used in clinical diagnosis of TB in recent years. The ADA assay is simple, rapid, and inexpensive.

ADA level should be used as a screening test to guide further management. Countries with a high prevalence of tuberculous pleural effusions have a high degree of specificity and sensitivity for the ADA test, which makes it an integral part of a diagnostic workup of lymphocyte rich exudative body fluid. The negative predictive value of the ADA test remains high, which provides an excellent means to rule out tuberculous etiology.

The use of CSF-ADA facilitates early differentiation of TB meningitis from other causes and timely initiation of ATT and potentially improves the patient's outcome when TB is highly suspected in the absence of positive microbiological investigations.

The consequence of a false-positive test result that may cause delay in the diagnosis of malignant pleural effusion, an incurable condition, is not as significant as missing a potentially curable tuberculous effusion.

The optimal cut off value of ADA for diagnosis of TB varies. The values vary according to the incident of TB in the population, specimen tested, proportional frequency of the diseases included in the diagnosis of the community in which the measurements have been taken and the test kits used.

Pleural ADA levels may increase in parapneumonic effusions, lymphomas, solid tumors, connective tissue diseases and other infectious diseases.

References

1. European Centre for Disease Prevention and Control, 2016; Handbook on TB laboratory diagnostic methods for the European Union.

2. WHO consolidated guidelines on tuberculosis, Module 3: Diagnosis, Rapid diagnostics for tuberculosis detection - 2021 update.

09 LABORATORY SAFETY

Laboratory health workers are responsible for their own safety and that of their co-workers. Strict adherence to safety regulations in the laboratory is very important.

Tuberculosis is transmitted through air. Therefore every effort must be made to avoid or reduce the production of aerosols in the laboratory to minimize the risk of disease transmission.

Hand washing, application of correct techniques and safe laboratory practices are mandatory for preventing TB transmission in laboratory settings.

Appropriate safety labels and signage should be in place. E.g. Biohazard label.

Figure 9.1 - Biohazard labels

9.1 Risk assessment of tuberculosis laboratories

TB laboratory facilities can be classified into three main levels of procedural risk, based on the activities being performed and their associated risks:

- Low TB risk
- Moderate TB risk
- High TB risk (such as a TB-containment laboratory)

Table 9.1 - Risk levels of TB laboratory

Risk level of TB laboratory	Laboratory activities	Assessment of risk
Low risk	Direct sputum-smear microscopy; preparation of specimens for use in an automated NAAT cartridge (such as the Xpert MTB/RIF assay).	Low risk of generating infectious aerosols from specimens; Low concentration of infectious particles.
Moderate risk	Processing and concentration of specimens for inoculation on primary culture media;	Moderate risk of generating infectious aerosols from specimens; Low concentration of infectious particles.

	Direct DST (E.g. LPA on processed sputum).	
Risk level of TB laboratory	**Laboratory activities**	**Assessment of risk**
High risk (TB-containment laboratory)	Culture manipulation for identification; DST or LPA on cultured isolates.	High risk of generating infectious aerosols from specimens; high concentration of infectious particles.

9.2 Safety in sputum collection

- Never collect sputum specimens inside the laboratory, toilets, waiting rooms, reception rooms, or any other enclosed spaces.
- Instruct the patients to cover their hair.
- Once collected, allow a sputum specimen to stand undisturbed for at least 20 minutes before opening (to settle any aerosols).
- Reject broken or leaking containers. Request another specimen.
- Assume ALL specimens are potentially infectious and handle them carefully when you open the sputum containers and during smear preparation.
- Cover sputum containers with their lids at all times except when removing for smear preparation.

9.3 Safety practices in the microscopy laboratory

- Entry to the laboratory should be restricted only to the laboratory staff.
- Establish airflow in working areas that will direct potentially infectious particles away from personnel.
- Refrain from eating, drinking, smoking and applying makeup inside the laboratory.
- Wear the relevant personal protective equipment when working inside the laboratory.
- Do not use the same desk for smear making and microscopy work.
- Wash hands with soap and water always after performing any procedure.
- Handle accidental spillages according to the protocols.
- Adhere to proper waste management practices.

9.4 Safety in sputum smears preparation

- Disinfect the working area before and after smear preparation.
- Gently open the sputum container, especially if the lid clicks or snaps on. Open the containers with care keeping them away from the face.
- Do not forcefully shake or stir the sputum in the container.
- Avoid any rapid motion when making the smear as infectious aerosols may be produced.
- Where available, use disposable wooden sticks for smear preparation. Discard it into a receptacle immediately after use.
- If wire loops are used, remove residual sputum on the wire loop before flaming by inserting the wire loop into a sand-lysol jar.

- Never heat a wire loop in a flame when sputum is still attached to it as sputum containing live AFB will produce infectious aerosols.
- Always keep a discard receptacle containing disinfectant in the working area.
- Fix smears by flaming only after they have dried completely. Wet slides can produce aerosols if disturbed. Do not flame slides to expedite drying. This too can produce aerosols.

9.5 Occupational safety

A baseline medical check-up is recommended for all staff prior to commencing work in a TB laboratory. Refer annex 26.

Annual checkups should be arranged thereafter.

Following should be included in the initial/follow-up assessment,

- An individual TB risk/symptom evaluation
- Chest X-ray
- Mantoux or Interferon-gamma release assay (IGRA) on enrollment
- If IGRA is available it should be performed
    - On enrollment for all categories of laboratory staff.
    - Recommended annually for the staff working in the TB culture laboratories.
    - Recommend ever 5 years for laboratory staff working in the microscopy laboratories.

9.6 Personal protective equipment in the TB laboratory

Personal protective equipment and clothing may act as barriers to minimize the risk of exposure to aerosols, splashes and accidental inoculation. Protective clothing should be worn whenever the staff works in the laboratory. Before leaving the laboratory, staff should remove their protective clothing, and wash their hands with soap and water thoroughly.

Table 9.2 - Personal protective equipment in the TB laboratory

PPE item	Comments
Laboratory coats	• Laboratory coats usually have long sleeves and fasten in the front to cover street clothes. • Coats should be used for activities where there is a low-risk of becoming infected with TB.
Laboratory gowns	• Gowns must be worn when working in a laboratory where there is a high risk of TB infection. • Laboratory gowns should have long sleeves and an elasticized cuff (at least 30mm long). • Gowns should open in the back. • Gowns should cover street clothing.
Respirators	• Should be used in the laboratories where TB cultures are performed and manipulated. • N95/KN95 are recommended for the protection against aerosols.

PPE item	Comments
	• Respirators should never be used as substitute for a properly maintained and functioning BSC. • Should undergo fit test before each use.
Gloves	• Disposal microbiologically approved latex, vinyl or nitrile. • Gloves must be worn for all procedures that involve direct contact or may involve accidental contact with sputum, blood, body fluid and other potentially infectious material. • After use, glove should be removed aseptically and hands washed.

- Protective clothing must not be worn outside the laboratory area (e.g. in canteens, coffee rooms, offices, libraries, staff rooms and toilets).
- Laboratory coats and gowns must be stored separately from personal clothing. Clean gowns and used gowns must be stored in different areas of the laboratory.
- Laboratory coats and gowns should be changed at least weekly.
- Eating, drinking, smoking, applying cosmetics and handling contact lenses are prohibited in the laboratory.
- Storing food or drink anywhere in the laboratory's working areas is prohibited.
- Covered footwear is recommended in the laboratory.
- Mobile telephones better not to be used in the laboratory.

9.7 Precautions against COVID-19

- Minimum safety attire consisting of single-use polythene apron in addition to laboratory coat, surgical mask, face shield, and gloves are recommended for all laboratory workers handling specimens in the context of COVID-19 pandemic.
- Respirators (N95 or equivalent) should be used during all procedures with potential aerosol generation (see table 9.1).

