LABORATORY MANUAL
FOR
SPUTUM MICROSCOPY

Government of Nepal
Ministry of Health
Department of Health Services
National Tuberculosis Centre
Fourth Edition Revised 2017

नेपाल सरकार
स्वास्थ्य मन्त्रालय
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मन्तव्य

क्षयरोग एक प्रमुख जनस्वास्थ्य समस्याको रुपमा रही आएको छ । क्षयरोग नियन्त्रणमा उपलब्ध श्रोत र साधनको समुचित परिचालन गरिरहेको वर्तमान अवस्थामा नेपालले विश्व स्वास्थ्य संघले तोकेको सुचाङ्क भन्दा बढि सफलता हासिल गरिरेहको कुरा सर्वविदितै छ । यो सफलता हासिल गर्नमा नेपाल सरकारको कार्यक्रम प्रतिको प्रतिबद्धता, विभिन्न दातृ संस्थाहरु जस्तै : ग्लोवल फण्ड, विश्व स्वास्थ्य संघ, नर्वेजियन एशोसिएसन फर हर्ट एण्ड लङ्गस प्यासेन्ट लगाएत विभिन्न संघ संस्थाको सहयोग उल्लेखनिय रहेको छ । जसको फलस्वरुप क्षयरोग प्रति समाजमा रहेको नकारात्मक धारणा परिर्वतन भई सकारात्मक धारणा बन्दै गएको छ । क्षयरोग नियन्त्रण कार्यक्रमको लागि भविष्यमा पनि ति दातृ संघ संस्थाहरुको सहयोगको अपेक्षा गरिएको छ ।

नेपाल सरकारले क्षयरोग कार्यक्रमलाई प्राथमिकता प्राप्त कार्यक्रमको रुपमा राखेको छ । हालका दिनहरुमा क्षयरोग नियन्त्रण कार्यक्रममा टीवी एचआइभीको सह संक्रमणको बढ्दो प्रकोप, बहुऔषधि प्रतिरोधी क्षयरोगको बृद्धि र वसाईसराई जस्ता चुनौतिहरु सामना गर्न क्षयरोग नियन्त्रण कार्यक्रमले बिगतका नीति तथा कार्यक्रमहरुलाई समयानुकूल परिमार्जन गर्दै विश्व स्वास्थ्य संगठनको रणनीति अनरुप नयाँ रणनीति अवलम्बन गरेको छ ।

यो Laboratory Manual for Sputum Microscopy पुस्तिका राष्टिय क्षयरोग नियन्त्रण कार्यक्रममा संलग्न सरकारी, गैर सरकारी, निजी क्षेत्रमा सञ्चालित स्वास्थ्य संस्थाका प्रयोगशालामा कार्यरत कर्मचारीहरु सबैको प्रयोगको लागि तयार पारिएको हो । यस कार्य पुस्तिकाको माध्यमबाट प्रयोगशालाको माध्यमबाट क्षयरोगीको निदान र उपचारमा उचित व्यवस्थापन गर्न मद्दत पुग्नेछ । विश्व स्वास्थ्य संगठनले सिफारिस गरेको परिमार्जित नीति अनुसार, क्षयरोग उपचारको लागि अन्तराष्ट्रिय मापदण्ड, The Stop TB Strategy र क्षयरोगका विरामीको बडापत्र अंगिकार गरी विश्व स्वास्थ्य संगठनको परिमार्जित निर्देशिका अनुसार नेपालको परिप्रेक्ष्यमा Laboratory Manual for Sputum Microscopy पुस्तिका तयार पारिएको छ ।

यस चौथों संस्करणलाई तयार गर्नको लागी रा.क्ष.केन्द्रका तालीम संयोजक यशोदा राजभण्डारीको संयोजकत्व र ल्याब प्रमुख गोर्कणराज धिमीरेले नेतृत्व गरी ल्या.टे.अ रामबाबु श्रेष्ठ, ल्या.टे.अ कृष्ण अधिकारी म.प.क्षे.स्वा.नी सुर्खेतका क्वालीटी कन्ट्रोल अधिकृत ओमराज आचार्य, जेनेटप कालीमाटीका माईकोवायोलोजीष्ट भगवान महर्जन, आईओएमका फिल्ड कोर्डिनेटर लालमणी अधिकारी लगायत क्षयरोग नियन्त्रण कार्यक्रममा आबद्ध बिभिन्न सरकारी तथा गैर सरकारी संघ संस्थाका प्रतिनिधीहरु तथा अन्य सरोकारवालाहरुको अथक प्रयास बाट तयार गरीएको हो । यसका लागी म सबैलाई धन्यवादका दिन चाहन्छु ।

यो कार्य पुस्तीकाको बिषयमा केही सुझावहरु एवं सल्लाह भएमा तलको ठेगानामा पत्रचार गर्नुहुन हार्दिक अनुरोध गर्दछु ।

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डा. केदार नरसिह के सी
निर्देशक
पो.ब.नं. ९५१७

Contents

Chapter I

1. INTRODUCTION

Tuberculosis is one of the most prevalent infectious disease and significant public health problem in Nepal and continues to pose serious threat to the health of the population and development of the country. Currently nearly 80,000 people have tuberculosis in Nepal, with more than 44,000 new cases arising every year. About half of these are infectious (sputum smear-positive) cases which continue the chain of transmission. The majority of TB patients belong to the economically active age groups of 15–45 years.

