Laboratory Manual for Tuberculosis Control

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National Programme for Tuberculosis Control and Chest Diseases
Ministry of Health - Sri Lanka

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Editorial Board

Chief Editor
Dr. (Mrs) Jayanthi Elvitigala

Edit Board
Dr. Sunil De Alwis
Dr. Sudath Samaraweera
Dr. (Mrs).G.H.Wickramanayake
Dr. K.D.J.H.Manilka Jayawardana

CONTRIBUTORS
Dr. Amitha Fernando
Dr. A. Ramachandran
Dr. Kamani Abeydeera
Dr. Chathura Hingalagoda
Miss.M.I.S.K. Munasinghe
Mrs J.Hettige
Mr. G.R. Udayakumara
Mrs R. Weerasingha
Mr.P.A.L.D.Bandara
Mrs.L.N.K.Dirokshika
# Content Page

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 01</td>
<td>INTRODUCTION</td>
<td>01</td>
</tr>
<tr>
<td>Chapter 02</td>
<td>NATIONAL PROGRAME FOR TUBERCULOSIS</td>
<td>03-04</td>
</tr>
<tr>
<td>Chapter 03</td>
<td>MICROSCOPY NETWORK OF THE NPTCCD</td>
<td>05-06</td>
</tr>
<tr>
<td>Chapter 04</td>
<td>GENERAL INFORMATION ON TUBERCULOSIS</td>
<td>07-10</td>
</tr>
<tr>
<td>Chapter 05</td>
<td>SAFETY PRECAUTIONS AND MANAGEMENT OF LABORATORY ACCIDENTS</td>
<td>11-14</td>
</tr>
<tr>
<td>Chapter 06</td>
<td>SMEAR MICROSCOPY FOR AFB</td>
<td>15-37</td>
</tr>
<tr>
<td>Chapter 07</td>
<td>QUALITY ASSURANCE OF SPUTUM SMEAR MICROSCOPY</td>
<td>39-45</td>
</tr>
<tr>
<td>Chapter 08</td>
<td>LABORATORY DOCUMENTS</td>
<td>47-50</td>
</tr>
<tr>
<td>Chapter 09</td>
<td>CULTURE AND DRUG SENSITIVITY TESTING FOR MYCOBACTERIA</td>
<td>51-56</td>
</tr>
<tr>
<td>Chapter 10</td>
<td>LABORATORY WASTE DISPOSAL</td>
<td>57-61</td>
</tr>
<tr>
<td>REFERENCES</td>
<td></td>
<td>63</td>
</tr>
<tr>
<td>ANNEXURES</td>
<td></td>
<td>65-87</td>
</tr>
</tbody>
</table>
FOREWORD

Laboratory service is an essential component of a Tuberculosis Control Programme.

It is a well-known and well accepted fact that the successful outcome of Tuberculosis management, as an individual or public health measure, substantially depend on accurate and early laboratory diagnosis. Therefore it is essential that all laboratory diagnostic settings, methods, procedures and performance are not only complies to the current international requirements, but also to be standard, nationally.

Laboratory service to the National Programme for Tuberculosis Control & Chest Diseases is provided by well-established network of microscopy centers, Laboratories of District Chest Clinics, Chest Hospital Welisara and National Tuberculosis Reference Laboratory at Welisara.

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

It is a great pleasure to mention here that NTRL under guidance of NPTCCD has launched some innovative actions recently, which includes the extension of its services to the private sector, particularly in quality assurance. In addition, many technological advancements and physical expansions are also to be integrated into National Tuberculosis Laboratory network very soon, in order to provide standard, quality assured, and efficient diagnostic service to the Tuberculosis control efforts of the country.

The formulation of national manual in Tuberculosis diagnostic service also can be considered as a land mark in the history of Tuberculosis control in Sri Lanka. In this service endeavor, I am thankful to Dr. (Mrs.) Jayanthi Elvitigala, Consultant Microbiologist for her commitment and enthusiasm in formulating this manual. Secondly my appreciation also extended to Dr. Chathura Hingalagoda, Medical Officer of NPTCCD who had coordinated this activity.

I do sincerely hope that this manual will help considerably in on our efforts aiming at elimination Of Tuberculosis from Sri Lanka.

Dr. Sunil De Alwis
Director, NPTCCD
A successful TB control in a country is heavily dependent on proper case detection supported by bacteriological confirmation. In Sri Lanka a network of laboratories technically guided by the National TB Reference Laboratory provide this laboratory support for the TB control programme by diagnosing the TB patients as well as by monitoring them till the end of the treatment period.

Performing standard techniques in TB microbiology is very essential for having accurate and reliable results in tuberculosis diagnosis and monitoring since some of the treatment options depend solely upon the lab diagnosis. Therefore implementation of such testing in a uniform fashion throughout the country is a must. A Laboratory manual plays a key role in keeping up the uniformity of the laboratory practices and at the same time bringing them up to the required standards.

As such The National Programme for Tuberculosis Control and Chest Diseases decided to update the lab manual which was printed nearly a decade ago.

This laboratory manual has evolved over the years to meet the needs of TB Laboratory diagnosis while ensuring the safety in laboratory environment. Quality assurance of the laboratory activities is also addressed in here to encourage and facilitate the internal quality control activities as well as external quality assurance programmes.

The intention of this is to provide the laboratory technicians & trainees with an organized, user-friendly standard tools to better enable them to understand and perform laboratory activities pertaining to TB.

All who extended their support and cooperation for this manual to see the day light is appreciated here. Publishing this manual was financially supported by GFATM.

I hope you enjoy this laboratory manual and also hope it makes your work of TB microbiology a bit easier.

Dr Jayanthi. P . Elwitigala
Consultant Microbiologist, NTRL/ Welisera
2012/ 07/ 20
Tuberculosis is an infectious disease caused by the bacillus Mycobacterium tuberculosis. Tuberculosis is still a significant public health problem in Sri Lanka. In recognition of its public health importance, TB was declared a global emergency in 1993 by the WHO. Annually, 1.7 million people still die from the disease, including 380,000 women, while increased deaths are due to drug-resistant TB. MDR-TB is a global threat, with a case-fatality rate of 50%. Recently, TB with extensively drug-resistant strains (XDR) has emerged as a new global threat. In addition, co-infection of TB and human immunodeficiency virus (HIV) is threatening world health still further. (WHO 2010/2011)

In Sri Lanka, 9118 new cases of tuberculosis were notified in year 2009. In 2007, the incidence of all forms of TB was: 43.5/100,000 with smear positive Pulmonary TB: accounting to 23.7/100,000. Tuberculosis commonly affects the lungs, although it can affect any other organ. In Pulmonary tuberculosis, tubercle bacilli can be excreted in the sputum by coughing or sneezing. This is the most infectious form of tuberculosis and the most important source of transmission of infection in the community. Therefore the highest priority of tuberculosis control is the identification and cure of these infectious cases i.e. patients with sputum smears positive for TB bacilli. The diagnosis of tuberculosis (TB), management of patients with the disease, and public health TB control services rely on accurate laboratory tests. Laboratory services are an essential component of effective TB control, providing key information to clinicians for patient management and to public health agencies for control services.

Purpose of the manual

Direct smear sputum examination is the most suitable cost effective tool for diagnosing infectious tuberculosis and for monitoring of cases on treatment, in a resource poor setting. It is essential to have clear guidelines on the standard techniques and procedures. Therefore this manual is prepared for the use of laboratory technicians and other health personnel, who are involved in tuberculosis laboratory activities. The guidelines in this manual are based on the national and international policies for TB control. The manual provides the technical details for sputum microscopy, procedure for culture and DST, laboratory management & External Quality Assurance (EQA) of sputum microscopy. All personnel involved with tuberculosis control activities in the country are expected to follow these.
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National Programme for Tuberculosis Control and Chest Diseases (NPTCCD) is an integral part of the national health service which functions under the Director General of Health Services through Deputy Director General, Public Health Services (DDG PHS), through Director, NPTCCD. NPTCCD is responsible for the TB control activities in the entire country. Its activities are closely integrated with the general health services.

Chapter
NATIONAL PROGRAMME FOR TUBERCULOSIS

National Policy in TB control
• Notification of all diagnosed TB patients
• Treat all diagnosed TB patients according to the national policy
• Registration of all diagnosed TB patients at chest clinics
• Services and drugs free to all patients
• DOTS at least in the initial phase of the treatment

The Goal of NPTCCD
To reduce morbidity, mortality, and transmission of TB until it is no longer a public health problem in Sri Lanka. Long-term goal - Elimination of Tuberculosis by year 2050. (WHO definition of TB elimination: -Less than one case per 100,000 population)

Vision of NPTCCD
TB free Sri Lanka

Mission of NPTCCD
To contribute to the socioeconomic development of the nation by committing ourselves to create a TB free Sri Lanka by formulation of policies, planning, coordinating, and monitoring of all TB and chest disease activities in the country.

Objectives of NPTCCD
• To ensure that every TB patient has access to effective diagnosis, treatment and cure of all TB patients, according to the national policy and guideline.
• To interrupt the transmission of TB.
• To prevent the emergence of Drug resistant TB.
• To reduce the social economic toll caused by TB.
• To reduce the disease burden from other respiratory diseases to the health system of Sri Lanka.
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**Objectives of NPTCCD**
- To ensure that every TB patient has access to effective diagnosis.
- Treatment and cure of all TB patients, according to the national policy and guideline.
- To interrupt the transmission of TB.
- To prevent the emergence of Drug resistant TB.
- To reduce the social economic toll caused by TB.
- To reduce the disease burden from other respiratory diseases to the health system of Sri Lanka.
Basic strategy for TB control

The basic strategy is to identify and treat all TB cases until they are cured. The most effective step is curing the infectious cases to break the chain of transmission. DOTS is the WHO accepted strategy adopted by NPTCCD for this purpose.