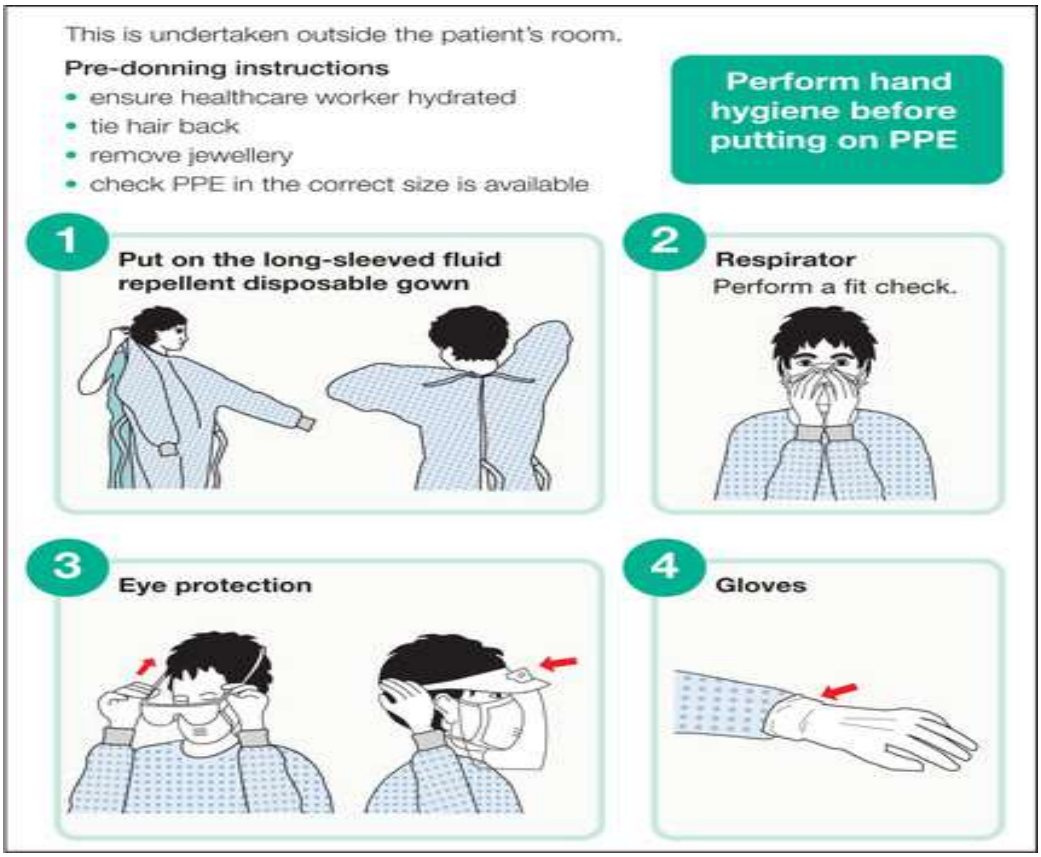
9.8 Respirator seal check

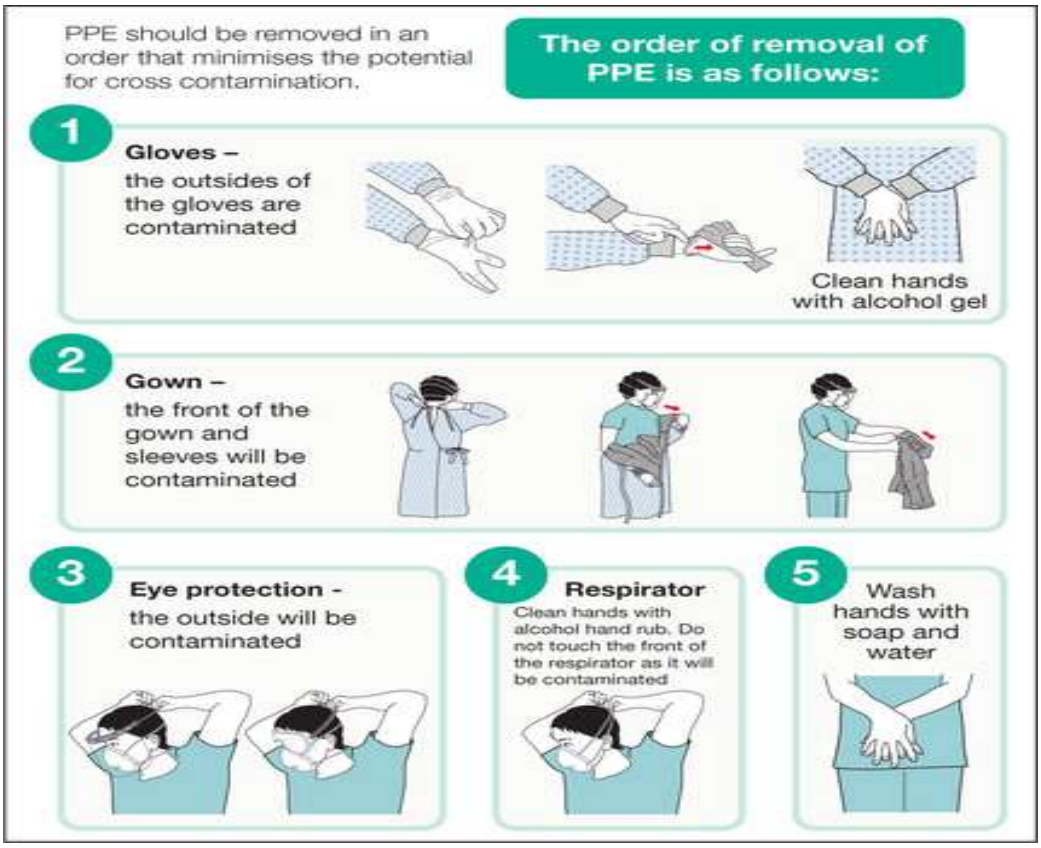
- All the staff who use respirators should be trained in seal testing.
- Cup the respirator in one hand, with the nosepiece at the fingertips; they should allow the headbands to hang freely (Figure 9.2)
- Position the respirator under the chin with the nosepiece upwards; pull the top strap over their head and place it high at the back of the head; pull the bottom strap over the head and position it around the neck below the ears.

- Place the fingertips of both hands at the top of the metal nosepiece; using two hands, mold the nose area to the shape of their nose by pushing inward while moving their fingertips down both sides of the nosepiece.
- Pinching the nosepiece with only one hand may result in an improper fit and less effective respirator performance; they should always use two hands.

Figure 9.2 – N95 Mask Fitting

9.9 Donning and doffing of PPE





9.10 Use of Biological safety cabinets during TB diagnostic procedures

BSC play a critical role in ensuring safety in TB laboratories performing high risk procedures. Class II type A2 BSCs with a moveable sash are recommended for TB laboratories. Proper use of BSC is important to provide worker safety as well as to minimize contamination of TB cultures.

A. Positioning of the BSC

BSCs can be used in BSL2 or BSL3 level laboratories. BSC should be transported, positioned inside the laboratory and installed by authorized personnel. When it is used inside a BSL3 laboratory exhaust air should be ducted out through a thimble system.

The integrity of the directional air inflow is fragile and can be easily disrupted by air currents generated by people walking close to the BSC, by open windows or by the opening and shutting of doors. Therefore, BSCs should be situated in a location away from traffic and from potentially disruptive air currents. Whenever possible, a clearance of 30cm should be provided behind and on each side of the cabinet to allow easy access for maintenance. A clearance of 30–35cm above the cabinet may be required to accurately measure air velocity across the exhaust filter, and to change exhaust filters. A diagram on positioning a BSC inside a laboratory is given below (Figure 9.3).

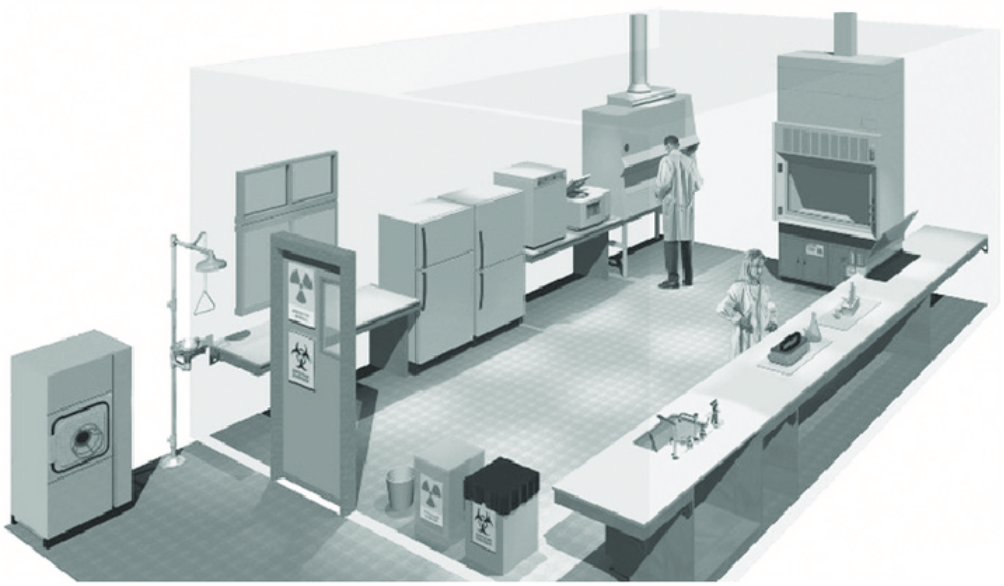


Figure 9.3 - Positioning a biological safety cabinet inside a laboratory.

B. Operators

If BSCs are not used properly, their protective benefits may be greatly diminished; in some instances, improper use can even result in increased risk to the laboratory worker.

All individuals working in BSCs should be trained and observed to ensure they follow correct working practices before they routinely perform testing in the BSC. They should be given regular refresher training to improve competency in using BSCs.

Turn the BSC on and check the functional status and wait for 5-10 minutes before starting work.

Maintain a BSC user log.

Operators need to maintain the integrity of air flowing through the front opening when moving their arms into and out of cabinets. They should move their arms slowly and ensure they are perpendicular to the front opening. Staff should wait about 2 minutes after placing their hands and arms inside the BSC before they begin manipulating materials; this will allow the airflow within the cabinet to adjust and the air to sweep the surface of their hands and arms.

The number of movements made across the front opening should be minimized by placing all necessary items into the cabinet before beginning manipulations. Preparing a written checklist of materials necessary for a particular activity will be useful.

C. Material placement

The front intake grill of Class II BSCs must not be blocked with paper, equipment, or other items. It is recommended that all work be performed on disinfectant-soaked absorbent towels arranged to capture splatters and splashes. All materials should be placed as far back in the cabinet as practical – that is, towards the rear of the work surface – without blocking the rear grill. Aerosol-generating equipment (such as vortexes and centrifuges) should be placed towards the rear of the cabinet. Bulky

items (such as biohazard bags and discard containers) should be placed to one side of the interior of the cabinet.

Active work should flow from clean areas to contaminated areas across the work surface.

Paperwork should never be placed inside BSCs.

The cabinet must not be overloaded because overloading may affect the efficiency of the air flow.

Clean materials are placed to the left of the cabinet; specimens are inoculated in the center of the cabinet; and contaminated pipettes and other materials are placed into waste containers on the right side of the cabinet. This arrangement can be reversed for people who are left-handed.

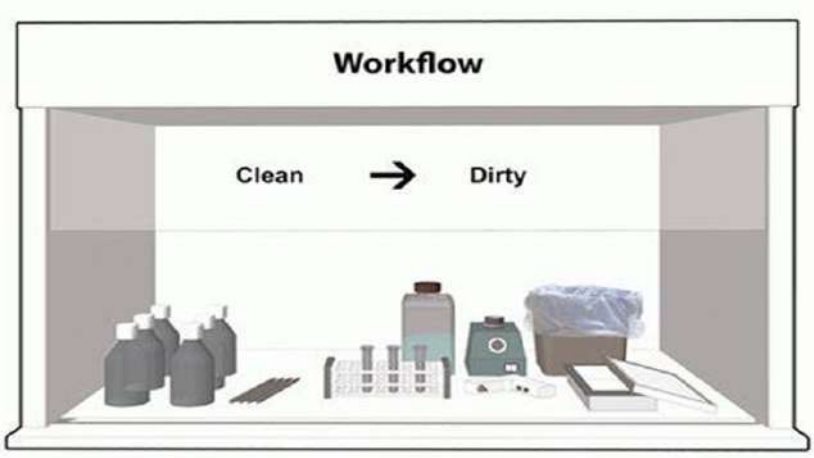


Figure 9.4 – Work flow organization inside a biological safety cabinet (for a right-handed person)

Open flames

Open flames disrupt the patterns of airflow within the cabinets. To sterilize bacteriological loops, micro incinerators or electric furnaces are available, and their use is preferable to open flames. The use of disposable loops and disposable transfer pipettes is preferred.