Bacteriological examination of sputum is the reliable method for diagnosing infectious TB in Nepal. Moreover, people with sputum smear positive TB is much more infectious than people with sputum smear negative TB. Sputum microscopy is not only reliable; it is also very simple and cost effective. Sputum microscopy is therefore the highest priority in the National Tuberculosis Programme (NTP) for the diagnosis of TB. Besides microscopy, Culture and Molecular techniques are also available as diagnostic tools.

This manual is prepared based on the guidelines of National Tuberculosis Programme. It should be followed by all government, nongovernment and any other organizations involved in TB control activities within the country.

1.1 Purpose of this manual

This manual is prepared for the laboratory workers in Nepal who are involved in sputum smear examination.

- It will help you to understand your role in the NTP.
- It will show you how to process the sputum smear examination for TB.
- It will show you how to use or maintain the NTP recording and reporting system.
- It will inform you about quality control for the sputum smear examination.
- It will help you how to prevent from laboratory infection and proper disposal system.
- It will teach you about occupational safety.

Chapter II THE NATIONAL TUBERCULOSIS PROGRAMME

2. Introduction of National Tuberculosis Program (NTP):

The National Tuberculosis Programme (NTP) is an approach within the national health system to prevention, control and management of TB. It has policies, plans and activities to achieve good case finding and treatment of tuberculosis patients. The NTP is covering countrywide, continuous, permanent and integrated with the general health services. It is relevant to the needs of the population. The NTP is a joint effort of the government and the community aimed at reducing, and in the long term eliminating, suffering due to TB.

2.1 Evolution of TB Strategy

2.1.1 The DOTS strategy (1995-2005)

a. Government commitment
b. Case detection through passive case finding
c. Standardised chemotherapy to all sputum smear positive TB cases of under proper case management conditions
d. Establishment of a system of regular supply of anti-TB drugs
e. Establishment of a monitoring system, for programme supervision and evaluation.

2.1.2 Stop TB Strategy (2006-2015)

a. Pursue high quality DOTS expansion and enhancement
b. Address TB/HIV,MDR-TB and other challenges
c. Contributing to health system strengthening
d. Engage all care provider
e. Empower people with TB and communities
f. Enable and promote research

2.1.3 The End TB Strategy (2016-2035)

In May 2014, the world health Assembly in its resolution WHA 67.1 adopted the global strategy and targets for tuberculosis prevention, care and control after 2015 based on a bold vision of a world without tuberculosis and targets of ending the global tuberculosis epidemic, elimination of associated catastrophic cost for tuberculosis affected households. The three pillars of the strategy include-integrated, patient centred care and prevention, bold policies and supportive systems; and intensified research and innovation. The strategy is based on principles of government stewardship and accountability; with monitoring and evaluation; strong coalition with civil society organizations and community; protection and promotion of human rights, ethics and equity; and adaptation of the strategy and targets at the country level, with global collaboration.

Vision:	**A world free of tuberculosis-zero deaths, disease and suffering due to tuberculosis**			
Goal:	**End the global tuberculosis epidemic**			
INDICATORS	**MILESTONES**		**TARGETS**	
	2020	**2015**	**SDG 2030**	**End TB 2035**
Reduction in number of TB deaths compared with 2015	**35%**	**75%**	**90%**	**95%**
Reduction in TB incidence rate compared with 2015 (%)	**20% (<85/100000)**	**50% (<55/100000)**	**80% (<20/100000)**	**90% (<10/100000)**
TB-affected families facing catastrophic costs due to TB	**0%**	**0%**	**0%**	**0%**

Principles:

1. Government Stewardship and accountability, with monitoring and evaluation
2. Strong coalition with civil society organizations and communities
3. Protection and promotion of human rights , ethics and equity
4. Adaptation of the strategy and targets at country level with global collaboration

Pillars and components:

2.1.3.1 Integrated, patients centred TB care and prevention

A. Early diagnosis of TB including universal drug-susceptibility testing, and systematic screening of contacts and high-risk groups
B. Treatment of all people with TB including drug-resistant TB, and patient support
C. Collaborative TB/HIV activities and management of comorbidities
D. Preventive treatment of persons at high risk, and vaccination against TB

2.1.3.2 Bold policies and supportive systems

A. Political commitment with adequate resources for TB care and prevention
B. Engagement of communities, civil society organizations, and public and private care Providers
C. Universal health coverage policy and regulatory frameworks for case notification, vital Registration, quality and rational use of medicines, and infection control
D. Social protection, poverty alleviation and actions on other determinants of TB

2.1.3.3 Intensified research and innovation

A. Discovery, development and rapid uptake of new tools, interventions and strategies
B. Research to optimize implementation and impact, and promote innovations

2.2 Burden of Tuberculosis in Nepal

Tuberculosis is one of the most prevalent infectious disease and significant public health problem in Nepal and continues to pose serious threat to the health of the population and development of the country. Currently nearly 80,000 people have tuberculosis in Nepal, with more than 44,000 new cases arising every year. About half of these are infectious (sputum smear-positive) cases which continue the chain of transmission. Over 225,000 people will develop tuberculosis during next five years which is equivalent to inhabitants of a densely populated hill district of the country. The majority of TB patients belong to the economically active age groups of 15–45 years. Without appropriate TB treatment, nearly 94,992 people would die in Nepal over the next five years. Given that National TB Programme remains well functioning the number of deaths in the next five year period will be reduced by 75% to 24,770, with a saving of around 70,222 lives.