What is DOTS?

Directly Observed Treatment Short course

It has five components.

1. Government commitment to sustain TB control.
2. Case detection by sputum microscopy of symptomatics.
3. Regular and uninterrupted supply of good quality anti-TB drugs
4. Short Course chemotherapy under direct supervision.
5. Good recording and reporting mechanism to monitor treatment outcome and the overall performance of the programme.
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Chapter 03

MICROSCOPY NETWORK OF THE NPTCCD

Tuberculosis microscopy network consists of laboratory services organized at three levels/layers which are closely interlinked.

Central Level - National TB Reference Laboratory (NTRL)

District level - Microscopy laboratories based at District Chest Clinics

Peripheral level - Microscopy Centres based at health Institutions other than District Chest Clinics.

The NTRL provides the technical guidance to the network and coordinate all the laboratory activities related to TB control.

District Chest clinics: These laboratories are functioning under administrative purview of respective Provincial Health Services with technical guidance of NTRL of NPTCCD. Colombo and Gampaha Chest Clinics are under administrative and technical purview of NPTCCD. In addition to the above-mentioned laboratories, there are sputum collection centres at identified peripheral institutions in each district.

Functions at each level

Microscopy Centres

- Perform sputum smear microscopy using Ziehl-Neelsen method
- Receive, process, and report on sputum samples
- Ensure 3 sputum specimens are examined for diagnosis and 2 during follow up
- Participate in EQA
- Maintain the records and statistics

District Chest Clinic Laboratories

- Perform sputum smear microscopy.
- Prepare and distribute reagents and other laboratory requirements to the microscopy centres
- Estimate the reagent requirement for the DCC and the Microscopy centres and maintain the equipment in the DCC and the Microscopy centres
- Quality assures the sputum microscopy performed at the microscopy centres and participate in EQA conducted by NRTL
- Provision of laboratory statistics to the NTRL

ORGANIZATION OF THE NPTCCD

DGHS

DDGPHS

DNPTCCD 08 PDHS

26 DPDHS

Central Unit

NPTCCD

Natoonal

TB

Reference

Laboratory

C.H

Welisara

C.H

KKS

C.C

Gampaha

Disitrict

C.C

Branch

Colombo

Branch

Gampaha

Branch
National TB Reference Laboratory

The main tasks

• Coordination of TB laboratory network, formulation of laboratory policies, and guidelines, establish standard techniques to use in the network and provision of technical guidance to the network laboratories.

• Supervision of all Lab services under the NPTCCD network.

• Quality Assurance of Smear Microscopy in the National laboratory network and some of the private sector laboratories.

• Organise and coordinate human resource development, capacity building of personnel in the network.

• Develop links with Supra Reference Laboratory (SRL) and participate in EQA by SRL.

• Perform TB Culture and Drug susceptibility testing and a limited number of sputum smear microscopy.

• Expansion of diagnostic services & EQA services.

• Implement surveillance of anti-tuberculosis drug resistance.

• Maintenance of national TB laboratory information system.

• Develop and carry out operational research and make recommendation for introduction of new technology to the network laboratories where relevant.
Chapter 3
National TB Reference Laboratory

The main tasks
- Coordination of TB laboratory network, formulation of laboratory policies, and guidelines, establish standard techniques to use in the network and provision of technical guidance to the network laboratories.
- Supervision of all Lab services under the NPTCCD network.
- Quality Assurance of Smear Microscopy in the National laboratory network and some of the private sector laboratories.
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- Expansion of diagnostic services & EQA services.
- Implement surveillance of anti-tuberculosis drug resistance.
- Maintenance of national TB laboratory information system.
- Develop and carry out operational research and make recommendation for introduction of new technology to the network laboratories where relevant.

Introduction

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

Multidrug-resistant tuberculosis (MDR-TB) is a form of TB caused by mycobacteria strains resistant to Isoniazid and Rifampicin.

Extensively drug-resistant strains, XDR TB is defined as TB strains resistant to isoniazid, Rifampicin and to:

a) any Fluoroquinolone, and quinolone, and
b) At least 1 of the 3 injectable second-line drugs (Capreomycin, Kanamycin, and Amikacin).

XDR TB has a very high mortality rate.

Tuberculosis bacteriology for the detection of sources of infection, the diagnosis of clinical suspects, and the follow up of the effect and results of treatment, are essential components of tuberculosis control.

Natural history of tuberculosis

The main reservoir of infection is a patient with pulmonary tuberculosis. When such a 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.

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 twenty five 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 5%, 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 through out the body by the lymphatic and blood systems. A limited number of bacilli may survive at these extra-pulmonary sites and give rise to the various forms of extra-pulmonary tuberculosis months or years later.

In those with infectious pulmonary tuberculosis, 30 % are spontaneously cured by the body’s defence 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.
- 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.

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

Investigations

Sputum Smear Microscopy

The first screening test for a PTB suspect is sputum microscopy. This is the most suitable reliable and cost effective method of diagnosing infectious cases of PTB in resource poor settings. Finding TB bacilli is more likely with three samples than with one or two samples.
A sample should contain at least 5000-10,000 bacilli per ml of sputum to be positive on microscopy.

**Role of the Chest Radiograph**

No chest radiographic pattern is characteristic or diagnostic of PTB. However certain changes such as upper lobe infiltrates (patchy shadows), patchy shadows that becomes confluent and bilateral with the spread of the disease, cavitations may suggest tuberculosis. If radiographic criteria alone are used for the diagnosis, PTB will be misdiagnosed 50 % of the time.

Many other conditions such as bacterial and fungal pneumonias, bronchiectasis, malignancies, alveolar haemorrhage, pulmonary oedema, lung abscess, interstitial lung diseases, pneumoconiosis mimics radiological changes of PTB.

**Tuberculin Skin Test**

Tuberculin is a Purified Protein Derivative (PPD) from the tubercle bacilli. When injected intradermally to an infected person the immune system mounts a type IV hypersensitivity reaction 24 – 48 hours later. This is characterized by a “wheal” and “flare.”

**In an adult, results are recorded and interpreted as follows:**

- **0-9 mm – Negative**
- **>10 mm- Positive**
- **>15mm- strongly positive**

A positive tuberculin test only indicates past infection with Mycobacterium tuberculosis or with mycobacteria other than tuberculosis (MOTT). The test is also positive in those who have been BCG vaccinated. In these individuals the reaction is usually weaker and less than 10 mm.

**A tuberculin test may be false negative in the**

- HIV infection.
- Malnutrition.
- Severe bacterial infection including tuberculosis.
- In the presence of viral infections such as measles, chickenpox.
- Malignancies.
- Immunosuppressive drugs, long term oral steroids.

**Sputum culture for Acid Fast Bacilli (AFB)**

Culture of Mycobacterium tuberculosis from clinical specimens provide the gold standard for the diagnosis of tuberculosis.

Routine cultures for Tuberculosis are performed only according to the National policy.

**Standard case definitions and treatment categories of TB patients**

**Patient types**

- **New:** Patient who has never taken anti TB treatment before or has taken treatment for less than 1 month.
- **Relapse:** a patient who was previously treated and declared cured or treatment completed and once gain developed smear or culture positive TB
- **Treatment after failure:** a sputum positive pulmonary TB patient whose follow up
sputum smears at five months are positive or a patient who was smear negative at the start of anti-TB treatment but smear positive while on treatment at follow up.

**Treatment after default:** TB patient who return to treatment, bacteriologically positive following interruption of treatment for 2 months or more

**Transfer in:** a patient transferred from another TB District register

**Transferred out:** patient was transferred out to another district and the treatment outcome was not known

**Other:** all patients who do not fit into above

### Standard treatment categories

<table>
<thead>
<tr>
<th>TB treatment category (CAT)</th>
<th>Type of patient</th>
<th>Intensive phase</th>
<th>Continuation phase</th>
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<tbody>
<tr>
<td>CAT 1</td>
<td>New smear Positive or negative pulmonary /extra pulmonary+/HIV positive</td>
<td>2HREZ (FDC4)</td>
<td>4HR (FDC 2)</td>
</tr>
<tr>
<td>CAT 2</td>
<td>Previously treated</td>
<td>2SHREZ (FDC4 +S)</td>
<td>5HRE (FDC3)</td>
</tr>
</tbody>
</table>

H=isoniazid, R=Rifampicin E= Ethambutol P=Pyrazinamide S= Streptomycin 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.

### Treatment outcome

**Cured**- initially sputum positive patient converting to negative on 2 occasions at completion of treatment

**Treatment completed** - patient has successfully completed treatment but do not fulfil criteria for cure or failure

**Died**- who dies for any reason while on TB treatment

**Defaulted**- treatment was interrupted for 2 consecutive months or more

### 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 tuberculosis 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. Tuberculosis of the eye (Tuberculous Uveitis), abdominal tuberculosis (Tuberculous peritonitis, Appendicular and other bowel masses) genitourinary 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 sample and / or by histopathological examination of relevant biopsy specimens of affected organs or tissue.
SAFETY PRECAUTIONS AND MANAGEMENT OF LABORATORY ACCIDENTS

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.