D. Certification of BSC

The functional operation and integrity of each BSC should be certified to national or international performance standards at the time it is installed, following any relocation in the laboratory, and regularly thereafter (at least annually) by qualified service technicians, according to the manufacturer's specifications.

An evaluation of the effectiveness of the cabinet's containment capability should include tests of the cabinet's integrity; tests for HEPA filter leaks (by particle counter); assessments of the down flow velocity profile, face velocity, negative pressure and ventilation rate, airflow smoke pattern, and alarms and interlocks.

The velocity of air flowing through the front opening into a BSC should meet the manufacturer's specifications.

A report on regular maintenance should be provided by the service agent.

Acceptable airflow characteristics of Class IIA2 BSC are as follows,

- Minimum intake (face) velocity 0.51 m/s
- Percentage recirculated airflow - 70%
- Percentage exhausted airflow - 30%
- Exhaust system - exhaust through HEPA filters to room or thimble connection

E. Cleaning and disinfecting the work area

When work is completed, all items within a BSC, including equipment, should have surfaces decontaminated and be removed from the cabinet.

The interior surfaces of BSCs should be decontaminated before and after each use. Work surfaces and interior walls should be wiped with a disinfectant (5% phenolic disinfectant and 70% alcohol) that will kill any microorganisms that might be found inside the cabinet.

At the end of the workday, the final surface decontamination should include wiping down the work surface, and the sides, back and interior of the glass. A second wiping with sterile water is needed when a corrosive disinfectant, such as bleach, is used.

Before it is switched off, the BSC should be left to run for 15 minutes after work is completed in order to purge the atmosphere inside.

Decontamination

BSCs must be thoroughly decontaminated before filters are changed and before the cabinet is moved; decontamination must include plenums and filters. Decontamination should be performed by a qualified professional.

F. Alarms

BSCs can be equipped with one of two audible alarms.

Sash alarms are found only on cabinets with sliding sashes. The alarm sounds when the laboratory worker has moved the sash to an improper position. When this alarm sounds, the sash must be returned to the proper position.

Airflow alarms indicate a disruption in the cabinet's normal airflow pattern. This alarm represents an immediate danger to the worker or product. When an airflow alarm sounds, work should cease immediately, and the laboratory manager should be notified. Manufacturers' instruction manuals should provide further details about how to address this type of alarm.

G. Spills

A copy of the laboratory's protocol for handling spills should be posted, read and understood by all laboratory staff. When a spill occurs inside a BSC, clean up should begin immediately and the cabinet should continue to operate. An effective disinfectant should be used and applied in a manner that minimizes the generation of aerosols. All materials that come into contact with the spilled agent should be disinfected and disposed of properly.

Instructions for managing a spill inside BSC

- Any laboratory worker in the vicinity of the spill must immediately wash his/her hands and forearms, even if not overtly contaminated by the spill.
- Use water and soap for cleaning. In case of wounds, cuts or abrasions, medical care should be sought immediately.
- Place absorbent tissue over the spill area and apply disinfectant solution (5% phenolic disinfectant) liberally. If BSC walls have been splashed, disinfectant solution should be sprayed or poured onto the contaminated surfaces, which are then covered with a layer of absorbent tissue.
- Leave affected areas covered for a minimum of 02 hours.
- Pick up the contaminated material using forceps and thick rubber gloves for protection against cuts from glass debris, if any, and place in an autoclavable bag.
- Any equipment or reusable material (homogenizer, blender, shaker, centrifuge buckets, etc.) that has been splashed should be swabbed with the same disinfectant. Removable parts should be washed with water and dried. Electrical equipment should be checked carefully (integrity of circuit-breakers and earth-fault-interrupters) before use.
- In case of major spillage (large volumes of liquid cultures of tubercle bacilli), the BSC HEPA filters should be fumigated according to manufacturer's instructions.

Reporting of spills

Notify the head of the laboratory of the incident. Every incident/accident must be documented, and records must be kept in the laboratory archives. All corrective action must be similarly documented.

Further work in the BSC must be authorized by the laboratory supervisor. If a large volume (>20 ml) of infectious material is spilled, an inspection of the BSC may be necessary.

All materials used in the clean-up should be treated as infectious waste.

Contaminated waste containers should be autoclaved. Those containing broken glass should be specifically labelled "CAUTION: GLASS DEBRIS" to prevent further risks of cuts and wounds.

9.11 Procedural safety

- All procedures must be performed in such a way as to minimize or prevent the formation of aerosols and droplets.
- Mouth pipetting is strictly prohibited.
- Written documentation that may be removed from the laboratory must be protected from contamination.
- All contaminated materials, specimens and cultures must be decontaminated appropriately before disposal or cleaning for reuse.
- All accidents, spills and potential exposures to infectious materials must be reported to the infection control team, Consultant in-charge and the medical laboratory technologist in-charge of the laboratory. All such incidents should be properly documented along with the corrective action taken.
- Standard operating procedures should be developed for all procedures including handling accidents and spills. The staff should be properly trained and assessed frequently.
- Packing and transportation of specimens must follow applicable national regulations. (Refer chapter 3).

9.12 Spill management in the TB laboratory

- All the workers should leave the laboratory and close the door. One person enters the lab after wearing a mask and cover the spill immediately. Use any available absorbent material. E.g. Paper towels, newspaper, cotton wool, cloth etc.
- Soak the cover with the appropriate disinfectant and completely wet the area. Leave the laboratory closing the door.
- Let it stand at least for 30 minutes, keeping the area wet during this period.
- Enter the laboratory attired in protective wear. Place all broken tubes / containers and clean up material in an appropriate container and discard by one of the waste disposal options described later.
- Mop the spillage area, floor and the laboratory benches with disinfectant. E.g. 5% phenolic disinfectant.

9.13 Chemical safety

- Always wear laboratory coats, gloves, and safety glasses when handling strong acids.
- Take particular care in diluting concentrated acids. Always add the concentrated acid to water. This avoids splashes of acid causing burns to the skin or eyes.
- Do not handle alcohol near an open flame as they are flammable.
- When using phenol avoid direct contact with the skin or mucus membranes. Minimize exposure to fumes by staining smears in a well-ventilated area.
- A batch of slides for staining should preferably contain a maximum of 12 slides.

9.14 Fire safety

Prevention of fire:

- The majority of lab fires have resulted from procedural errors or carelessness. Therefore plan your work.
- Minimize materials. Keep only the essentials on the bench.
- Observe proper housekeeping. Keep work areas uncluttered, and clean frequently.
- Keep aisles/corridors, doors, and access to emergency exit unobstructed at all times.
- Observe proper safety practices.
- Store solvents properly.
- Wear proper clothing and personal protective equipment.
- A written emergency evacuation plan should be available. E.g. emergency evacuation.
- Training - Exercise the emergency plan and learn to use the emergency equipment provided.
- Know where things are: the nearest fire extinguisher, fire alarm, exit(s), telephone, emergency shower/eyewash, and first aid kit, etc.

In case of fire:

- If SAFE TO DO SO, attempt to extinguish (only for small/isolated fires).
- Know how to operate the extinguishers –
    - Depending on the size, 10 seconds to 30 seconds of spray,
    - Keep yourself between the fire and your exit,
    - Remember the acronym " **P A S S**"
        - **P** – Pull the pin
        - **A** – Aim extinguisher nozzle at the base of the flames,
        - **S** – Squeeze trigger while holding the extinguisher upright,
        - **S** – Sweep the extinguisher from side to side, covering the area of the fire with the extinguisher agent.

9.15 Electrical safety

- Inspect wiring of equipment before each use.
- Replace all frayed or damaged electrical cords immediately.
- Better if equipment with three prongs (ground) are used in the laboratory.
- Fix a voltage stabilizer to the power grid.
- Limit the use of extension cords. Use only for temporary operations. In all other cases, request installation of a new electrical outlet.
- Minimize the potential for water or chemical spills on or near electrical equipment.
- Effective earthing system should be in place as per the specifications of Ceylon Electricity Board (CEB).
- Know the location and how to operate shut-off switches and/or circuit breaker panels. Use these devices to shut off equipment in the event of a fire or electrocution.

9.16 Laboratory register for accidents (Accident book)

- Should be kept by the laboratory supervisor.
- Should contain details about accidents and the measures taken.

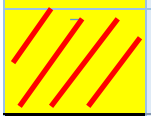
References

1. Centers for Disease Control and Prevention; Biosafety in Microbiological and Biomedical Laboratories, 6th Edition; June 2020.
2. Global Laboratory Initiative; Standard Operating Procedure for Use and maintenance of class I and class II biological safety cabinets; Accessed in June 2021.
3. World Health Organization; Tuberculosis Laboratory Biosafety Manual- 2012, Geneva.

10 LABORATORY WASTE DISPOSAL

Ministry of Health, Sri Lanka has developed National guidelines on waste management in order to implement a uniform waste management system in health care institutions. National colour code has been circulated to all the government health care institutions. It identifies 7 specific categories.

Table 10.1 - Segregation of waste- National colour code

Colour	Colour	Category	Contents
	Yellow	Infectious waste	Cultures or stocks from microbiology, tissues from surgeries/autopsies, material or equipment in contact with blood or body fluids, soiled linen, dialysis equipment such as tubing and filters.
	Yellow with red stripes	Sharps waste	Sharps, needles and IV sets contaminated with body fluids.
	Black	General waste	General or municipal waste that is uncontaminated.
	Green	Biodegradable waste	Garden, kitchen and food waste.
	Red	Glass waste	Uncontaminated drink bottles and water bottles.
	Blue	Paper waste	Paper, cardboard and office stationary.
	Orange	Plastic waste	Uncontaminated plastic medicine bottles, saline bottles without IV sets and plastic bags.