2.3 History of Tuberculosis Control in Nepal

1951 Tuberculosis Control Programme (TBCP) was launched by Government of Nepal.

1953 Tokha Sanatorium and Central Chest Clinic (CCC) came into existence offering diagnosis and treatment services.

1955 Nepal Anti-TB Association (NATA) established

1965 NATA outpatient clinic became operational

1970 TBCP was reorganized with tripartite agreement between Government of Nepal, WHO and UNICEF. TBCP provided nationwide TB control services in selected districts. NATA Chest Hospital came in to operation

1989 National Tuberculosis Centre Thimi, Bhaktapur at the central level and Regional Tuberculosis Centre (RTC) Pokhara were established with cooperation of Japan International Cooperation Agency (JICA).

1993 Till this date unsupervised Short Course Chemotherapy (SCC) was provided in selected districts with the support of INGOs and bilateral partners. Unfortunately this resulted in high defaulter rate and resistant TB cases.

1994 Joint review by Government of Nepal, WHO and other International and National partners recommended DOTS strategy for TB control in the country.

1995 DOTS strategy was adopted by MOHP/NTP

1996 DOTS strategy based programme started in four pilot districts covering 1.7% of the population.

2001 Nationwide DOTS coverage achieved. 315 Centres and 1,050 Sub-centres in all 75 districts of the country provided DOTS based services.

2005 MDR TB Management Programme started

2006 MOHP/NTP adopted new STOP TB Strategy

2007 PAL initiative launched in two pilot districts

2007 Fixed Dose Combination Adopted

2008 International Standards of TB Care (ISTC) endorsed and adopted by Nepal Medical Association and Professional Societies

2008 DOTS programme services were expanded through 4,323 sites including 1,088 Treatment Centres, 3,147 Sub-Centres and 88 Urban DOTS centres covering all health institutions in the country.

2009 TB HIV Co-infection Programme and Expansion

2012 Isoniazid Preventive Therapy (IPT) started.

2012 Gene-Xpert Technology started.

2013 Universal Coverage on TB treatment.

2014 HIV screening for all new TB cases

2015 Convert all Sub-Treatment centre to Treatment Centre

2016 Implement of Community DOTs

2.4 Introduction to Directly Observed Treatment Short-Course (DOTS)

DOTS is a result oriented and effective strategy of NTP. Through DOTS, treatment of TB patients under the direct supervision of trained health personnel is ensured. The DOTS strategy is based on the five essential components. They are;

2.4.1 Political Commitment

Political Commitment denotes the commitment of all GoN, GOs/ NGOs, people and community.

2.4.2 Case detection through Quality Assured Bacteriology

Quality Assured Bacteriology refers to case detection through Gene-Xpert and culture technique. Laboratory service is the back-bone of NTP. Diagnosis and treatment follow-up of tuberculosis is done by sputum examination which should be easily available, simple, qualitative and sustainable.

2.4.3 Standardized treatment with supervision and patient support

The main aim of NTP is to provide high quality treatment services to patient. It applies Fixed Dose Combination (FDC) Drug Regimen as recommended by WHO.

2.4.4 An Effective drug supply and management system

Basic element of NTP is to provide regular and sustainable quality drugs supply.

2.4.5 Monitoring and Evaluation System, and Impact Measurement

There should be a regular monitoring, evaluation and Impact measurement system to make NTP sustainable and effective.

2.5 Microscopy Network:

The microscopy network consists of multipurpose laboratories at hospitals and primary health centres (PHCs) throughout the country. They carry out microscopic examination of sputum smears stained by the Ziehl-Neelsen method.

At the central level, NTC (National Tuberculosis Centre) collaborates with NPHL (National Public Health Laboratory) and NHTC (National Health Training Centre) for implementing Microscopy service in NTP.

The Reference Laboratory for Tuberculosis at the NTC develops laboratory policies for the NTP. It also prepares training programme for Laboratory personals provides culture and drug susceptibility testing services, and carries out quality control of Regional Tuberculosis Quality Control Centres (RTQCC).

From the central level, the TB control programme is conducted through the RHD (Regional Health Directorate) and the DHO (District Health Office) to the Microscopy Centres and Treatment Centres for case finding, monitoring of treatment and Quality Control (QC) of sputum smear microscopy examination.

The Regional Tuberculosis Quality Control Centres (RTQCC) provides training, supervision, logistic support and quality control for peripheral microscopy centres.