<table>
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<tr>
<th>SAFETY PRACTICES IN THE TB MICROSCOPY LABORATORY</th>
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<tr>
<td>- Entry to the laboratory should be restricted only to the laboratory staff</td>
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<tr>
<td>- Establish airflow in working areas that will direct potentially infectious particles away from personnel</td>
</tr>
<tr>
<td>- Refrain from eating, drinking, smoking and applying make up inside the laboratory</td>
</tr>
<tr>
<td>- Wear the relevant personal protective equipment when working inside the laboratory</td>
</tr>
<tr>
<td>- Do not use the same desk for smear making and microscopy work</td>
</tr>
<tr>
<td>- Wash hands with soap and water always after performing any procedure</td>
</tr>
<tr>
<td>- Handle accidental spillages according to the protocols</td>
</tr>
<tr>
<td>- Adhere to proper waste management practices</td>
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</table>

Surgical masks do NOT protect you fully against TB infection as TB bacilli can pass through these masks.

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 mouths while coughing to collect sputum
- 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.

Safety in sputum smear 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.
- 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.
- Always keep a discard receptacle containing disinfectant in the working area.

Chemical Safety

Take the following precautions when working with chemicals in the TB microscopy laboratory:

- 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.
- Phenol is a toxic chemical. Avoid direct contact with the skin or mucus membranes. Reduce exposure to phenolic fumes by staining smears in a well-ventilated area and by limiting the number of slides in each staining batch to a maximum of 12.
Laboratory Register for Accidents (accident book)

This is a document which should be kept by the Laboratory supervisor. The accident book should contain details about laboratory accidents and the measures taken. Each laboratory accident should be reported to the person in charge.

The details to be entered in the book -

- Date of accident
- Name of person concerned
- Description of accident

Both the Laboratory supervisor and the person who faced the accident should sign the statement in the laboratory accident register.

Chemical safety

Chapter 5

Laboratory accidents

Plan of action for a Laboratory accident – Spillage

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, news paper, 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 for at least 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. eg : 5% lysol
Chapter 06

SMEAR MICROSCOPY FOR AFB

Direct smear microscopy for AFB is the primary diagnostic tool for the detection and control of TB.

6.1 Aims of sputum microscopy

6.1.1 The aims of sputum microscopy are to:
• Diagnose patients with infectious tuberculosis
• Monitor progress of tuberculosis patients who are on treatment

6.1.2 Advantages of sputum microscopy
1. Much more reliable diagnostic tool than X-ray for the diagnosis of infectious TB
2. Simple to perform
3. Easy to read
4. Quick results
5. Inexpensive
6. High sensitivity and specificity for detecting infectious cases.
7. Minimal infrastructure is required to set up a microscopy services

6.1.3 Staining for microscopy

Ziehl-Neelsen staining for acid fast bacilli
The method of choice for sputum smear microscopy is the Ziehl-Neelsen technique. In this method, when stained with Carbol Fuchsin the acid-fast organisms appear red against a blue background.

Fluorochrome staining -
For this method a fluorescent dye (Auramine O, Auramine-rhodamine) is used and when examined under a special fluorescent microscope the organisms appear bright yellow against a dark background. The advantage of fluorescent 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 Ziehl Neelsen technique. If the work load is high it may be more cost effective to use fluorescent microscopy. However the main disadvantage is the high initial cost of the fluorescent microscope and the running expenditure and the need for uninterrupted power supply.

![Clump of AFB seen in a sputum smear. (Ziehl-Neelsen stain, examined at x1000.](image1)]
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Value of smear examination for AFB on extra-pulmonary specimens

Because Mycobacterium tuberculosis may infect almost any organ in the body, the laboratory may receive a variety of extra-pulmonary specimens, e.g. body fluids, tissues, pus, and urine. The benefit of microscopy on these specimens is limited because of their paucibacillary nature and due to the presence of mycobacteria other than tubercle bacilli in some of these specimens’ e.g. Urine, gastric aspirates. Therefore extra-pulmonary specimens should always be referred for culture.
6.2 PROCEDURE FOR SPUTUM EXAMINATION

**SPUTUM COLLECTION**
- Registration
- Labelling

**SMEARING**
- Spreading
- Drying
- Fixing

**STAINING**
- Staining
- Washing
- Decolourization
- Washing
- Counter staining
- Washing
- Drying

**MICROSCOPIC EXAMINATION**

**RECORDING**

**REPORTING**
6.2.1 Sputum Collection

**For Diagnosis**

Collection of three samples is recommended.

**Spot-Early morning-Spot**

**For follow up**

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. Minimum of two samples (one early morning sample and one spot specimen) should be examined at the end of intensive phase, at 5-months and at the end of treatment.

### Schedule for follow up sputum examinations

<table>
<thead>
<tr>
<th>Category</th>
<th>When to do</th>
</tr>
</thead>
<tbody>
<tr>
<td>New smear-positive PTB cases</td>
<td>• End of 2nd month (End of 3rd month if smear is positive at 2nd month)</td>
</tr>
<tr>
<td></td>
<td>• End of 5th month</td>
</tr>
<tr>
<td></td>
<td>• End of 6th month</td>
</tr>
<tr>
<td>New smear-negative PTB cases</td>
<td>• End of 2nd month</td>
</tr>
<tr>
<td></td>
<td>• End of 6th month</td>
</tr>
<tr>
<td>Re-treatment cases</td>
<td>• End of 3rd month (End of 4th month if smear is positive at 3rd month)</td>
</tr>
<tr>
<td>- Relapse</td>
<td>• End of 5th month</td>
</tr>
<tr>
<td>- Failures</td>
<td>• End of 8th month</td>
</tr>
<tr>
<td>- Return after default</td>
<td></td>
</tr>
</tbody>
</table>
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</tr>
<tr>
<td>- End of 6th month</td>
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</tr>
</tbody>
</table>

For Diagnosis

Spot - Early morning

Collection of three samples is recommended.

How to collect the sputum sample?

When a new patient is referred to the laboratory for sputum examination, three sputum samples should be collected for examination.

1. Supervised spot specimen at first visit
2. Early morning sample on the next day
3. Supervised spot specimen when he comes with the morning sample.

Place of sputum collection -

The risk of infection is very high when the patient coughs. Therefore sputum is preferably collected in a well-ventilated room with good sun light entering the room, with washing facilities and water drainage into a pit. (cough area) Alternatively the open air can be used and the collection area should be away from other people and as far as possible.

Sputum Containers*

Fig 6.3

A good sputum container should be:
- Wide mouthed
- Leak proof
- Provided with a tight fitting lid (preferably screw-capped)
- Easily disposable by burning
- Clean
- Transparent
- Unbreakable.

6.2.2 REGISTRATION

How to record information before sputum collection?

* Check Laboratory Form for completeness and accuracy (Form (TB 05).

The following data should be checked
- Name of the patient
- Age, and sex
- Address of patient
- Name of referring health unit
- Reason for examination
- Date of collection

If the data is not complete take necessary measures to fill it up.

Enter the data in the TB laboratory Register: Accurate entry of the patient data in the laboratory register is of high priority as this data is used to trace the patients at times.

Register the patient in the TB Laboratory Register (TB 04) and assign a Laboratory Serial Number to the patient. Write the Laboratory Serial Number on the Laboratory Request Form and on the side of the sputum container.

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.

* The containers can be collected from central drug stores at Chest hospital, Welisara.
When a new patient comes for sputum examination:-

For diagnosis - give **one Laboratory Serial Number** for three samples

Follow up at 2nd month- Give a new Laboratory Serial Number.

Subsequent follow up - new Laboratory Serial Number at each visit.

Write the Laboratory Serial Number on the Laboratory Request Form and on the side of the sputum container using a permanent marker and never label on the lid. This is because the lid from one container may be placed on another container causing incorrect labelling of specimens. (Refer Fig 6.3.)

How to obtain a good sputum sample

**Procedure for sputum collection**

- Explain to the patient, the reason for sputum examination.
- Explain how many samples are needed.
- Give the patient the labelled sputum container and explain the importance of not rubbing off the number written on the container.
- Explain the difference between sputum and saliva and the importance of bringing out sputum deep from the lungs.

**Spot Specimen**

Instructing the patient by demonstrating with actual actions is very successful in producing good quality sputum.

**Method**

- Place both hands on the hip.
- Inhale deeply 2–3 times and cough
- Open the container; keep it close to the mouth and,
- Bring the sputum out into it;
- Close the container.

Before the patient leaves the laboratory, visually examine the sputum sample for quality. If the sample is not good, ask the patient to cough again until a good sample is obtained.
When a new patient comes for sputum examination:

- For diagnosis: Give one Laboratory Serial Number for three samples.
- Follow up at 2 months: Give a new Laboratory Serial Number.
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**Fig. 6.4**

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**20**

**Thick sputum coughed out deeply from the lungs**

**Purulent Yellowish mucoid**

**A good sputum sample**

**amount – 3-5 ml**

**Fig. 6.5**

**6.2.3 Preparation and Staining of Sputum Smears**

Assess and record the visual appearance of the sample. Enter the information on the Laboratory Request Form by ticking the appropriate box.

- Make sure the Laboratory Serial Number on the Form matches the Laboratory Serial Number on the container.
- Arrange the specimen containers in serial order.
Steps in the preparation of smears

Step 1 - Labelling the slides

- Select a new clean unscratched slide and write the Laboratory Serial Number and the Sample 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 sample) E.g.325/A (Fig.6.6)

- Break a wooden stick into two pieces with rough ends (Fig 6.7)

- A wire loop may be used. When a wire loop is used, it should be flamed until red-hot and allowed to cool.

- 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 (Fig.6.8)

Step 2 - Smearing

- 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 (Fig. 6.10).

- The size of the smear should be approximately 2 x 1 cm (Fig 6.11).

- The smear should be spread evenly and not too thick nor too thin.

- It should be thin enough to read newsprint through.