Waste categorization includes;

- Non-infectious waste – for reuse, recycle or dispose as general waste.
- Infectious waste – To be disposed by burying, incinerating or autoclaving
    - Clinical Specimen.
    - Contaminated items.
    - Contaminated sharps: such as broken glass, syringes and slides.

10.1 Disposal of different types of waste

All infectious waste should be rendered non-infectious prior to disposal. It is important to note that infectious waste should not be taken out of the premises without making it noninfectious autoclaving or chemical disinfection. Every laboratory should have a dedicated autoclave for decontamination and protocols for pretreatment of infectious waste.

Table 10.2 - Recommended disinfectants

Disinfectant	Uses
Phenol (5% in water)	Phenol solutions are used for decontaminating equipment and single-use items prior to disposal.
Chlorine - Sodium hypochlorite 1% (10,000ppm)	Chlorine (Bleach) can be used as a general purpose disinfectant and for soaking contaminated metal-free materials; because it is highly alkaline, it can corrode metal.
Alcohol 70%	A solution of 70% alcohol can be used on laboratory benches and BSCs for routine decontamination.

A. Infectious Waste

1. Sputum cups:
   - Incineration is recommended. In the absence of incineration facilities, making it non-infectious by autoclaving can be practiced before sending it out of the laboratory.
   - In the absence of both as an alternative method of making the material non-infectious, by adding a disinfectant e.g. 5% phenolic disinfectant or 1% Sodium hypochlorite to the specimen containers can be practiced).

2. Discard jars used inside the BSC:
   - Unbreakable discard containers (e.g. plastic) to be used with appropriate disinfectants effective against MTB.
   - Disinfectants recommended as suitable for use in TB laboratories are those containing phenols, chlorine or alcohol. These are usually selected depending on the material to be disinfected.
   - Discard containers should be decontaminated and washed before reuse.

3. Universal Bottles:
   - Used glass sputum containers can be recycled after autoclaving at 121^{o}C and thorough washing.

4. Applicator Sticks:
   - Collect Applicator Sticks into a discard jar with disinfectant (e.g. 5% phenolic disinfectant). Leave it overnight before discarding.

Infectious waste should be collected into yellow medical waste bags kept inside the yellow bins with lid, and international biohazard sign on it.

Specification for yellow waste bags for infectious waste

- Preferably biodegradable.
- Leak proof.
- Thickness 75 microns/300 gauge.
- Polyethylene or polypropylene.
- Heat resistance up to 121°C.
- Marked with the bio-hazardous sign.
- Should be available in different sizes.

Yellow bins with foot-operated lid are preferred.

Figure 10.1 – Sharp Bin and a foot Operated Lid Waste Bins

B. Laboratory Sharps

A sharp is any device/item having acute corners, edges, or projections capable of cutting or piercing the skin.

Microscope slides & cover slips are categorized under sharp waste. Therefore, they should be collected in to sharp boxes.

ALWAYS collect these items in approved sharps disposal containers.

Essential features of a sharp bin

- Puncture resistant.
- Cardboard which is incinerable.
- Leak proof.
- Yellow bin with a red stripes on it.
- Clearly marked with a bio hazard symbol.
- Designed with a small opening so that items can be dropped in but no item can be removed within easy reach of the work station.

C. Liquid Infectious Waste

This should be disposed in to a closed drainage system which does not get connected to any other system or water source. If there is no closed drainage system, waste should be decontaminated with 5% phenolic solution or 1% hypochlorite solution over night before disposing in to a drainage system using a designated sink.

D. General waste

Should be collected to black polythene bags (preferably biodegradable) kept inside the black bins.

E. Biodegradable waste

Should be collected to green polythene bags (preferably biodegradable) kept inside the green bins.

10.2 Handing over waste to the waste collector/collection points

When the waste bag is approximately two thirds full, seal the bag as follows,

1. Gather the tops of the bag.
2. Fold the neck of the bag over.
3. Tie the neck by forming a loop and passing the end through the loop, creating a knot.
4. Tighten the knot to ensure an effective seal.

Sharps Bins - Dispose when the bin is filled to no more than ¾ capacity; never overfill a sharps disposal container.

10.3 Transportation of waste from the laboratory to the collection site

- The segregated infectious waste should be transported separately from non-infectious waste.
- Carts, trolleys or wheeled bins, which are easy to load and clean, should be used to transport waste, within the institution.
- These items should not be used for any other purpose.
- Waste should not be carried in hand.
- Porters who collect and transport clinical waste must be provided with protective clothing.
- The collection route from the laboratory should be direct from the point of collection/ waste/ storage area/ disposal area depending on the circumstances.

10.4 Waste storage

- Storage should be done in an area not subjected to floods.
- Storage area should be well secured & should be inaccessible for unauthorized people.

- Storage place should have adequate ventilation & light, a continuous supply of running water, and it should be a place which is easy to clean & disinfect.
- Infectious waste should not be stored together with non-infectious waste.
- Waste should not be stored for more than 48 hrs.

10.5 Waste disposal

A. Infectious Waste

- Ideally Infectious waste should be disposed of by incineration.
- Making the waste non-infectious before disposal should be given high priority when there are no incineration facilities.
- Autoclaving the waste makes it non-infectious.
- All positive TB cultures must be autoclaved before disposal. An autoclave should be available close to or in the laboratory where TB culture is performed.
- The best method of disposal of infectious waste is high temperature incinerator (800 - 1200^{o}C).
- In the absence of that drum incineration can be used as an alternate.

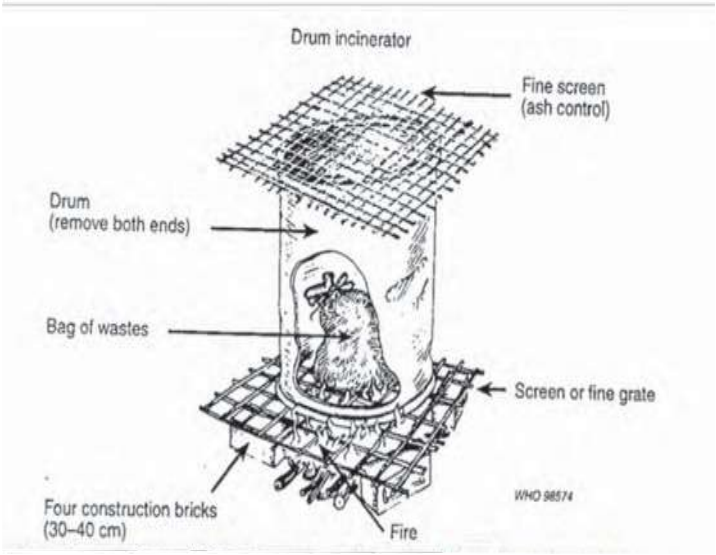


Figure 10.2 - Drum incinerator.

B. Glass slides

Positive slides: All positive slides should be,

- Incinerated/Autoclaved.
- Broken and discarded by deep burial to prevent their re-use (If not incinerated).
- It should be at least 50 feet away from a water source (e.g. well, river).
- After disposing slides, they should be covered with a layer of soil.
- When one pit fills the next should be dug adjacent to it.
- A map of pits should be available at the laboratory.

Negative Slides:

- Negative slides can be incinerated/autoclaved, broken and discarded by deep burial to prevent their re-use for TB work. They can be used for non-TB work.

❖ Each institution should have a written Standard Operating Procedure with a proper time schedule for waste collection and transport depending on the amount of waste generated.

Features of a deep burial pit

- It should be at least 50 feet away from water source (e.g. well, river).
- After disposing slides, they should be covered with a layer of soil.
- When one pit fills the next should be dug adjacent to it.
- A map of pits should be available at the laboratory.

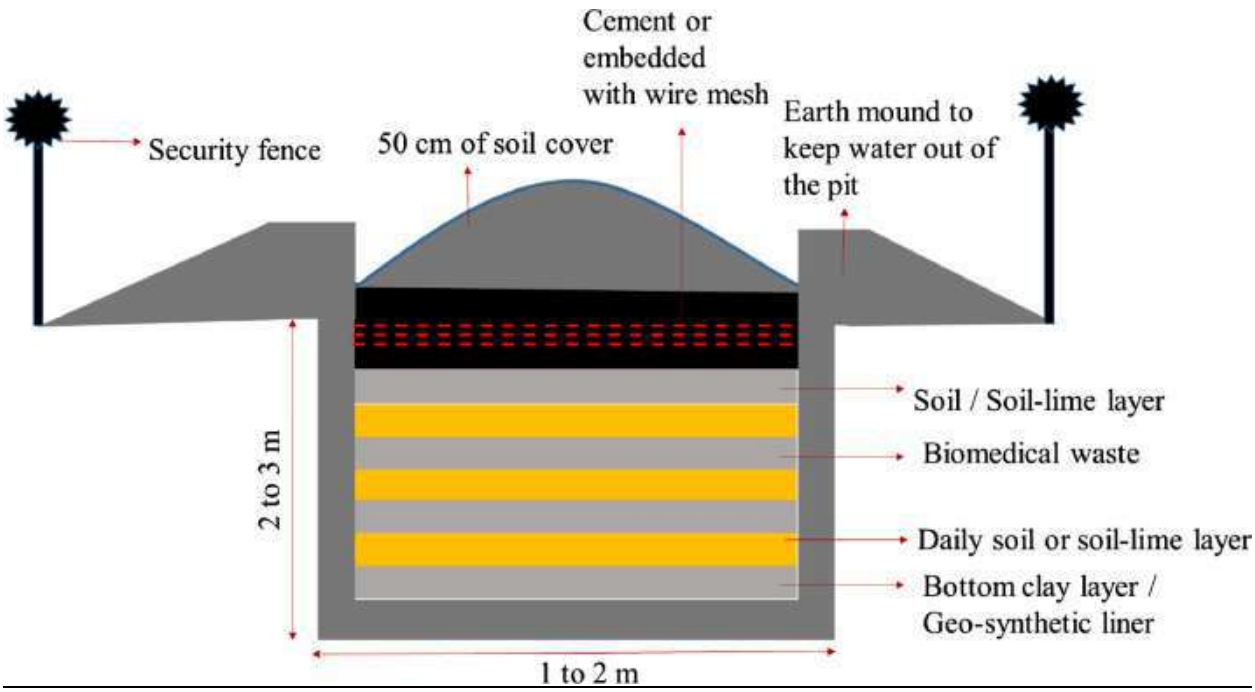


Figure 10.3 - Design for Deep Burial Pits

References

1. Tuberculosis Laboratory Biosafety Manual , World Health Organization, 2012, Geneva.

11 LABORATORY DOCUMENTS

Maintenance of documents in the DCC laboratories and MCs is of greatest importance in maintaining the quality and standard of the laboratories. These documents need to be available to inspect during onsite evaluations.