The Microscopy Centres and Treatment Centres are linked each other by the process of the sputum smear examinations.

Sputum smear microscopy remains the key tool for diagnosis of infectious tuberculosis. National Tuberculosis Control Programme operates a network of laboratories with permanent External Quality Assessment System.

Central Laboratory at National Tuberculosis Centre is responsible for planning, training, monitoring, supervision and evaluation of the laboratory network in the country. Central Laboratory also provides quality control services for the Central Region.

Centre	**National TB Reference Laboratory** **Planning, Monitoring, Supervision, Training, Logistics, EQA, Smear Microscopy, Culture & Drug Susceptibility Test**				
	Regional TB Quality Control Centre				
Region	FWDR	MWDR	WDR	CDR	**EDR**
	Regional level planning, implementation, training, supervision, QC, logistic and supply				
	DPHO / DHO **Smear Microscopy**				
District					
	Microscopy Centres **Smear microscopy**				

Five Regional Health Directorates are supposed to do EQA. Regional quality control centres are now operating independently under NTC and Regional Health Directorates, these includes QC Labs in the Western and Mid-Western Regions.

EQA for Smear Microscopy **National TB Center**					
Region	**FWDR** GoN	**MWDR** GoN	**WDR** RTC	**CDR** NTC	**EDR** GoN
			▼ ▲		
			DPHO / DHO		
District			▼ ▲		
			Microscopy Centres		

The microscopy network has two main functions in the NTP:

1. **Diagnosis of new TB**
2. **Monitoring of the TB treatment**

2.5.1 Role of laboratory personals in microscopy center:

In Nepal, most of the bacteriological diagnosis of tuberculosis is carried out in peripheral or local laboratories whose major responsibility is to provide diagnostic microscopy for the NTP based on sputum smear examination by Ziehl-Neelsen (ZN) staining. So laboratory personals should be well cable of performing sputum smear microscopy. The responsibilities of the laboratory personals in microscopy centre are:

- Collecting adequate number and good quality sputum specimens.
- Preparing sputum smear according to Standard Operating Procedures (SOPs).
- Performing microscopy examination promptly and accurately.
- Recording and reporting correctly with all patient's details and results in the laboratory register book.
- Promptly sending microscopy results to the treatment centre.
- Complete filling of all the laboratory forms needed for the NTP program evaluation.
- Keeping all the examined slides serially and prepare them according to Lot Quality Assurance Sampling (LQAS) System for external quality assessment (EQA).

2.5.2 Who are the suspects for sputum smear examination?

Anyone who has had **a cough for more than 2 weeks**, **fever, unexplained weight loss (more than 1.5 kg in one month)** should have a sputum examination. Extra-pulmonary tuberculosis suspects should also have a sputum examination, as they may also have tuberculosis in their lungs.

Two examinations from different sputum specimens of one TB suspects are recommended, because multiple sputum examination has higher sensitivity yield. If we only collect 1 specimen, we will miss some smear positive cases. A series of two examinations can detect almost all sputum positive cases.

Collect 2 Sputum Samples as follows:

1. Supervised **spot sputum** specimen at the first visit

   Early **morning sputum**

   specimen in the next day

First Spot

Morning

2.5.3 Why is sputum examination important?

The sputum examination is much more reliable diagnostic tool than an X-ray. Because tuberculosis is an infectious disease caused by a type of bacteria, to identify bacteria is only the best method of diagnosing TB. It is also simple, cost effective and available at many medical facilities.

People with sputum smear positive pulmonary TB are 10 times more infectious than people with sputum smear negative TB.