- 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. (Fig6.9)

- Place the used wooden sticks in a container with disinfectant (e.g.5%phenol).
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Step 3 – Drying

- Place the smeared slides on the drying rack (Fig 6.12).
- Let the slides dry in the air for about 15 – 30 minutes.
- Do not use the flame for drying.
- 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

- Make sure the slide is completely dry.
- Hold the dry slide using forceps with the smeared side facing upwards (Fig.6.13).
- 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.
- Do not heat the slide for too long or keep it stationary over the flame. It could damage the bacilli.

Step 5 – Staining

- Place the slides in serial order on the staining rack with the smeared side facing upwards (Fig 6.14).
- Leave space between the slides so that they do not touch each other.
- Never stain more than 12 slides at a time.
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Step 5 – Staining

- Heat the slide gently by passing flame underneath the slide, until vapour rises (Fig 6.16).
- 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.
- Include positive and negative controls with each day's reading.
- Pour filtered 1% Carbol Fuchsin to cover the entire surface of the slide (Fig.6.15).
- Tip off excess staining solution (Fig. 6.17).
• Rinse the slides under a gentle stream of running water until all excess stain is washed off. (Fig 6.18).

• Tilt the slides to drain off excess water (Fig.6.19).

Step 6 - Decolourization

• Sputum smears appear red in colour.
• Replace slides on the staining rack.
• Pour 3% acid alcohol onto the slides (Fig 6.20)
• Let it stand for 2 minutes.
• Gently rinse the slides with water to remove the acid alcohol and excess stain. (Fig. 6.21).

• If the slides are still red repeat the process by adding more acid alcohol until the red colour has disappeared, but do not over-decolourize.

• Tilt the slides to drain off the water (Fig. 6.22).

• Pour 0.1% Methylene blue on the slide (Fig. 6.23).

• Allow standing for 1 minute.
• Tilt the slide to drain off the water (Fig 6.25).

• Gently rinse the slide with tap water (Fig 6.24).

• Tilt the slide to drain off the water (Fig 6.25).

• Allow the slide to dry and then examine it under the microscope.

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Staining Procedures should be posted above the staining area
6.2.4 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 on 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.

- Examine at least 100 microscopic fields.

- For an experienced laboratory technician this will take at least 5 minutes.

- Examination must be systematic and standardized. Begin examining the slide at the left end of the smear.

- Systematically examine each field thoroughly beginning at the periphery of the field and ending at the centre.

- 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.

- Move the slide a small distance vertically so that a second length can be read from right to left.
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.

Count the number of AFB seen per field and record the results in the Laboratory Request Form (Annex 2).

### 6.2.4.2 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>
<thead>
<tr>
<th>Number of bacilli seen</th>
<th>Result</th>
<th>Grading</th>
<th>No. of fields to be examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AFB seen per 100 fields</td>
<td>Negative</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>1-9 AFB per 100 fields</td>
<td>Positive (Scanty)</td>
<td>Record the exact No. Seen</td>
<td>100</td>
</tr>
<tr>
<td>10-99 AFB per 100 fields</td>
<td>Positive</td>
<td>1 +</td>
<td>100</td>
</tr>
<tr>
<td>1-10 AFB per field</td>
<td>Positive</td>
<td>2 +</td>
<td>50</td>
</tr>
<tr>
<td>More than 10 AFB per field</td>
<td>Positive</td>
<td>3 +</td>
<td>20</td>
</tr>
</tbody>
</table>

After completing the examination, remove the slide carefully from the microscope stage.
Clean the slides to remove the immersion oil.
Before examining the next slide, wipe the immersion 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.
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.

6.2.5 RECORDING AND REPORTING OF RESULTS

6.2.5.1 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 District Chest Clinic with the report.

6.2.5.1 Recording the Results

Recording in TB Laboratory Register (TB 04 Annex 01)

- 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 Number**
  - Date received
  - Patient’s name, sex, age, Address
  - Name of health institution
  - Reason for examination (for diagnosis or follow up)
  - District TB No. of follow up cases

- 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, fibres, and pollen
- Saprophytic acid-fast bacilli
- Scratch marks on the slides
  Precipitated stains (using unfiltered Carbol fuchsin)
- Inadequate decolourization
- 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
- Labelling the sputum containers and slides with errors

Causes of false negative results

- Poor sputum sample - 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-decolourization
- Not examining at least 100 microscopic fields
- Errors in labelling 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 positives results

- Presence of food particles, fibres, and pollen
- Saprophytic acid-fast bacilli
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- Errors in labelling sputum containers, slides and Laboratory Request Forms
- Errors in recording and reporting.

6.3 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 labelled and stored appropriately after preparation.

Maintain proper records with regard to stain preparation. (Refer Lab Documents)

Always Quality Control the batch of stains 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.

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.

Preparation of Reagents

<table>
<thead>
<tr>
<th>1% Carbol Fuchsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic Fuchsin</td>
</tr>
<tr>
<td>Absolute alcohol</td>
</tr>
<tr>
<td>Phenol</td>
</tr>
<tr>
<td>Distilled water</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1% CARBOL FUCHSIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ml</td>
</tr>
<tr>
<td>Prepared: 2009 – 01 – 01</td>
</tr>
<tr>
<td>Date of Exp: 2009 – 06 – 30</td>
</tr>
</tbody>
</table>
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.

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.

### 3% Acid alcohol solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol 95%</td>
<td>970 ml</td>
</tr>
<tr>
<td>Concentrated hydrochloric acid</td>
<td>30 ml</td>
</tr>
</tbody>
</table>

3% ACID ALCOHOL

100ml
Prepared: 2009 - 01 - 01
Date of Exp: 2009 - 06 - 30

Carefully add concentrated hydrochloric acid to 95% alcohol. Always add acid slowly to alcohol and not vice versa.

Store in an amber coloured bottle. Label the bottle with the name of the reagent and dates of preparation and expiry.

### 0.1% Methylene blue

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene blue</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>500 ml</td>
</tr>
</tbody>
</table>

0.1% METHYLENE BLUE

100ml
Prepared: 2009-01-01
Date of Exp: 2009-06-30

Dissolve the Methylene blue in distilled water.

Store in an amber coloured bottle. Label the bottle with the name of the reagent and dates of preparation and expiry.
6.4 The Microscope
6.4.1 Parts of a binocular microscope

The main parts of a microscope are:

- Eye- pieces
- Microscope tube
- Nose piece
- Objective
- Mechanical stage
- Condenser
- Coarse and fine focusing knobs
- Light source

Fig. 6.27  Binocular microscope

6.4.2 Care and maintenance of the microscope

Proper handling and maintenance of the microscope is very important.

The following points should be observed.

- Handle the microscope with care.
  - 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.
- Also 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 re-used.

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 everyday, 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.

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 airbrush 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.
### 6.4.3 Common Problems with microscope.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible causes</th>
<th>Solution</th>
</tr>
</thead>
</table>
| 1. Field dim | - Condenser may be too low  
- Condenser iris may be closed | Raise the condenser  
Open the diaphragm |
| 2. Dark shadows in the field which move when eye piece is moved | - Eyepiece 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 |
| 3. 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 x100 lens from side to side  
Clean the lens.  
Use only good quality immersion oil. |
| 4. 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  
Clean the lens  
New lens will be needed |
QUALITY ASSURANCE OF SPUTUM SMEAR MICROSCOPY

Sputum smear microscopy is the cornerstone for both, the diagnosis and follow up of tuberculosis 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
- External Quality Assessment
- Quality improvement

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

Internal Quality Control
Internal Quality control 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 tuberculosis 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
External quality assessment 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.

7.1 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
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7.1 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:-

**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.

**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.

**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.

**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 is used first.

**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.
7.2 EXTERNAL QUALITY ASSESSMENT (EQA)

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

- EQA neither identify individual slide errors nor validate 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
- RANDOM BLINDED RE-CHECKING (RBRC)
- ON SITE EVALUATION
- PROFICIENCY TESTING or PANEL TESTING (PT)

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
1) No of slides performed per year by each laboratory.
2) Number of +ves detected by the laboratory
3) Slide positivity rate

Slide Positivity Rate
SPR = \( \frac{\text{Number of positive smears per year}}{\text{Annual slide volume}} \times 100 \)

7.2.1 RANDOM BLINDED RE-CHECKING (RBRC)
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 DCCLs and to the microscopy centers by the NTRL at the beginning of each year),

- 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. (eg: is shown below.)
A table for determining Annual Sample Size
(80% sensitivity, 100% Specificity and '0' Acceptance number, CI= 95%)

<table>
<thead>
<tr>
<th>Number of negative slides per year</th>
<th>% Slide positivity rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>200 - 350</td>
<td>107 (9)</td>
</tr>
<tr>
<td>351 - 750</td>
<td>154 (13)</td>
</tr>
<tr>
<td>751 - 3000</td>
<td>180 (15)</td>
</tr>
<tr>
<td>3001 - 5000</td>
<td>208 (18)</td>
</tr>
<tr>
<td>5000- 50000</td>
<td>216 (18)</td>
</tr>
</tbody>
</table>

* Monthly sample size is in(n)

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

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 District chest clinic 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.