11.1 Documents to be maintained at both DCC laboratories and MCs.

1. Laboratory staff qualification, service training records, results of proficiency testing.
2. Standard operating procedures (Filed and displayed on the wall)
3. Laboratory equipment maintenance records (Information to be included: date of receipt, suppliers information, model, serial number, country of make, calibration details, service records, malfunction and corrective measures records)
4. Laboratory equipment log books.
5. Stock book (Annex 12).
6. TB Laboratory Register - TB 04 (Annex 03)
7. Smear microscopy monthly summary (Annex 06)
8. Slide positivity charts.
9. Laboratory accident book (date of the accident, name of the officer concerned, description of the accident, laboratory number of the specimen involved, stain, extent of the accident, follow-up measures taken)
10. Deep burial site map.
11. Maintenance of internal quality control register of staining procedure.
12. Maintenance of internal quality control register of staining preparation.
13. Feedback reports of Random Blinded Rechecking (RBRC) – NTRL reports to DCC and DCC reports to MCs.
14. Onsite evaluation reports – Reports of visits from NTRL, Reports of visits from DCC to MCs.

11.2 Documents to be maintained at ITL.

1. TB culture register.
2. Quarterly laboratory culture report: Laboratory performance of culture including positive, negative and contamination in a given quarter.
3. Annual culture report: Laboratory performance of culture including positive, negative and contamination annually.

11.3 Documents to be maintained at Xpert sites.

1. Xpert register (Annex 19).
2. Xpert worksheet (Annex 20).
3. Xpert machine folder.
4. Xpert monthly summary (Annex 21).

11.4 Returns to be sent to NTRL

From DCC and MC laboratories

Monthly: 1. Smear microscopy monthly summary (Annex 6).
2. Information on specimen sent to central laboratory for culture (Annex 27).

Quarterly: RBRC of slides – Quarterly report of MCs.

From GXP sites,

Monthly: GeneXpert Monthly summary (Annex 21).

From culture laboratories including ITLS, other government and privet sector laboratories,

Quarterly: Quarterly laboratory culture report: Laboratory performance of culture including positive, negative and contamination in a given quarter (From ITLs).

Annually: Annual culture report: Laboratory performance of culture including positive, negative and contamination annually.

ANNEXURES

Annex 01 - Diagnostic Algorithm for TB – Adult
Annex 02 - Diagnostic Algorithm for TB – Pediatric
Annex 03 - Tuberculosis Laboratory Register – TB 04
Annex 04 - The laboratory form - Request for sputum examination – TB 05
Annex 05 - TB Culture Request form – TB 06
Annex 06 - Smear Microscopy Monthly summary
Annex 07 - Smear Result Sheet for Blinded Rechecking
Annex 08 - Slide Distribution Book for RBRC – For NTRL & DCC
Annex 09 - Random Blinded Rechecking for Smear Examination (Form A)
Annex 10 - Quality Assurance Report on Sputum Microscopy
Annex 11 - Quarterly Report Random Blinded Rechecking of Slides – For microscopy Centers
Annex 12 - Stock Book
Annex 13 - Laboratory Requirements
Annex 14 - Supervisory Visit to Microscopy Centers by District Chest Clinics Check list
Annex 15 - Quarterly Report – Laboratory Supervision of Microscopy Centers
Annex 16 - TB Culture Report
Annex 17 - TB Culture and DST Final Report
Annex 18 - Distribution of GeneXpert Sites in Sri Lanka, 2021
Annex 19 - Gene Xpert Register
Annex 20 - Gene Xpert Work Sheet
Annex 21 - Gene Xpert Monthly Summary
Annex 22 - Gene Xpert Report of MTBND
Annex 23 - Gene Xpert Report of MTBD, RRND
Annex 24 - Gene Xpert Report of MTBD, RRD
Annex 25 - Gene Xpert Report of MTBD, RID
Annex 26 - Laboratory Staff Medical Checkup
Annex 27 - Information on specimen sent to central laboratory for culture

Annex 01

Diagnostic Algorithm for Tuberculosis

Patients with signs and symptoms suggestive of Pulmonary Tuberculosis(PTB) [1,2]

Three sputum samples for AFB staining (AFB x 3) & Chest X Ray (CXR)

- Two or more sputum AFB positive OR One or more sputum AFB positive [3] & CXR positive [4]
- Sputum AFB x 3 negative & CXR positive [4]
- One sputum AFB positive & CXR negative
- Sputum AFB x 3 negative, CXR negative & Persistent symptoms

If facilities available repeat Sputum AFB, Xpert MTB/RIF and TB culture OR Refer to District Chest Clinic (DCC) for further investigation

- Xpert MTB detected / RR not detected
- Xpert MTB detected / RR detected
- Xpert MTB not detected

- Treatment center to start ATT by consultant respiratory physician / trained medical officer
- Refer Drug Resistant TB (DR-TB) Algorithm
- For further evaluation by specialist team

1. Key symptoms of TB are **cough of 2 weeks or more** and/or chest pain, shortness of breath, haemoptysis. In immunosuppressed individuals and in elderly people (60 years or more of age) cough of any duration should be considered.
   Other symptoms of TB are loss of appetite, tiredness, loss of weight or failure to gain weight in children, low grade fever, night sweats.
2. Pulmonary tuberculosis suspects with high risk should be referred urgently / early to District Chest Clinics or situation where no other diagnosis to be consider. These **High-risk categories** include Health care workers (HCW), Patients living with HIV (PLHIV), prisoners, drug addicts, close contacts of **Bacteriologically confirmed PTB** patients and patients with past tuberculosis or immunosuppression.
3. If two or more sputum samples are positive without chest x- ray , the patient should be referred for treatment
4. Positive chest X-ray is defined as any abnormality that could be consistent with TB

Abbreviations

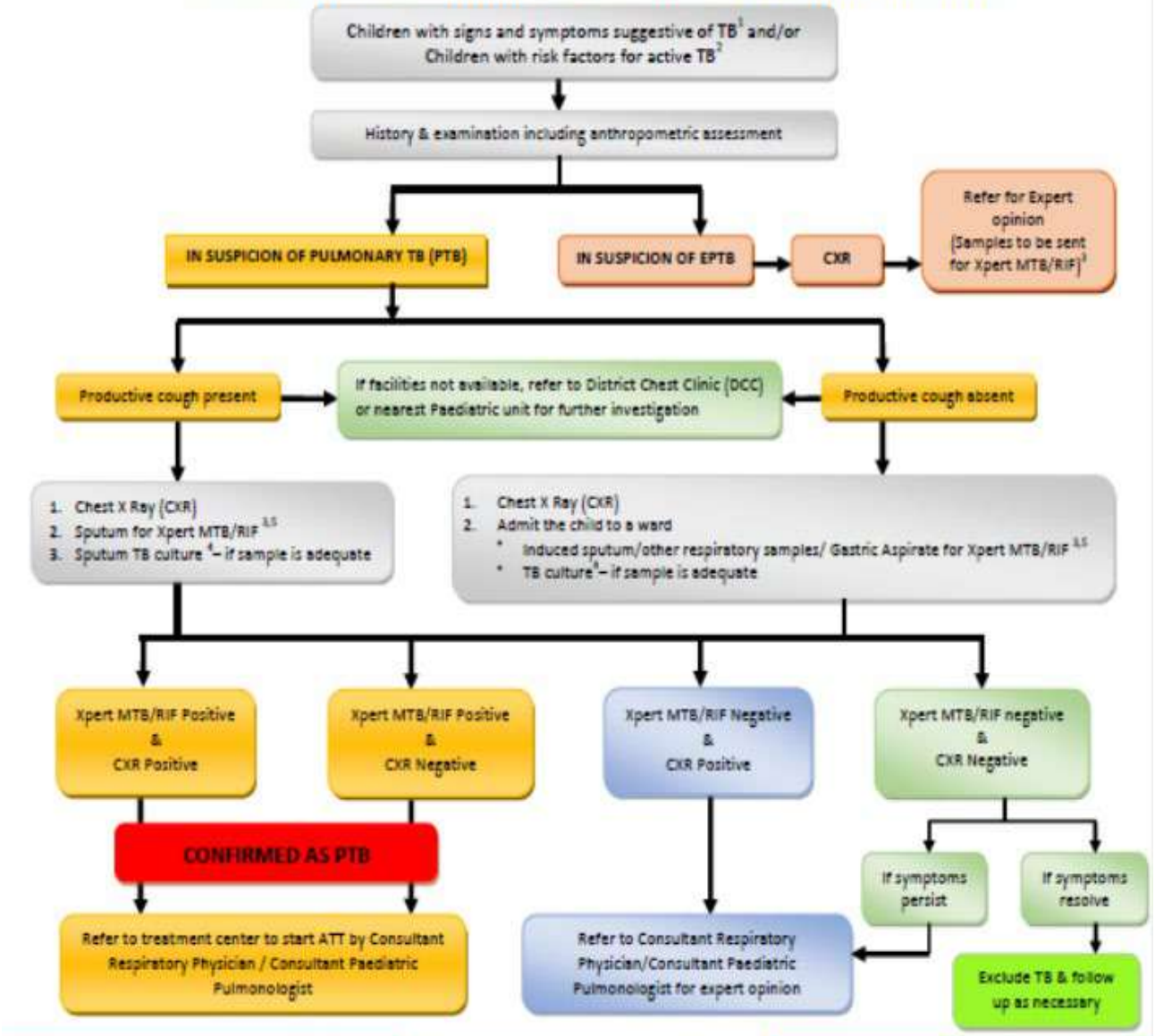
AFB - Acid Fast Bacilli , Xpert - Gene Xpert , MTB - Mycobacterium Tuberculosis, RR - Rifampicine Resistance, ATT - Anti Tuberculosis Treatment