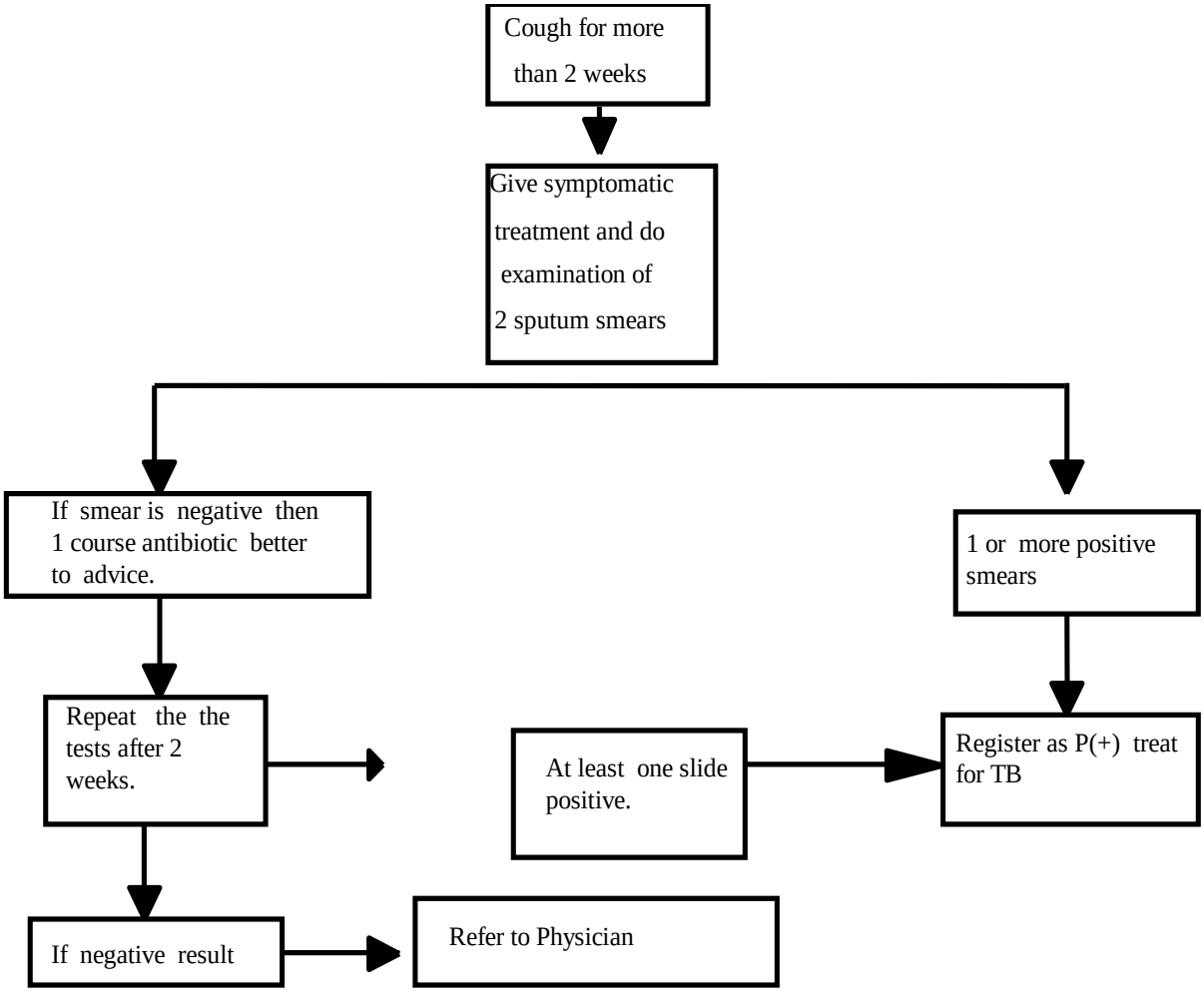
People with sputum smear positive TB have a higher mortality rate without treatment than people with sputum negative TB, so to find out sputum smear positive pulmonary TB is the main strategy of NTP.

2.5.4 Flow chart 1: Diagnosis of Pulmonary TB

Tuberculosis case findings

There are two ways of case findings:

1. Passive case finding: It means diagnosis of TB in Patients attending health facilities by themselves.
2. Active case finding: It means that health worker themselves seek out tuberculosis suspect eg. Collecting sputum from chest symptomatic in the community.



2.5.5 What is a diagnosis sputum examination?

The sputum specimens are examined to identify smear positive pulmonary TB before starting treatment are known as ***diagnosis sputum examinations***. Sputum examination is the best way of diagnosing pulmonary TB.

If smear examination failed to detect, then it should be performed by Gene Xpert.

We should examine the sputum of anyone who has a cough for more than 2 weeks. We must examine the sputum of those suspected to have extra-pulmonary tuberculosis, as they may also have tuberculosis in their lungs. We collect 2 specimens of sputum from a chest symptomatic suspect. If we collect 1 specimen only, we will miss some smear positive cases.

2.5.6 What is a follow up sputum examination?

Follow up sputum specimens are examined are during the treatment for checking the effectiveness of the treatment. **The patient must have the sputum examinations after 2(3*), 5 and last months of treatment.**

	Treatment			
Treatment duration	**0 month (Diagnosis)**	**2 (3*) months (follow-up)**	**5 months (follow-up)**	**Last month (follow-up)**
No. of sputum examination	**2**	**1**	**1**	**1**
Type of sputum sample	**-spot** **-morning**	**-spot**	**-spot**	**-spot**
	Diagnosis examination	**Follow-up examination**		

(3; Category 2 regimens patient*)

(For follow up examination, early morning sample is best preferable)

Chapter III GENERAL INFORMATION ON TUBERCULOSIS
3. Tuberculosis (TB):

Tuberculosis (TB) is an air borne infectious disease caused by Mycobacterium tuberculosis. *Mycobacterium tuberculosis* is also called acid fast bacilli (AFB). Tuberculosis is most commonly transmitted by inhalation of infected droplet nuclei which are discharged in the air when a sputum smear positive TB patient coughs or sneezes. Only about 5%–10% of infected persons (primary infection) develop active tuberculosis disease. Among the remaining 90% to 95 % of infected persons, initial infection usually goes without further consequences. Transmission of TB infection occurs almost exclusively through the respiratory route. The infection may then spread from the primary lung lesion to any part of the body via the blood stream, lymphatic and bronchial systems.