Example: 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.
Method of selection of slides for Random Blinded Rechecking from TB Laboratory Register

<table>
<thead>
<tr>
<th>Lab Serial No</th>
<th>Date</th>
<th>Name</th>
<th>Sex (M/F)</th>
<th>Address</th>
<th>Name of Treatment Unit</th>
<th>Reason for Examination</th>
<th>Result of specimen</th>
<th>Signature</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Blinded Rechecking Process:**
Selected slides are sent to the NTRL / DCCL 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.
Feedback

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

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

Classification of Errors of RBRC

<table>
<thead>
<tr>
<th>Result of Peripheral Laboratory</th>
<th>Result of controller</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Negative</td>
<td>Correct</td>
</tr>
<tr>
<td>1-9 AFB/100 fields</td>
<td>LFP</td>
</tr>
<tr>
<td>1+</td>
<td>HFP</td>
</tr>
<tr>
<td>2+</td>
<td>HFP</td>
</tr>
<tr>
<td>3+</td>
<td>HFP</td>
</tr>
</tbody>
</table>

- 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

7.2.2 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.

7.2.3 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 once a quarter.
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 eg: disposal of contaminated material.
- Practice of satisfactory turn around time in reporting
- Adherence to correct procedures in sputum collection, smearing and staining.
- Practice of internal QC
- Record keeping and documentation as per requirements of the NPTCCD 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 District Chest Clinic 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 settings.

*Quarterly report on supervisory visits to microscopy centers should be sent to the NTRL by the District Chest Clinic Laboratories.(Annex 15)
Chapter LABORATORY DOCUMENTS

Keeping proper laboratory documents is of paramount importance to maintain the laboratory standards and for the quality improvement of the laboratories. Documents related to internal quality control and external quality assessment should be available in the laboratories for inspection during the onsite evaluations. Lack of documentation is a major drawback to implement quality improvement activities in the laboratory settings.

Some of the important documents to be maintained in a laboratory performing sputum microscopy is discussed in this chapter. Documents related to INTERNAL QUALITY Administration:
- Staffing-
  - Current staff details with qualifications, designations and service experience
  - Current Training Record File (should contain the current training record for each Staff Member.)
  
  These files should be updated on a regular basis.

Laboratory Arrangement:
- Standard operating Procedures (SOP)
  - SOPs, manuals and posters must be located in files with easy access for all staff and displayed in the appropriate place in the laboratory when necessary.
  
  Eg:
  - Smearing & Staining procedure
  - Grading chart
  - Preparation and Internal Quality Control of reagents
  - Use and cleaning of the microscope
  - Cleaning the worksite and disposal of waste

Equipment
- Equipment Maintenance and Service Records
  - These should be available for each piece of equipment (Eg: Microscopes, Refrigerators, Balances, Bio-Safety Cabinets, Centrifuges, Incubators, Water Baths and Autoclaves)
  - In the file for each piece of equipment,
    - Date of receiving,
    - supplier name,
    - serial number,
    - model/make,
    - Periodic Efficiency Checks and Calibration,
    - Copies of the Letters sent to the Professional Servicing Company for Periodic Efficiency Checks and Calibration,
    - Service Records (eg: details of due date of service, Date of actual service done, Due date for next service)
  
  should be indicated.
Keeping proper laboratory documents is of paramount importance to maintain the laboratory standards and for the quality improvement of the laboratories. Documents related to internal quality control and external quality assessment should be available in the laboratories for inspection during the onsite evaluations. Lack of documentation is a major drawback to implement quality improvement activities in the laboratory settings.

Some of the important documents to be maintained in a laboratory performing sputum microscopy is discussed in this chapter.

**Documents related to INTERNAL QUALITY**

**Administration : Staffing**
- Current staff details with qualifications, designations and service experience
- Current Training Record File (should contain the current training record for each Staff Member.)
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**Laboratory Arrangement:**

**Standard operating Procedures (SOP)**
SOP's, manuals and posters must be located in files with easy access for all staff and displayed in the appropriate place in the laboratory when necessary.

Eg:
- Smearing & Staining procedure
- Grading chart
- Preparation and Internal Quality Control of reagents
- Use and cleaning of the microscope
- Cleaning the worksite and disposal of waste

**Equipment Maintenance and Service Records**

These should be available for each piece of equipment (Eg: Microscopes, Refrigerators, Balances, Bio-Safety Cabinets, Centrifuges, Incubators, Water Baths and Autoclaves)

In the file for each piece of equipment,
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- Copies of the Letters sent to the Professional Servicing Company for Periodic Efficiency Checks and Calibration,
- Service Records (eg: details of due date of service, Date of actual service done, Due date for next service) should be indicated
Daily Working Log Books

For each piece of equipment log books should be maintained to keep records.

Supplies – stock keeping

The Format of the Stock Book to be maintained is given in Annex12. Stock books should be updated regularly. Correct stock keeping is essential for correct estimations of the laboratory requirements. When the laboratory estimates are done, copies of them should be filed separately for the district chest clinic and the microscopy centres.

Laboratory Register

The register should be located in the laboratory work area at all times and stored in a secure location.

- Results should be written directly into the register rather than transcribed from a worksheet.
- Lab register should be complete. The register should be legible and up-to-date.
- All positive results should be entered in Red.
- 3 specimens per new patient should be examined.
- A Summary report (including statistics on workload and results) should be prepared in the TB Laboratory Register every month.
- All records should be retained for two years.

Data collection Files

- This should have the Monthly Summaries filed separately for the DCC Laboratory and for the Microscopy Centres.

The slide positivity charts

- The Slide Positivity Rate should be plotted in a graph against the months of the year for the DCC Laboratory and for the Microscopy Centres in separate Charts to see whether a marked difference is there.

Laboratory Safety

Laboratory accident book

This book should be kept by the Laboratory Safety Officer (Laboratory supervisor, Laboratory Senior Officer) and should contain details about laboratory accidents and the remedial measures taken. Each laboratory accident should be reported to the person in charge and all details entered in the book-

- Date of accident
- Name of person concerned
- Description of accident
- Laboratory number of specimen / strain involved.
- Extent of injury
- Containment and follow up measures taken

Waste Disposal – Sites of Deep Burial Pit - Map

If deep burial is used to dispose the used slides the places used /the places for future use should be mapped. A map of pits should be available at the laboratory indicating the
locations for deep burial. The places for deep burial should be identified minimizing waste of earth.

**Microscopy**

Maintenance of Internal Quality Control Registers for

**Staining QC (Staining procedure)**
The Staining procedure is checked with a known positive slide (preferably 1+) and a known negative slide daily, and results entered in the register.

**Stain QC (Stain preparation)**
Performance of reagents are checked with a known positive slide (preferably 1+) and a known negative slide when stains are prepared or prepared stains are received, and results entered in the register.

**Documents related to EXTERNAL QUALITY ASSESSMENT**

**Feedback Reports of Random Blinded Rechecking**
A file for Feedback Reports sent from the Reference Laboratory should be kept in each District chest clinic.

A file for each Microscopy centre with copies of the Feedback Reports generated by the District Chest Clinic Laboratory should also be kept separately.

**EQI Document File**
This should include

- Instructions received from the Reference Laboratory for Random Blinded Rechecking of Slides of District Chest Clinic Laboratories

**Quarterly reports** -
Copies of Quarterly Feedback Report of Random Blinded Rechecking of Slides of Microscopy Centres sent to the National Tuberculosis Reference Laboratory should be kept in a separate file.

**On-site Evaluation (Supervisory Visits)**
A file for Reports sent from the Reference Laboratory

A file for each Microscopy centre with copies of the On-site Evaluation Reports sent to the Microscopy Centres

A file for Copies of Quarterly Feedback Reports of On-site Evaluation of Microscopy Centres sent to the National Tuberculosis Reference Laboratory

**Panel Testing (Proficiency Testing)**
A file for Feedback Reports sent from the Reference Laboratory

A file for each Microscopy centre with copies of the Feedback Reports generated by the Laboratory
TB culture is highly recommended for bacterial diagnosis of TB and it is considered the gold standard of diagnosis. Due to the limited facilities available for culture, routine cultures are performed only according to the National policy.

National policy

- Pre-treatment cultures in Category I patients who have a high risk of drug resistance. E.g. HIV positives, contacts of known drug resistant TB patients, prisoners, health care workers, drug addicts.
- Pre-treatment cultures in all Category II patients.
- Pre-treatment cultures in sputum negative PTB patients.
- Patients whose sputum remains positive after three months of anti-TB treatment.

Expansion of TB culture services has been given high priority by NPTCCD as to provide this diagnostic service at easy access to the patients. Accordingly, few culture laboratories are set up to cover all the provinces. These laboratories are expected to perform TB cultures for the relevant provinces and send the culture isolates to the National reference Laboratory for confirmation and DST. Similar to sputum microscopy services, these laboratories too work in network fashion with the technical guidance of the NTRL of NPTCCD.

- MDR suspects – It is recommended to culture 2 specimens for diagnosis on two consecutive days.
- MDR Patients – Monitoring will be done by one sputum culture (one sample) and sputum smears (2 samples).

In the Intensive Phase, Sputum Smears and Cultures should be done monthly, starting from the 2nd month of treatment. (All samples should be sent at least 30 days apart.)

In the Continuation Phase, Sputum Smears and Cultures should be done at 2-month intervals.

Changing over from Intensive to Continuation Phase is done after Sputum conversion. Conversion is defined as two consecutive negative cultures, sent 30 days apart.
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**Changing over from Intensive to Continuation Phase is done after Sputum conversion. Conversion is defined as two consecutive negative cultures, sent 30 days apart.**
Advantages of Culture

- detects fewer bacilli (even 10) and increase the number of case detection
- provides definitive diagnosis;
- allow identify the organism,
- Provide material for DST

Disadvantages:

- Expensive
- *M tuberculosis* proliferates extremely slowly (generation time is 18-24 hours). Therefore results take a long time (6-8 weeks)in conventional cultures
- Needs high degree of training
- Needs special laboratory facilities

SAMPLE COLLECTION, STORAGE, DISPATCH FOR TB CULTURE

**Sputum**

Container - wide mouth

Screw capped

Leak proof

Sterile

Transparent

using sterile container issued by NTRL (central laboratory) is preferred

Sterile containers should be used within one month. Containers, which have not been used within one month from the date of sterilisation, should be returned to the NTRL.