TUBERCULOSIS is completely cured with proper treatment

National Programme for Tuberculosis Control & Chest Diseases
Ministry of Health

Annex 02

DIAGNOSTIC ALGORITHM FOR TUBERCULOSIS (TB) IN CHILDREN



1. CHILDREN WITH SIGNS AND SYMPTOMS SUGGESTIVE OF TB

- Cough for two weeks or more
- Weight loss–
  Reported or confirmed weight loss (>5%) since the last visit
- Poor weight gain –
  Very low weight for age (<-3 SD)
  Underweight (weight for age < -2 SD)
  Growth curve flattening
- Pneumonia not responding to antibiotics
- Poor control of 'asthma' / wheezing despite appropriate treatment
- Undiagnosed febrile illness continuing for > 2 weeks
- Poor feeding/anorexia

2. CHILDREN WITH RISK FACTORS FOR ACTIVE TB

- Children who are contacts of TB patients in past two years
- Children of all ages living with HIV
- Children living with HIV infected patients
- Children with Severe Acute Malnutrition (SAM)/ Failure to thrive
- Immunocompromised children
- Immigrant and refugee children
- Infants and children under 5 years from high risk settings

Abbreviations

- ATT – Anti tuberculosis treatment
- CXR – Chest X ray
- EPTB – Extra pulmonary tuberculosis
- PTB – Pulmonary tuberculosis
- Xpert – Gene expert

3. XPERT MTB/RIF (GENEXPERT®) – is offered to all paediatric TB patients

- Sputum obtained via – expectoration & induction
- Other respiratory specimens (2-3ml) (Bronchial wash, broncho-alveoler lavage, endotracheal aspirates) – patient should be admitted to ward to get these samples
- Extra-pulmonary samples
  - CSF, pus aspirates, lymph node aspirate and other fluids

4. TB culture is more sensitive & specific than direct smear microscopy & is recommended to be done in all paediatric patients.

5. If unable to send samples within 1 day, the samples should be refrigerated

NATIONAL PROGRAMME FOR TUBERCULOSIS CONTROL AND CHEST DISEASES
MINISTRY OF HEALTH

Annex – 03

TB 04

TUBERCULOSIS LABORATORY REGISTER

NATIONAL TUBERCULOSIS PROGRAMME

LAB SERIAL NO.	DATE	STD CARD NO/ BHT NO/ DISTRICT TB NO	NAME IN FULL	SEX M/F	AGE	NAME OF TREATMENT UNIT	ADDRES (FOR NEW PATIENTS)	REASON FOR EAMINATION		RESULTS OF EXAMINATION			SIGNATURE	REMARKS
								DIAGNOSIS	FOLLOW UP	1	2	3		

Annex – 04

NATIONAL TUBERCULOSIS PROGRAMME **TB 05**

THE LABORATORY FORM
REQUEST FOR SPUTUM EXAMINATION

Name of Treatment Unit: …… Date: ……

Name of Patient: …… Age: …… Sex: M ☐

Address in Full: ……F ☐

Disease classification District: ……

☐ Pulmonary

☐ Extra-Pulmonary Site: ……

Reason for Examination:

☐ Diagnosis

☐ Follow-up chemotherapy

OPD No./ BHT No./ CC/ DISTRICT TB No: ……..
DATE OF SPUTUM COLLECTION: ……

Signature: ……

RESULTS (To be completed in the laboratory)

LAB SERIAL NO: ……

a) Visual appearance of Sputum: ……..

Specimen	Mucopurulent	Blood stained	Saliva
1	☐	☐	☐
2	☐	☐	☐
3	☐	☐	☐

b) Microscopy

Date	Specimen	Results*	POSITIVE (grading)			
			+++	++	+	Scanty

*write negative or positive

Examined by: ……

The completed form (with results) should be sent to the treatment unit to record the results on the treatment card.

Annex – 05

REQUEST FORM
TB CULTURE, DRUG SUSCEPTIBILITY AND MOLECULAR TESTING
National TB Reference Laboratory, Welisara

Specimen		Date of Collection		
Sputum	Other (Specify)	dd	mm	yy

Lab Use Only			Serial No	
Date of Receipt			Lab No.	
dd	mm	yy	Culture	DST

Last Name of the Patient (In Block Letters)

Date of Birth		
yyyy	mm	dd

First Name/Initials of the Patient (In Block Letters)

Sex	M	F

Contact Number	NIC/ID of Patient/ Parent/Guardian	Patients Address:	Residential District:

Name of Sending Institution	Ward/ Clinic	BHT/Clinic No	Forwarding DCC	Standard Card No.	District TB NO	Report to be Sent to

CXR	Not Done	Done		
		Changes present	No Changes	Report pending

GeneXpert	Not Done	Done*						
		MTB					RR	
		ND	H	M	L	VL	D	I

Test/s Requested		Indication		
Culture & DST	GeneXpert (MTB/RIF)	For Diagnosis	Follow-up (Indicate month M1/M2/M3**)	Other (Specify)

Probable Site	PTB	Smear +		EPTB		If EPTB, Site/s	
		Smear -					

Treatment History	New	Previously Treated						
		Relapse	Failure	Loss to Follow Up	History Unknown	Other (Specify)	Known MDR	Known MOTT

Details of Treatment (Indicate drugs and duration)

	Drugs/Regime	Duration
Past ATT (Indicate periods of treatment)		
Present ATT (on date of specimen collection)	Not on ATT / On ATT (indicate regime)	Starting date

Current Sputum Smear Status of Follow Up Patients	
Positive	Negative

Does the patient belong to a Presumptive MDR group?	
Yes	No

*ND: Not Detected H: High M: Moderate L: Low VL: Very Low D: Detected I: Indeterminate
** Indicate month of follow-up E.g. M1/M2/M3...

Previous Cultures Done

Lab Serial No.	ABST No.	MDR No.	Year	Result

Other Relevant Clinical Details (e.g. HIV /Other Causes of Immune Suppression/X Ray/Mantoux)

.....

.....

.....

.....

.....

Signature of Medical Officer:.....

Name: .....

Designation: HO/ MO/DTCO/SHO/REG/SR/VP/VS/.....

Please Refer to Lists Given to District Chest Clinic for the Following

- *Indications for Culture - List 1*
- *Indications for Xpert MTB/RIF - List 2*
- *Presumptive MDR Groups –List 3*

Laboratory Use Only

Lab Serial No: .....

Smear	Positive 3+		Positive 2+		Positive 1+		Positive scanty		Negative	

Culture	Positive		Negative		Contaminated		Other	

Identification	MTB		Atypical		Other (Specify)	

Results of Sensitivity Test

Result	Streptomycin	Isoniazid	Rifampicin	Ethambutol
Sensitive				
Resistant				

.....
MLT /NTRL

Date:.....

.....
Consultant Microbiologist/NTRL

Date: .....

Contact No.: 011-2956702 or 011-2951428 or 011-2951751 or 011-2958271 Ext 409, 138 or 421

Annex – 06

Monthly Summaries to be sent to NTRL at the end of each month with RBRC slides.

Station: Month: Year:

MONTHLY SUMMARY

Please fill up the following data and send to the supervising Laboratory when you send the slides for Random Blinded Re-Checking.

SLIDES EXAMINED IN THE LABORATORY DURING THE MONTH						PATIENTS EXAMINED			
DIAGNOSIS			FOLLOWUP			DIAGNOSIS		FOLLOWUP	
Positive	Total	Slide Positivity Rate	Positive	Total	Slide Positivity Rate	Positive	Total	Positive	Total

Slide Positivity Rate = No of Positive Slides × 100
Total No of Slides

Station: Month: Year:

MONTHLY SUMMARY

Please fill up the following data and send to the supervising Laboratory when you send the slides for Random Blinded Re-Checking.

SLIDES EXAMINED IN THE LABORATORY DURING THE MONTH						PATIENTS EXAMINED			
DIAGNOSIS			FOLLOWUP			DIAGNOSIS		FOLLOWUP	
Positive	Total	Slide Positivity Rate	Positive	Total	Slide Positivity Rate	Positive	Total	Positive	Total

Slide Positivity Rate = No of Positive Slides × 100
Total No of Slides

Annex – 07

SMEAR RESULTS SHEET FOR BLINDED RECHECKING

Microscopy Center: …… Year: …….
Name of District: ……. Month: ……

Sl No	Lab Serial No	Result including grading for positive smears	Sl No	Lab Serial No	Result including grading for positive smears
1			31		
2			32		
3			33		
4			34		
5			35		
6			36		
7			37		
8			38		
9			39		
10			40		
11			41		
12			42		
13			43		
14			44		
15			45		
16			46		
17			47		
18			48		
19			49		
20			50		
21			51		
22			52		
23			53		
24			54		
25			55		
26			56		
27			57		
28			58		
29			59		
30			60		

Slides selected by DTCO/Medical Officer/ Supervising Laboratory technician

Name of Laboratory Technician of the Microscopy Center:

Signature: …… Date: …….