Pulmonary TB, sputum smear is positive, is highly infectious and should receive topmost priority for treatment. Sputum smear-negative cases are much less infectious than those who are smear-positive. Extra-pulmonary TB can affect the lymph nodes, pleura, bones and joints, the genito-urinary tract, the nervous system (meningitis), intestines, etc.

If untreated, TB leads to death within 5 years in at least half the patients. About 20 to 25% will naturally heal and 25 to 30% remain positive and continue to spread the disease in the community.

3.1Transmission of Tuberculosis

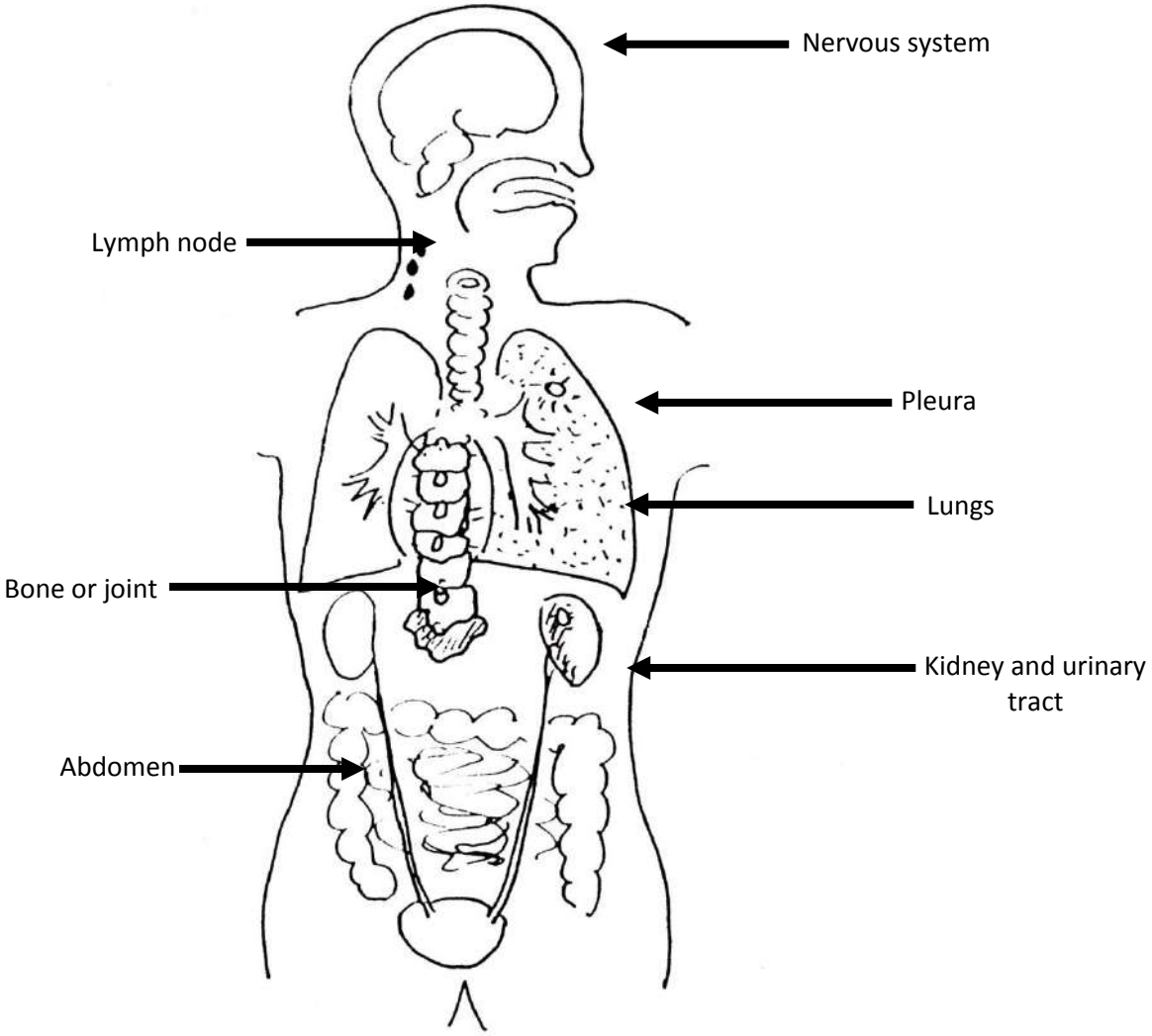
The bacteria that cause tuberculosis spread from the lungs of people with TB when they cough sneezes of spit. When a person inhales these bacteria it invades the lung. Macrophages surround and take up the bacteria. Immune cells try to kill the bacteria and cause an area of local inflammation in the lung , This is called a ‘primary focus’, The bacteria may also spread to hailer, lymph, glands, causing enlargement . The combination of a primary focus and the affected lymph nodes is called a ‘primary complex’.

Not every, who is infected with the bacteria gets TB. Only 10% of people who are infected get TB disease. If the infected person has good immunity disease may not occur, however if the immunity is weak (e.g. in malnutrition or people with HIV infection) then TB disease can develop soon after infection.