When collecting sputum

- Explain to the patient, the reason for sputum culture
- Early morning sample is preferred rather than a spot sample

Refer Chapter 6.2 - How to obtain a good sputum sample

A good sputum sample should be

- thick,
- purulent (yellowish mucoid and not saliva) and,
- of sufficient quantity (3-5 ml)
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- provides definitive diagnosis
- allows the identification of the organism
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**SAMPLE COLLECTION, STORAGE, DISPATCH FOR TB CULTURE**

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- Container - wide mouth, screw capped, leak proof, sterile, transparent
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- of sufficient quantity (3-5 ml)

**Label the bottle correctly as follows**

<table>
<thead>
<tr>
<th>TB CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital/Chest clinic ............................................</td>
</tr>
<tr>
<td>Name ..............................................................</td>
</tr>
<tr>
<td>Age .............................................................. WD</td>
</tr>
<tr>
<td>BHT/TB Reg. No .......................................................</td>
</tr>
<tr>
<td>Specimen ............................................................</td>
</tr>
<tr>
<td>Date of collection ...................................................</td>
</tr>
</tbody>
</table>

Specimen should be transported to the NTRL as soon as possible after collection. If this is not possible, specimens should be stored in a refrigerator at 4 °C, until it is dispatched to the NTRL. Culture yield is reduced when specimens are kept too long before processed. Therefore, it is advised to send the cultures to the laboratory within a maximum of 3 days. (If there is a delay in transporting the specimens 1% cetyl pyridinium chloride (CPC) in 2% NaCl, can be added to the specimens. If this is added the samples should not be refrigerated.)

Ideally, the specimens should be transported as follows.

Closed Specimen bottle should be put into a Zip-lock polythene/plastic bag and need to be put into a second container. In-between should be absorbent material. The second container should be placed in a third container along with the Request forms (3-layer packing). During transport, the specimens must be kept as cool as possible and protected from sunlight.

If dispatch is by mail, label the transport boxes correctly, mark the upside by arrows, label properly and sent to the NTRL. (One should adhere to the local postal regulations too)

Indicate the Address on the package as below

<table>
<thead>
<tr>
<th>NATIONAL TB REFERENCE LABORATORY</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.O. BOX 13,</td>
</tr>
<tr>
<td>WELISARA</td>
</tr>
</tbody>
</table>

If the specimens are hand delivered
- Request forms (TB 06) can be sent separately from the specimens.
- Send the laboratory Dispatch Book separately from the specimens.

**EXTRAPULMONARY SPECIMENS**

- **Body fluids:**
  - CSF
  - Pleural fluid
  - Aspirates – knee joint, FNA etc..
  - Gastric washing
    (preferably transport within 4 hours)
  - Bronchial washing
- **Biopsies:** Biopsy samples should be sent in sterile normal saline (not in formal saline or formalin)
  - immediately transport to the NTRL after resection.
- **urine:** 3 consecutive Early morning samples
  - Volume – 40 ml each
TB CULTURE
Preparation of the Lowenstein –Jensen (LJ) culture medium

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium dihydrogen phosphate anhydrous (KH2PO4)</td>
<td>2.40 g</td>
</tr>
<tr>
<td>Magnesium sulphate –anhydrous</td>
<td>0.24 g</td>
</tr>
<tr>
<td>Magnesium citrate</td>
<td>0.6 g</td>
</tr>
<tr>
<td>Asparagine</td>
<td>3.6 g</td>
</tr>
<tr>
<td>Glycerol (reagent grade)</td>
<td>12.0 ml</td>
</tr>
</tbody>
</table>

**Autoclave 121 ° C / 30 minutes Cool**

**+**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malachite green solution</td>
<td>20 ml</td>
</tr>
<tr>
<td>(2% aqueous solution freshly prepared)</td>
<td></td>
</tr>
</tbody>
</table>

**+**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenized whole eggs</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

**MIX WELL**

Dispense 6 – 8 ml to each sterile universal container

**Inspissation in slant position at 85 °C , 50 minutes**

**Sterility test at 37 °C , 48 Hours**

**Store in cool condition**

Chapter 9

54
Precautions during media preparation

Directions for preparing media must be followed precisely and without modification. Following points are important to obtain good quality media and avoid contamination.

- Keep the environment as clean as possible. Swab the work surface with a suitable disinfectant (eg. 70% alcohol, 5% phenol). Clean the floor with a wet mop to limit dust.
- Use clean, sterile glassware and equipment
- Use reagent grade chemicals and reagents
- Use freshly prepared distilled water
- Check the temperature of inspissators (85°C) and hot air ovens (160°C)
- Follow strict aseptic techniques when preparing media, e.g. flaming flasks and tubes
- When preparing egg-based media, carefully clean egg shells before breaking
- Do not overheat during inspissation
- Do not leave prepared media exposed to light, but store in the refrigerator in the dark when not in use.

Sputum culture procedure

To x ml of sputum add x ml of 4% NaOH (Equal volume of NaOH)

Tighten the cap of container (Universal container) & shake to digest

Shake for 15 minutes at room temperature

Centrifuge at 3000 g for 15 minutes

Pour off supernatant

Add distilled water up to neck and resuspend the sediment

Centrifuge at 3000 g for 15 minutes

Decant the supernatant in to discard bottle and inoculate the sediment on to 2 L-J medium
Inoculation and incubation

- The deposit is inoculated on to two slopes of Lowenstein- Jensen Medium. Each slope is inoculated with the centrifuged specimen with 5mm wire loop.
- The slopes are then incubated at 37˚ C for up to 08 weeks.
- The cultures are examined weekly up to 08 weeks for any growth. If there is no growth, the slopes are discarded as negative.
- Typical colonies of Mycobacterium tuberculosis appear as dry, rough, irregular heaped up colonies, which are buff or off-white in colour.

Culture examination

All cultures should be examined 48-72 hours after inoculation, to detect gross contaminants. Thereafter cultures are examined weekly, up to 8 weeks on a specified day of the week. It is useful to label containers with cultures with the dates of inoculation and to place containers in the incubator in chronological order. 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.

Reading of cultures

Typical colonies of M. tuberculosis are rough, crumbly, waxy, non-pigmented (buff coloured) and slow- growers, i.e., only appearing two to three weeks after inoculation. With doubtful cultures, the acid-fastness should be confirmed by Zeil-Nelsen (ZN) staining.

Culture reports should be qualitative (i.e., positive or negative) as well as quantitative (i.e., number of colonies isolated.) The following scheme is recommended:

<table>
<thead>
<tr>
<th>Reading</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>No growth</td>
<td>Negative</td>
</tr>
<tr>
<td>1-19 colonies</td>
<td>Positive (number of colonies)</td>
</tr>
<tr>
<td>20-100 colonies</td>
<td>Positive (1+)</td>
</tr>
<tr>
<td>&gt;100 discreet colonies</td>
<td>Positive (2+)</td>
</tr>
<tr>
<td>Confluent growth</td>
<td>Positive (3+)</td>
</tr>
<tr>
<td>Contaminated</td>
<td>Contaminated</td>
</tr>
</tbody>
</table>

Drug susceptibility Tests (DST)

There are three general methods used for determining drug susceptibility of mycobacteria:

- The absolute concentration method (MIC method)
- The resistance ratio method
- The proportion method

The method used in Sri-Lanka is the proportion method

The Proportion method enables precise estimation of the proportion of mutants resistant to a given drug. Several 10-fold dilutions of inoculum are planted on to both control and drug-containing media. At least one dilution should yield isolated countable (50-100) colonies. When these numbers are corrected by multiplying by the dilution of inoculum used, the total number of viable colonies observed on the control medium, and the number of mutant colonies resistant to the drug concentrations tested may be determined. The proportion of bacilli resistant to a given drug is then determined by expressing the resistant portion as a percentage of the total population tested.
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<table>
<thead>
<tr>
<th>Reading Report</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>No growth</td>
<td>Inoculation samples, or no growth at all</td>
</tr>
<tr>
<td>1-19 colonies</td>
<td>Contaminated cultures</td>
</tr>
<tr>
<td>20-100 colonies</td>
<td>Contaminated cultures</td>
</tr>
<tr>
<td>&gt;100 discreet colonies</td>
<td>Positive (1+) cultures</td>
</tr>
<tr>
<td>Confluent growth</td>
<td>Positive (2+) cultures</td>
</tr>
<tr>
<td>Contaminated</td>
<td>Positive (3+) cultures</td>
</tr>
</tbody>
</table>

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Chapter 9
56

LABORATORY WASTE DISPOSAL

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

1. Segregation of waste- National colour code

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

2. 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.

2.1. Infectious Waste

sputum cups

- Incineration is recommended. In the absence of incineration facilities, making it noninfectious 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 noninfectious, adding a disinfectant {e.g. 5% Lysol (a Phenolic compound) or few drops of formaldehyde} to the specimen containers can be practised.)
Universal Bottles

- Used glass sputum containers can be recycled after autoclaving at 121°C and thorough washing.

Applicator Sticks

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

Infectious waste should be collected into Yellow polythene bags (preferably biodegradable) kept inside the yellow bins with lid, and international biohazard sign on it.

Bins with foot-operated lid are preferred.

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.