Annex – 08

Slide Distribution Book for RBRC

Year: ……

Serial No of the Slide Bundle	Name/ Code of the LT (1st Controller)	Microscopy Center	Month	Total No of Slides in the Slide Bundle	Date of Issue to 1st Controller	Date of Results issued by the 1st Controller	No of Slides to be Rechecked by the 2nd Controller	Date of Issue to 2nd Controller	Date of Results issued by the 2nd Controller

Annex – 09

RANDOM BLINDED RECHECKING OF SMEAR EXAMINATION

(Form A)

Microscopy Center/ District Chest Clinic Laboratory: District:

Month: Year:

No of Positives:

No of Negatives:

Sl. No	Lab Serial No	AFB Result By		Specimen Quality		Staining		Cleanness		Size		Thickness		Evenness	
		Microscopy	Assessor	Good	Poor	Good	Poor	Good	Poor	Good	Poor	Good	Poor	Good	Poor
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															
11															
12															
13															
14															
15															
16															
17															
18															
19															
20															
21															
22															
23															
24															
25															
26															
27															
28															
29															
Percentage Poor Sides															

Problems/Comments:

Name of Laboratory Technician:

Signature: …… Date: ……..

Annex – 10

Quality Assurance Report on Sputum Microscopy

District Chest Clinic: …….. Month: …….

Microscopy Center: …….. Year: ……

		Final Counter Check result by LT/DCC					
		Negative	1-9 AFB/100 Fields	1+	2+	3+	TOTAL
Peripheral Results by LT/MC	Negative		*LFN*	*HFN*	*HFN*	*HFN*	
	1-9 AFB/100 Fields				*QE*	*QE*	
	1+	*HFP*				*QE*	
	2+	*HFP*	*QE*				
	3+	*HFP*	*QE*	*QE*			
	TOTAL						

Summary of Errors identified (Nos)				
Major Errors		**Minor Errors**		
High False Positive	High False Negative	Low False Positive	Low False Negative	Quantification Error
Total Major Errors		Total Minor Errors:		

Number Of False Results		Lab Serial No fo the Slide				
False Positive						
False Negative						
Quantification Error						

Comments:

Reporting date: …….. ……

LT/DCC

Sample Receipt Date: …….. ……..

DTCO

Annex – 11

Quarterly Report – Random Blinded Rechecking of Slides

Smear Microscopy of Microscopy Centers

District: ……. Year: …….. Quarter: ……

Microscopy Center	Number of Slides Examined uring the Quarter (Work load of MC)				Number of Slides Rechecked for EQA during the Quarter	HFP	HFN	LFN	LFP	QE	Total No of Errors	Remarks
	Positive	Negative	Total	Slide Positivity Rate								
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												

- This Format should be completed and sent to the Consultant Microbiologist, National Tuberculosis Reference Laboratory (Central Laboratory of NPTCCD), Welisara at the end of each Quarter.

Annex – 12

STOCK BOOK

Item name: ……

Date	Quantity Received	Quantity Issued to DCC	Quantity Issued to Microscopy Centers																Balance Stock in hand
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	

Annex – 13

Laboratory Requirements

District: ……..

Chest Clinic

Total No of smears done in the previous Quarter (A): ……..……

No of Laboratory Technicians (B): ……

Microscopy Centers

Total No of smears done in the previous Quarter I: ……

No of Laboratory Technicians (D): ……

Item	Quantity needed per smear	Requirement for				Total amount in hand (stock) at the end of previous Quarter in the Chest Clinic	Actual Amount requested	Remarks
		1 Quarter		3 Quarters				
		Chest Clinic (F)	Microscopy Centers (G)	Chest Clinic (H)	Microscopy Centers (I)			
	I	(F=A × E)	(G=C × E)	(H= 3XF)	(I=3XG)	(J)	(H+ I –J)	
Glass Slides (no.)	1							
Basic Fuchsin (g)	0.03							
Methylene Blue(g)	0.003							
Phenol(ml)	0.15							
Alcohol(ml)	5.15							
HCl (ml)	0.15							
Immersion Oil (ml)	0.05							
Methylated Spirit (ml)	1							
	I	(F=M × E)	(G=N × E)	(H =3XF)	(I=3XG)	(J)	(H+ I –J)	
Lens Tissue(No.)	92 per Microscope per quarter							
	I	(F=B × E)	(G=D × E)	(H= 3XF)	(I=3XG)	(J)	(H+ I –J)	
Gloves	92 per person Per quarter							
Masks	92 per person Per quarter							
Filter Paper								

Item	Amount in hand(stock)		If you need more mention the further requirement for the year		Remarks
	Chest Clinic	Microscopy Centers	Chest Clinic	Microscopy Centers	
Microscopes	(M)	(N)			
Diamond Pencils					
Spirit Lamps					
Slide Tray					
Slide Rack					
Staining Rack					
Slide Storage Boxes					
Slide Mailers					
Drop Bottles					
Wash Bottles					
Slide Forceps					
Laboratory Overcoats					

Annex – 14

SUPERVISRY VISIT TO MICROSCOPY CENTERS BY DISTRICT CHEST CLINICS CHECK LIST

1. General information

Microscopy center	
District	
Number of MLT/PHLT	MLT: PHLT:
Names and designations of the current staff	
Date of visit	
Names & designations of visiting team	

2. Action implemented as per previous visit:

3. Current Visit Particulars:

No			Item	Adequate/ Acceptable	Problems Identified
1.			**Infrasturcure:**		
	1.1		Separate area for TB laboratory work	Y/N	
	1.2		Separate bench/ tables for specimen receipt/ smear preparation/ microscopy	Y/N	
	1.3		Uninterrupted power supply	Y/N	
	1.4		Running water supply	Y/N	
2.			**Availability of Standard Operating Procedures:**		
	2.1		Staining procedure	Y/N	
	2.2		Grading chart	Y/N	
	2.3		QC of reagents & staining procedure	Y/N	
	2.4		Use and cleaning of the microscope	Y/N	
	2.5		Cleaning worksite and disposal of waste	Y/N	
	2.6		EQA Protocol	Y/N	
3.			**Availability of Items (Adequate stocks & Supply)**		
	3.1		**Consumables**		
		3.1.1	Slides	Y/N	
		3.1.2	Slides re-used	Y/N	
		3.1.3	Sputum cups	Y/N	
		3.1.4	Lens tissue	Y/N	
		3.1.5	Immersion oil	Y/N	
		3.1.6	Disinfectants	Y/N	
		3.1.7	Smearing/ Staining equipment (Staining racks, Loops, Sticks, etc)	Y/N	
	3.2		**Staining Reagents within Expiry date**		
		3.2.1	CarbolFuchsin	Y/N	
		3.2.2	Methyelene Blue	Y/N	
		3.2.3	3% Acid Alcohol	Y/N	
		3.2.4	Distilled water	Y/N	
	3.3		**Equipment – Binocular Microscopes**		
		3.3.1	Adequate number	Y/N	
		3.3.2	Functioning well	Y/N	
		3.3.3	Adequate maintenance – Service done once a year	Y/N	
		3.3.4	Spare bulb	Y/N	

4.		**EQA**		
	4.1	Slide storage according to lab register	Y/N	
	4.2	All slides are stored in box and no missing slides	Y/N	
	4.3	Slides stored without touching each other	Y/N	
	4.4	EQA forms available	Y/N	
	4.5	Reports filed	Y/N	
5.		**Documentation**		
	5.1	Laboratory Request Form used	Y/N	
	5.2	Request form is completed	Y/N	
	5.3	Lab register in available and completed	Y/N	
	5.4	Results in the lab register entered daily	Y/N	
	5.5	Results provided within 24hrs	Y/N	
	5.6	3 specimen per new patient examined	Y/N	
6.		**Safety**		
	6.1	Adequate ventilation – Exhaust Fan	Y/N	
	6.2	Smear not prepared near open window	Y/N	
	6.3	Sand and Lysol containing jar for loop cleaning	Y/N	
	6.4	Lab coats worn while working	Y/N	
	6.5	Gloves used during work and cleaning	Y/N	
	6.6	Flowing water for hand washing	Y/N	
7.		**Waste Disposal**		
	7.1	Work area cleaned daily	Y/N	
	7.2	Waste bin with lid available	Y/N	
	7.3	Yellow bin	Y/N	
	7.4	Infectious sputum/ Waste disposal by	Y/N	
		- Autoclave	Y/N	
		- Burning	Y/N	
		- Disinfection	Y/N	
		- Burial	Y/N	
	7.5	General order/ cleanliness	Y/N	
8.		**Staff Training**		
	8.1	Has all new staff had smear EQA training during the past 2 years?	Y/N	

4. Observation check list for collection, smearing and staining procedures

No	Observations	Adequate/ Acceptable	Problems identified
1.	Instructions for collecting sputum is given to the patient	Y/N	
2.	Correct labeling of the boy of the container	Y/N	
3.	Are the slides 19 labeled with a diamond pencil correctly?	Y/N	
4.	Is the wire loop cleaned in a sand/Lysol bath after each use?	Y/N	
5.	Is the smear air dried completely before fixing?	Y/N	
6.	Fixed adequately by sending reverse slide of slide over flame 3-4 times	Y/N	
7.	Number of slides stained in a rack ≤ 12	Y/N	
8.	Staining procedure is done correctly	Y/N	
9.	Microscope lens is cleaned after each slide examination	Y/N	
10.	Is reporting of slides as per the NPTCCD guidelines (Scanty with number, 1+, 2+, 3+)?	Y/N	
11.	Internal QC slide box has adequate number of positives and negative slides	Y/N	

5. Assessment of the workload (Last 3 months)

Slide volume	
Negative slide no	
Positive slide no	
Slide positivity rate (SPR)	

1) Onsite Evaluation Summary

a) Operational problems (E.g.: Infrastructure- power supply, etc.)

b) Technical problems

c) Overall remarks

d) Action required

Format for recording results of panel testing during the Supervisory visit to the microscopy centers:

(Each laboratory technician of the microscopy center should complete a separate sheet)

To be entered by MLT/PHLT of the MC		For use by laboratory technician of the DCC		
Slide No	Result of MLT/PHLT of MC	Expected result	Error Type	Remarks
1.				
2.				
3.				
4.				
5.				

Name of the PHLT/MLT:

Name of the MC:

Date of tested:

Annex – 15

Quarterly Report – Laboratory Supervision of Microscopy Centers

DCC: …….. Year & Quarter: ……

1. Operation problems

Microscopy center	Problems identified	Action required
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		

2. Technical problems

Microscopy center	Problems identified	Action required
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		

3. Overall remarks:

Date: …… Signature: ……

If further space is needed, please attach another sheet.