3.2 Common site of tuberculosis in the body:

The most common site of tuberculosis infection in the body is lungs and secondly in lymph node. In addition to that TB can infect any part of body except nail and hair (*Ref.- John Crofton*).

Some common sites for tuberculosis are shown in the diagram below:



3.3 What is Tubercle Bacillus?

Tubercle bacillus in sputum stained by Ziehl-Neelsen method at the magnification of x1000 is shown on page no. 98 which is also known as **Acid Fast Bacilli**.

Mycobacterium tuberculosis was discovered by German Scientist Robert KOCH on 24th March 1882.

3.3.1 Physical and chemical characteristics

Mycobacterium tuberculosis is generally a slightly curved, thin rod, measuring 2-6 micro metre (µm) in length and 0.3-0.6 micro metre (µm) in thickness (1 µm = 1/1000 mm). This organism is non-motile, non-capsulated and having no true branching. It is difficult to stain this organism by usual staining method because the cell wall of ***M. tuberculosis*** is composed of higher waxes and a high content of mycolic acid. It can retain the pink-red colour when stained by Carbol Fuchsine solution, and it resists decolourization by acids and alcohol. Therefore it is called *Acid Fast Bacillus (AFB).*In a sputum smear stained by Ziehl-Neelsen method, AFB appears as a red or pink rod under microscope.

- Mycobacterium tuberculosis
  - slightly curved thin rod shaped bacilli
  - 2-6 µm in length and 0.3- 0.6 µm in width
  - Is non-motile, non-capsulated and without true branching
  - Difficult to stain by usual staining method because of its cell wall is composed of higher waxes and a high content of mycolic acid.
  - Can retain the pink-red colour when stained by Carbol Fuchsine solution and it resists decolourization by acids and alcohol. Therefore it is called *Acid Fast Bacillus (AFB).*

- In Ziehl-Neelsen staining method, AFB appears as a red or pink rod under microscope.

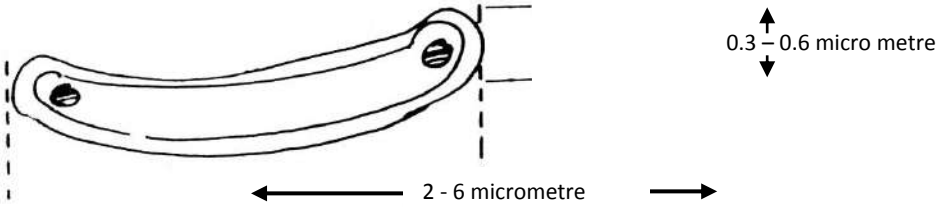


Fig:- Rod shaped Mycobacterium Tuberculosis(image under electron microscope)

3.3.2 Nutrition and Growth

In the laboratory, ***M. tuberculosis*** grows in an egg enriched selective media with very slow rate of growth. The generation time of the TB bacterial cells, under ideal culturing conditions, is 17-20 hrs.

3.4 What is drug resistance?

Among the millions of tuberculosis bacteria found in the lungs of a sputum smear positive pulmonary TB patient, a few will be resistant to one or more anti-tuberculosis drugs. If such patients are treated with a single drug, the sensitive bacteria will be killed but the resistant bacteria will increase their numbers. Fortunately, these resistant bacteria are usually sensitive to at least one or other drugs used in the recommended regimen against tuberculosis. So, we give an appropriate combination of drugs for a sufficient period of time, and the bacteria resistant to a single drug will also be killed or TB will be cured.

3.4.1 Multi - Drug Resistant Tuberculosis (MDR TB):

When two or more drugs showing no effect to a TB patient due to the presence of resistance bacteria, such condition is called drug resistance. Usually a TB patient resistant to ***Isoniazid and Rifampicin*** is said to be as ***MDR TB Case***.

There are three types of resistance of TB bacilli to anti-tuberculosis drugs: (Mechanism only)

1. **Natural drug resistance:**
   A naturally drugs resistant strain is a wild strain resistant to particular drug without ever having been in contact with it. This is known as ***Natural drug resistance.***

2. **Acquired drug resistance:**
   Acquired drug resistance is developed due to the incorrect chemotherapy. If the patient does not take the drugs for long enough, or takes the drugs irregularly or if the dose is inadequate, these resistant bacteria will not be killed or drugs will not work and the patient will develop acquired drug resistance.

3. Primary drug resistance:

If a patient with acquired resistance infects another person, that person will develop ***primary drug resistance***, even though he has not taken anti-tuberculosis drugs before.