Laboratory Sharps

Definition of 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 Sharps bins

- Puncture resistant
- Cardboard which is incinerable
- leak proof
- Yellow bin with a red stripe on it
- Clearly marked with a biohazard 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
Universal Bottles

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- Within easy reach of the work station

**Chapter 10**

2.2 General waste - collect to

• Black polythene bags (preferably biodegradable) kept inside the black bins

2.3 Biodegradable waste - collect to

• Green polythene bags (preferably biodegradable) kept inside the green bins

3. Handing over of Waste to Waste collecting Porter

Waste Bags

When the bag is approximately two thirds full, exchange it for an empty bag.

Seal the bag as follows:

• gather the top of the bag;
• fold the neck of the bag over;
• Tie the neck by forming a loop and passing the end through the loop, creating a knot; or tie the neck by forming a “swan neck”, twisting the top of the bag and sealing it with a cable-tie fastening to form a watertight seal;
• 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.

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 should be direct from the point of collection to the waste storage area or to the disposal area depending on the circumstances.

4. Waste Storage

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

5. **Waste disposal**

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.

The best method of disposal of infectious waste is high temperature incinerator. (800-1200 °C) In the absence of that drum incineration can be used as an alternate.

Drum Incineration.
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)

Design for Deep Burial Pits

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.
1. Priorities for Tuberculosis Bacteriology Services in Low-Income Countries; Second Edition 2007; IUATLD
2. Tuberculosis laboratory assessment tool (3rd draft); world health organization
3. Manual for laboratory technicians; smear microscopy for detection of acid-fast bacilli; revised national tuberculosis control programme India
4. Washington state tuberculosis services manual; updated 04/30/2009
5. TB CAP Focus; Laboratory Tools; Issue 1 | March 2009
References

1. Priorities for Tuberculosis Bacteriology Services in Low-Income Countries; Second Edition 2007; IUATLD

2. Tuberculosis laboratory assessment tool (3rd draft); world health organization

3. Manual for laboratory technicians; smear microscopy for detection of acid-fast bacilli; revised national tuberculosis control programme India

4. Washington state tuberculosis services manual; updated 04/30/2009

5. TB CAP Focus; Laboratory Tools; Issue 1 | March 2009


ANNEXURES
# Tuberculosis Laboratory Register

<table>
<thead>
<tr>
<th>Lab Serial No.</th>
<th>Date</th>
<th>Std Card No./BHT No./District TB No.</th>
<th>Name in Full</th>
<th>Sex (M/F)</th>
<th>Age</th>
<th>Name of Treatment Unit</th>
<th>Address (For New Patients)</th>
<th>Reason for Examination</th>
<th>Results of Examination</th>
<th>Signature</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>
THE LABORATORY FORM
REQUEST FOR SPUTUM EXAMINATION

Name of Treatment Unit: ............................................................ Date: ....................

Name of Patient: ................................................................. Age: ........... Sex M □ F □

Address in Full: .................................................................

Disease Classification:
[ ] Pulmonary District: .........................
[ ] 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:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Mucopurulent</th>
<th>Blood stained</th>
<th>Saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>2</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>3</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

b) Microscopy:

<table>
<thead>
<tr>
<th>Date</th>
<th>Specimen</th>
<th>Results*</th>
<th>POSITIVE (grading)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>+++</td>
</tr>
</tbody>
</table>

*Write negative or positive.

Examined by (Signature): ..........................................................

The completed form (with results) should be sent to the treatment unit to record the results on the treatment card.
REQUEST FORM
TB CULTURE & DRUG SUSCEPTIBILITY TESTING
National TB Reference Laboratory, Welisara
(Central Laboratory of NPTCCD)

Name of the Patient (in block letters)

Age: __________ yrs. Sex: M [ ] F [ ]

Patients address: ........................................................................................................
Residential district: _______________

---

1. HISTORY

1. Relevant clinical details .................................................................
.................................................................

2. Probable diagnosis

PTB Y [ ] N [ ]
EPTB Y [ ] N [ ]

If PTB Sputum smear Positive [ ] Negative [ ]

3. P/H of TB

Yes [ ] No [ ] not known [ ]

4. Diagnosed case of MDR TB

Yes [ ] No [ ] not known [ ]

5. Anti TB treatment (ATT) given

Past Y [ ] N [ ] Present Y [ ]
( on treatment) N [ ]

6. Category of patient

CAT 1 [ ] CAT 2 [ ]

NEW
Never exposed to anti TB Rx
ATT started within one month

Others
(specify)

---

7. If on 2nd line drugs specify: .................................................................
......................................................................

LAB USE ONLY
Date of Receipt LAB NO
CULTURE DST

Name of the Institution WD/ Clinic
BHT/Clinic No/Standard Card No.
District TB NO Report should be forwarded to

---

P.T.O.
2. **REASON FOR CULTURE**

1. For diagnosis  │ Y □  │ N □  
   Pre treatment - Y  │  │  
   On treatment - Y  │  │  
   Others (specify)  │  │  

2. For follow up  │ Y □  │ N □  
   If yes, Date of starting anti TB therapy  

3. **PREVIOUS CULTURES DONE**

<table>
<thead>
<tr>
<th>LAB SERIAL NO</th>
<th>ABST NO</th>
<th>MDR NO</th>
<th>YEAR</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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</tr>
</tbody>
</table>

Signature of Medical Officer: ............................................
Name: .............................................................................
Designation: HO/ MO/DTCO/SHO/REG/SR/VP/VS/..........................................
..............................................................................

---

**Laboratory use only**

Lab serial No. : .................................

Smear
- Positive  □  
- Negative  □

Culture
- Positive  □  
- Contaminated  □
- Other  □
- Negative  □

Identification:
- MTB  □
- Atypical  □
- Other  □
- Specify:  ....................

**Results of Sensitivity Test**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifampicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethambutol</td>
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MLT /NTRL  Consultant Microbiologist/NTRL

Date:...............  Date: ......................
National TB reference Laboratory
(Central Laboratory of NPTCCD)
Welisara

TB Culture Report

To: Lab Serial No:

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Name of Patient

Age: Sex:

District TB No / BHT No / Clinic No

Ward:

Specimen:

Culture Results:

Culture Method:

Culture Results:

.......................................................... ..........................................................

MLT Consultant Microbiologist

Date:
National TB Reference Laboratory  
(Central Laboratory of NPTCCD)  
Welisara

**TB Culture and DST Final Report**

To:  

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Name:  

Age:  

Sex:  

Address:  
BHT No:/ Clinic No :  

District:  
Ward No :  

Specimen:  

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**Culture Results:**

Culture Method:  
Culture Results:  

**Culture Identification:**

Culture Identified as  

---

**Antibiotic Sensitivity**

Streptomycin:  
Isoniazid:  
Rifampicin:  
Ethambutol:  

Comments  

---

MLT  
Consultant Microbiologist  
Date:
Monthly summaries to be sent to NTRL at the end of each month with RBRC's 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.

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<th>PATIENTS EXAMINED</th>
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| Slide Positivity Rate = \[
\frac{\text{No. of Positive Slides}}{\text{Total No. of Slides}} \times 100\%
\]

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.

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| Slide Positivity Rate = \[
\frac{\text{No. of Positive Slides}}{\text{Total No. of Slides}} \times 100\%
\]

Prepared by National Tuberculosis Reference Laboratory, Welisara
### SMEAR RESULTS SHEET FOR BLINDED RECHECKING

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Slides selected by: DTCO/ Medical Officer / Supervising Laboratory Technician

Name of Laboratory Technician of the Microscopy Centre: 

Signature: ............................................... Date: .................................

Prepared by National Tuberculosis Reference Laboratory, Welisara
# Slide Distribution Book For RBRC

**Year:** ............

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*Prepared by National Tuberculosis Reference Laboratory, Welisara*
### RANDOM BLINDED RECHECKING OF SMEAR EXAMINATION

(FORM A)

Microscopy centre/District Chest Clinic Laboratory:  
District:  
Month:  
Year:  
No. of Positives:  
No. of Negatives:

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Percentage of Poor Slides

**Problems/comments:**

Name of Laboratory Technician:............................

Signature:.................................................  
Date: .............................................
Quality Assurance Report on Sputum Microscopy

District Chest Clinic……………………
Microscopy Centre:……………………

Month:………………..  
Year :………………..

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Summary of errors identified (nos.)

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Total Major Errors:  
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Comments:

Reporting Date……………………………..  
LT/DCC
Sample Receipt Date: ……………………..  
DTCO

Prepared by National Tuberculosis Reference Laboratory, Welisara
Quarterly Report – Random Blinded Rechecking of Slides

Smear Microscopy of Microscopy Centres

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<td>9.</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>10.</td>
<td></td>
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</tr>
<tr>
<td>11.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 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.