Annex – 16

Name of the laboratory
Address of the laboratory
Contact information of the Laboratory (Telephone No, Email Address)

Name:

Age:

Sex:

BHT No:

Ward:

Institute:

Clinic No:

Received From:

Residential District:

Specimen:

Lab no
Date of Received
Date of Collection

Microscopy

Deposit:

Mycobacterial Culture Report

Culture Result

Culture Method:

Culture Result:

Culture identification:

Comments

…… ……

MLT Consultant Microbiologist

Date :

Annex – 17

Name of the laboratory
Address of the laboratory
Contact information of the Laboratory (Telephone No, Email Address)

Name:

Age:

Sex:

BHT No:

Ward:

Institute:

Clinic No:

Received From:

Residential District:

Specimen:

Lab no

Date of Collection

Date of Received

Mycobacterial Culture & DST Final Report

Culture result

Culture method:

Culture result:

Culture identification:

Drug Susceptibility

Streptomycin:

INAH:

Rifampicin:

Ethambutol:

Pyrazinamide:

Comments

…… ……

MLT Consultant Microbiologist

Date :

Annex - 18

Distribution of GeneXpert Sites in Sri Lanka, 2021

	Institute	Module	Number of machines
1	CC Anuradhapura	IV Module	1
2	PGH Badulla	IV Module	1
3	TH Batticaloa	IV Module	1
4	TH Karapitiya	IV Module	1
5	CC Kandy	IV Module	1
6	TH Kurunegala	IV Module	1
7	NHSL, Colombo	IV Module	1
8	NTRL	IV Module and XVI Module	2
9	THRathnapura	IV Module	1
10	DGH Kegalle	IV Module	1
11	NIHS, Kalutara	IV Module	1
12	TH Jaffna	IV Module	1
13	LRH	IV Module	1
14	Prison Hospital, Colombo	IV Module	1
15	DGH Polonnaruwa	IV Module	1
16	DGH NuwaraEliya	IV Module	1
17	DGH Hambantota	IV Module	1
18	AMH - Kalmunai	IV Module	1
19	CC Colombo	IV Module	2
20	CSTH, Kalubowila	IV Module	1
21	DGH Vavuniya	IV Module	1
22	DGH Ampara	IV Module	1
23	BHPuttalam	IV Module	1
24	DGH Trincomalee	IV Module	1
25	DGH Mannar	IV Module	1
26	DGH Matale	IV Module	1
27	DGH Matara	IV Module	1
28	DGH Monaragala	IV Module	1
29	Faculty Of Medicine -Colombo	IV Module	1
Total			**31**

Annex – 19

GXPERT REGISTER

-Page 1-

Lab No	GXP No	Patients name	Age			Sex	BHT No	Ward	Institution	Clinic No	Clinic	Specimen	Smear		Category	MDRS
			Y	M	D								DS	Dep		

-Page 2-

Lab No	GXP No	Date Processed	Mycobacterium tuberculosis Detection	Rifampicin resistance	Reporting			Signature	Date	Specimen	Smear		Remarks
					DD	MM	YY				DS	Dep	

Annex – 20

Gene Xpert Work Sheet

Date	Lab No	GXP No	MTB Detection	Amount – VL/L/M/H	Rif Resistance

Annex – 21

GENE XPERT MONTHLY SUMMARY

Laboratory: …… Month: …… Year: ……

Total tests done			
MTB detected	**Total**		
	RRD		
	RRND		
	RR Ind		
MTBND			
Error			
Invalid			
Repeat			

Cartridge Lot	**Expiry Date**
1.	
2.	

Stock Available at the end of the month	**Expiry Date**

	Name	**Designation**
Prepared by		
Approved by		

Total Pulmonary specimen		**AFB Smear +**	**AFB Smear -**	**Smear result not known**	**Total no**
MTB Detected	**Total**				
	RRD				
	RRND				
	RR Ind				
MTBND					
Error					
Invalid					
Repeat					

Total Extra Pulmonary Specimen			
MTB detected	**Total**		
	RRD		
	RRND		
	RR Ind		
MTBND			
Error			
Invalid			
Repeat			

Total CSF Specimens			
MTB detected	**Total**		
	RRD		
	RRND		
	RR Ind		
MTBND			
Error			
Invalid			
Repeat			

Annex – 22

Name of the laboratory
Address of the laboratory
Contact information of the Laboratory (Telephone No, Email Address)

Name :
Age : Y
Sex :
BHT No. :
Ward :
Institute :
Clinic No. :
Received From :
Residential District :
Specimen :

Lab No.	
X-Pert No.	
Date Collected	//
Date Received	//

Molecular Diagnostic Test Report

Test : Xpert MTB /RIF

Result : *Mycobacterium tuberculosis* NOT detected

Note - X pert MTB/RIF is an automated NAAT (GENE X-pert) recommended by WHO. The test identifies the MTB in the specimens and detects the genotypic resistance for Rifampicin. Rifampicin resistance is considered as the surrogate marker of MDR. However it is suggested to exclude Rifampicin mono resistance before commencing the therapy for MDR.

P. S. Genotypic resistance might differ from phenotypic resistance due to the variations of genetic expression.

Comments

…… ……

MLT Consultant Microbiologist

Date :

Annex-23

Name of the laboratory
Address of the laboratory
Contact information of the Laboratory (Telephone No, Email Address)

Name :
Age : Y
Sex :
BHT No. :
Ward :
Institute :
Clinic No. :
Received From :
Residential District :
Specimen :

Lab No.	
X-Pert No.	
Date Collected	//
Date Received	//

Molecular Diagnostic Test Report

Test : Xpert MTB /RIF

Result : *Mycobacterium tuberculosis* DETECTED;(HIGH)

Rifampicin resistance NOT DETECTED

Note - X pert MTB/RIF is an automated NAAT (GENE X-pert) recommended by WHO. The test identifies the MTB in the specimens and detects the genotypic resistance for Rifampicin. Rifampicin resistance is considered as the surrogate marker of MDR. However it is suggested to exclude Rifampicin mono resistance before commencing the therapy for MDR.

P. S. Genotypic resistance might differ from phenotypic resistance due to the variations of genetic expression.

Comments

…… ……

MLT Consultant Microbiologist

Date :

Annex-24

Name of the laboratory
Address of the laboratory
Contact information of the Laboratory (Telephone No, Email Address)

Name :
Age : Y
Sex :
BHT No. :
Ward :
Institute :
Clinic No. :
Received From :
Residential District :
Specimen :

Lab No.	
X-Pert No.	
Date Collected	//
Date Received	//

Molecular Diagnostic Test Report

Test : Xpert MTB /RIF

Result : *Mycobacterium tuberculosis* DETECTED; (VERY LOW)

Rifampicin resistance DETECTED

Note - X pert MTB/RIF is an automated NAAT (GENE X-pert) recommended by WHO. The test identifies the MTB in the specimens and detects the genotypic resistance for Rifampicin. Rifampicin resistance is considered as the surrogate marker of MDR. However it is suggested to exclude Rifampicin mono resistance before commencing the therapy for MDR.

P. S. Genotypic resistance might differ from phenotypic resistance due to the variations of genetic expression.

Comments

…… ……

MLT Consultant Microbiologist

Date :

Annex-25

Name of the laboratory
Address of the laboratory
Contact information of the Laboratory (Telephone No, Email Address)

Name :
Age : Y
Sex :
BHT No. :
Ward :
Institute :
Clinic No. :
Received From :
Residential District :
Specimen :

Lab No.	
X-Pert No.	
Date Collected	//
Date Received	//

Molecular Diagnostic Test Report

Test **:** Xpert MTB /RIF

Result **:** *Mycobacterium tuberculosis* DETECTED; (VERY LOW)

Rifampicin resistance INDETERMINATE

Note - X pert MTB/RIF is an automated NAAT (GENE X-pert) recommended by WHO. The test identifies the MTB in the specimens and detects the genotypic resistance for Rifampicin. Rifampicin resistance is considered as the surrogate marker of MDR. However it is suggested to exclude Rifampicin mono resistance before commencing the therapy for MDR.

P. S. Genotypic resistance might differ from phenotypic resistance due to the variations of genetic expression.

Comments

…… ……

MLT Consultant Microbiologist

Date :

Annex - 26

LABORATORY STAFF MEDICAL RECORD

Name	:	
Age	:	
Sex	:	
Marital Status	:	
Designation	:	
Date of appointment to NTRL	:	
Permanent Address	:	
Telephone No	: Mobile	
	Residence	

Medical Screening of Laboratory Staff on enrollment

Tuberculosis risk symptom evaluation:

Cough: >3w …….. LOA: …… LOW: …… Night sweats: …….. Mild fever: ……

Past medical history:

……

Past surgical history:

……

Drug history:

……

Vaccination History:

……

History of drug or food allergy:

……

Family history: Contact history of TB ☐

……

Social History:

……

Examination Findings:

Height: …… Weight: …… BMI: ……..

Wasting ☐ Pallor ☐ Jaundice ☐ SOB ☐

Oedema ☐ BCG Scar ☐ Clubbing ☐

PR: ……..bpm Blood pressure: …… mmHg

Respiratory system:

……

Basic Investigations:

Investigation	Date	Results
FBC		
ESR		
S.Cr		
FBS		

Other Investigations:

Investigation	Date	Results
Mantoux/ IGRA		
Chest Xray - PA		

Problem list:

No	Problem	Action taken

Annex 27

INFORMATION ON SPECIMEN SENT TO CENTRAL LABORATORY

To be submitted by the 1st week of the next month.

(Data of the specimens sent to intermediate culture laboratory, need to be submitted separately).

Month: Year:

District:

Week	Number of specimens sent for culture			
	Sputum		Other	
	No of patients	No of specimens	No of patients	No of specimens
1st week				
2nd week				
3rd week				
4th week				
5th week				
Total				

National Programme for Tuberculosis Control and
Chest Diseases, Sri Lanka