Prepared by National Tuberculosis Reference Laboratory, Welisara
# STOCK BOOK

**Item Name:** ________________

<table>
<thead>
<tr>
<th>Date</th>
<th>Quantity Received</th>
<th>Quantity Issued to DCC</th>
<th>Quantity Issued to Microscopy Centres</th>
<th>Balance Stock in Hand</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16</td>
<td></td>
</tr>
</tbody>
</table>
# Laboratory Requirements

**District:** ___________________

**Chest Clinic**
- Total No. of Smears done in the Previous Quarter (A) =
- No. of Laboratory Technicians (B) =

**Microscopy Centres**
- Total No. of Smears done in the Previous Quarter (C) =
- No. of Laboratory Technicians (D) =

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

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount in hand (stock)</th>
<th>If you need more, mention the further requirement for the year</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chest Clinic</td>
<td>Microscopy Centres</td>
<td>Chest Clinic</td>
</tr>
<tr>
<td>Microscopes</td>
<td>(M)</td>
<td>(N)</td>
<td></td>
</tr>
<tr>
<td>Diamond Pencils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spirit Lamps</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slide Tray</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slide Rack</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staining Rack</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slide Storage Boxes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slide Mailers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drop Bottles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash Bottles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slide Forceps</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory Overcoats</td>
<td></td>
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</table>

Prepared by National Tuberculosis Reference Laboratory, Welisara
**SUPERVISORY VISITS TO MICROSCOPY CENTERS**  
**BY DISTRICT CHEST CLINICS**

**Checklist**

### 1. General Information

<table>
<thead>
<tr>
<th>Microscopy centre</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>District</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of MLT/PHLT</th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>Names &amp; Designation of current staff</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>--------------------------------------</td>
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<td>--------------------------------------</td>
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<table>
<thead>
<tr>
<th>Date of Visit</th>
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</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Names &amp; Designation of Visiting team</th>
<th></th>
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<tr>
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<td>--------------------------------------</td>
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<td>--------------------------------------</td>
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</table>

### 2. Action implemented as per previous visit:

<p>| | |</p>
<table>
<thead>
<tr>
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<td></td>
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</tbody>
</table>

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*contd.*
3. Current visit particulars –

<table>
<thead>
<tr>
<th>No.</th>
<th>Item</th>
<th>Adequate / Acceptable</th>
<th>Problems identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><strong>Infrastructure:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1.</td>
<td>Separate Area for TB Laboratory Work</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>1.2.</td>
<td>Separate Bench/Tables for Specimen Receipt/Smear Preparation/Microscopy</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>1.3.</td>
<td>Uninterrupted Power Supply</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>1.4.</td>
<td>Running Water Supply</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td><strong>Availability of Standard Operating Procedure:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1.</td>
<td>Staining Procedure</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>2.2.</td>
<td>Grading Chart</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>2.3.</td>
<td>QC of Reagents &amp; Staining Procedure</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>2.4.</td>
<td>Use and Cleaning of the Microscope</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>2.5.</td>
<td>Cleaning Worksite and Disposal of Waste</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>2.6.</td>
<td>EQA Protocol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td><strong>Availability of Items (Adequate Stocks &amp; Supply):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1.</td>
<td><strong>Consumables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1.1.</td>
<td>Slides</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3.1.2.</td>
<td>Slides re–used</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3.1.3.</td>
<td>Sputum cups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1.4.</td>
<td>Lens tissue</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3.1.5.</td>
<td>Immersion oil</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3.1.6.</td>
<td>Disinfectants</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3.1.7.</td>
<td>Smearing/staining equipment (staining racks, loops, sticks etc)</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3.1.8.</td>
<td>Diamond pencil</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3.1.9.</td>
<td>Spirit lamp/ Bunsen burner</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3.1.10.</td>
<td>Gas for burner</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3.1.11.</td>
<td>Slide boxes</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3.2.</td>
<td><strong>Staining Reagents within Expiry Date</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.2.1.</td>
<td>Carbol Fuchsin</td>
<td>Y / N</td>
<td></td>
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<tr>
<td>3.2.2.</td>
<td>Methylene Blue</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3.2.3.</td>
<td>3% Acid Alcohol</td>
<td>Y / N</td>
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</tr>
<tr>
<td>3.2.4.</td>
<td>Distilled Water</td>
<td>Y / N</td>
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</tr>
<tr>
<td>3.3.</td>
<td><strong>Equipment –Binocular Microscopes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.3.1.</td>
<td>Adequate number</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3.3.2.</td>
<td>Functioning well</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3.3.3.</td>
<td>Adequate Maintenance– Service done once a year</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3.3.4.</td>
<td>Spare Bulb</td>
<td>Y / N</td>
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</tbody>
</table>

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contd.
<table>
<thead>
<tr>
<th></th>
<th>EQA</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1.</td>
<td>Slide Storage according to Lab Register</td>
</tr>
<tr>
<td>4.2.</td>
<td>All slides are stored in Box and no Missing Slides</td>
</tr>
<tr>
<td>4.3.</td>
<td>Slides stored without touching each other</td>
</tr>
<tr>
<td>4.4.</td>
<td>EQA Forms available</td>
</tr>
<tr>
<td>4.5.</td>
<td>Reports filed</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Documentation</th>
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<tbody>
<tr>
<td>5.1.</td>
<td>Laboratory Request Form used</td>
</tr>
<tr>
<td>5.2.</td>
<td>Request Form is completed</td>
</tr>
<tr>
<td>5.3.</td>
<td>Lab Register is available and completed</td>
</tr>
<tr>
<td>5.4.</td>
<td>Results in the Lab Register entered daily</td>
</tr>
<tr>
<td>5.5.</td>
<td>Results provided within 24 hours</td>
</tr>
<tr>
<td>5.6.</td>
<td>3 Specimens per New Patient examined</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Safety</th>
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</thead>
<tbody>
<tr>
<td>6.1.</td>
<td>Adequate Ventilation – Exhaust Fan</td>
</tr>
<tr>
<td>6.2.</td>
<td>Smears not prepared near open window</td>
</tr>
<tr>
<td>6.3.</td>
<td>Sand and Lysol containing Jar for Loop cleaning</td>
</tr>
<tr>
<td>6.4.</td>
<td>Lab coats worn while working</td>
</tr>
<tr>
<td>6.5.</td>
<td>Gloves used during work and cleaning</td>
</tr>
<tr>
<td>6.6.</td>
<td>Flowing Water for Hand Washing</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Waste Disposal</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1.</td>
<td>Work Area cleaned daily</td>
</tr>
<tr>
<td>7.2.</td>
<td>Waste Bin with Lid available</td>
</tr>
<tr>
<td>7.3.</td>
<td>Yellow Bin</td>
</tr>
<tr>
<td>7.4.</td>
<td>Infectious Sputum/Waste Disposal by:</td>
</tr>
<tr>
<td></td>
<td>• Autoclave</td>
</tr>
<tr>
<td></td>
<td>• Burning</td>
</tr>
<tr>
<td></td>
<td>• Disinfection</td>
</tr>
<tr>
<td></td>
<td>• Burial</td>
</tr>
<tr>
<td>7.5.</td>
<td>General Order/Cleanliness</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Staff training:</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1.</td>
<td>Has all New Staff had Smear EQA Training during the past 2 years?</td>
</tr>
</tbody>
</table>

contd.

Prepared by National Tuberculosis Reference Laboratory, Welisara
### 4. Observation Checklist for collection, smearing and staining procedures

<table>
<thead>
<tr>
<th>No</th>
<th>Observation</th>
<th>Adequate/Acceptable</th>
<th>Problems Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>Instructions for collecting sputum is given to the patient</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>2)</td>
<td>Correct labelling of the body of the container</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>3)</td>
<td>Are the Slides labelled with a diamond pencil correctly?</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>4)</td>
<td>Is the Wire-loop cleaned in a Sand/Lysol Bath after each use?</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>5)</td>
<td>Is the smear air dried completely before fixing?</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>6)</td>
<td>Fixed adequately by sending reverse side of slide over flame 3–4 times</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>7)</td>
<td>Number of slides stained in a rack –12 or less</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>8)</td>
<td>Staining procedure is done correctly</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>9)</td>
<td>Microscope lens is cleaned after each Slide Examination</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>10)</td>
<td>Is Reporting of Slides as per NPTCCD Guidelines (Scanty with number, 1+,2+,3+)?</td>
<td>Y / N</td>
<td></td>
</tr>
<tr>
<td>11)</td>
<td>Internal QC slide box has adequate number of positive and negative slides</td>
<td>Y / N</td>
<td></td>
</tr>
</tbody>
</table>

### 5. Assessment of the workload (last 3 months)

<table>
<thead>
<tr>
<th>Slide Volume</th>
<th>Neg. slide No.</th>
<th>Pos. slide No.</th>
<th>Slide Positivity Rate(SPR)</th>
</tr>
</thead>
</table>

*contd.*

*Prepared by National Tuberculosis Reference Laboratory, Welisara*
1) **On-site Evaluation Summary**

a) Operational Problems (e.g. Infrastructure – Power supply, etc.)

b) Technical Problems

c) Overall Remarks

d) Action Required

*Prepared by National Tuberculosis Reference Laboratory, Welisara*
- Format for recording Results of Panel Testing during the Supervisory Visit to the Microscopy Centres:

(Each Laboratory Technician of the Microscopy Centre should complete a separate sheet.)

<table>
<thead>
<tr>
<th>To be entered by MLT/PHLT of the MC</th>
<th>For use by Laboratory Technician of the DCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slide No.</td>
<td>Result of MLT/PHLT of MC</td>
</tr>
<tr>
<td>1.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
</tr>
</tbody>
</table>

Name of the PHLT/MLT:

Name of the MC:

Date tested:

Prepared by National Tuberculosis Reference Laboratory, Welisara
# Quarterly Report – Laboratory Supervision of Microscopy Centres

DCC: …………………………… Year and Quarter:…………………………

## 1. Operational Problems

<table>
<thead>
<tr>
<th>Microscopy Centre</th>
<th>Problems Identified</th>
<th>Action Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td></td>
<td></td>
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Plate 1

Mycobacterium Tuberculosis seen in a sputum smear stained by Ziehl-Neelsen method at magnification x 1000
Glossary and Abbreviations

DTCO - District Tuberculosis Control Officer

LT - Laboratory Technician (Medical Laboratory Technologists and Public Health Laboratory Technicians)

SO - Supervisory Officer

LT/NTRL - Laboratory Technician, National TB Reference Laboratory

NTRL - National TB Reference Laboratory

MO/NTRL - Medical Officer, National TB Reference Laboratory

QA - Quality Assurance

MC - Microscopy Centre