3.4.2 Extensively drug-resistant TB (XDR-TB):

It is defined as MDR-TB that is resistant as well as to any one of the fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin etc) and to at least one of three injectable second-line drugs aminoglycosides (Kanamycin, Capreomycin or Amikacin)

3.5 How does acquired drug resistance occur?

Drug resistance could develop due to the following reasons:

- Inadequate treatment
- Inappropriate treatment regiment
- Inadequately formulated drugs
- TB treatment not following DOTS
- Defaults drug taking
- Irregular drug taking

3.6 What are culture and drug susceptibility test (DST)?

Sputum culture examination is a bacteriological method of detecting live TB Bacilli. Culture is the process of growing the numbers of organism in artificial media. It can be done by using different types of culture media. Most common culture media is Lowenstein Jensen (LJ), which is egg based solid enriched media. Another media is liquid media (eg. Middle brook). Culture is the gold standard method in tuberculosis diagnosis. Result turn out time is longer in solid media (8weeks) than liquid media (4weeks). Colony characters are observed in solid media and other bio-chemical tests are used in liquid media. TB bacilli grow very slowly in an egg enriched selective media (Ogawa, Lowenstein-Jensen media). It takes four to eight weeks before the colonies of bacteria can be seen with the naked eyes.

The drug susceptibility test is done to know the characteristics of TB bacilli whether they are susceptible or resistant to anti TB drugs. Drug susceptibility test is important not only to choose the most effective drug regimen for the treatment of individual patient but also for the epidemiological purpose to assess the efficiency of the treatment service in NTP. The laboratories performing Mycobacterial culture and drug susceptibility tests in Nepal are as follows;

- The National Tuberculosis Centre, National Tuberculosis Reference Laboratory, Thimi, Bhaktapur
- German Nepal TB Project (GENETUP), National Tuberculosis Reference Laboratory, Kalimati, Kathmandu

3.7 Laboratory diagnosis of TB

Laboratory diagnosis can classify into two types.

1. Conventional diagnostic techniques
2. New diagnostic techniques

3.7.1 Conventional diagnostic techniques

Conventional diagnostic techniques consists microscopy examination and solid culture.

3.7.1.1 Microscopy Examination:-

It detects the character of Acid fastness of bacilli. Ziehl Neelsen technique is the widely used technique for microscopy.

3.7.1.2 Culture Technique:-

This method detects the viable TB bacilli in culture media. It is a process of growing the numbers of microorganism in artificial media. There are different types of solid culture media. Lowenstein and Jensen (L-J) media is the most commonly used solid culture for growing TB bacilli. It is a confirmative diagnostic technique but slow technique. Usually TB bacilli grow in three to four weeks.

3.7.2 New diagnostic techniques:

- LED Fluorescence Microscopy
- Liquid culture (Bactec MGIT-960)
- Molecular techniques (PCR) :- Gene Xpert, MTBDR*plus* Line Probe Assay (LPA), Loop Mediated Isothermal Amplification (LAMP)
- Lipoarabinomannan Glycoprotein (LAM) Antigen in urine
- IGRA:- Interferon Gama Release Assay, it detects latent TB in adults and children.

3.7.2.1 LED Fluorescence Microscopy:

A **fluorescence microscope** is an optical microscope used to study properties of organic or inorganic substances using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption.

3.7.2.2 Liquid Culture MGIT-960:-

The BACTEC MGIT 960 System is an *in vitro* diagnostic instrument for rapid detection of Mycobacteria in clinical specimens other than blood. This system is simple, efficient, safe to use and occupies small laboratory space. The MGIT 7ml tube contains modified middle brook 7H9 broth. Culture tubes contain a fluorescent sensor at the bottom which responds to the concentration of oxygen Initial concentration of dissolved oxygen quenches the emission from the compound, and little fluorescence can be detected. Actively respiring microorganisms consume the oxygen which allows the compound to fluorescence and detect as a positive. It is completely automatic system. In liquid culture, TB bacilli usually grow in 7-8 days and DST takes about one more week.

3.7.2.3 Gene-Xpert MTB/RIF:

Gene–Xpert MTB/RIF is novel technology for the diagnosis of tuberculosis. It is a real time multiplex PCR technology with integrated and closed fluid transfer system. It is a single hands free step technology.

It detects mycobacterium tuberculosis complex and its resistance to rifampicin by PCR amplification of the *rpoB* gene. Rifampicin is the most effective and powerful first line anti-tuberculosis drug. Most of the rifampicin resistant patients are also resistant to isoniazid but isoniazid resistant patient may not resistance to rifampicin. The sensitivity and specificity is much higher than sputum microscopy. It gives result within two hours, so it had also called as Rapid Diagnostic Test (RDT). It is Point of Care Test (POC).

Sample reagent

Cartridge

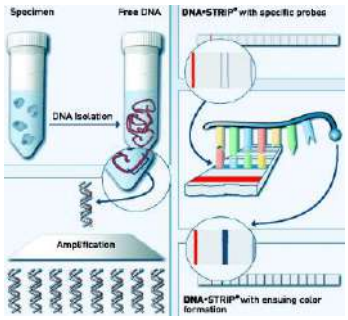
Computer

Gene-Xpert machine

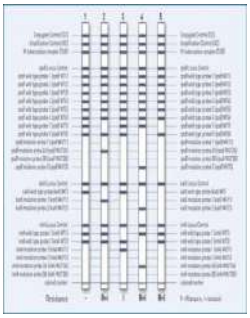
3.7.2.4 Molecular Line Probe Assay (LPA):

LPA is a multiplex PCR with reverse hybridization technique. It detects genetic identification of the *M. tuberculosis* complex (MTBC) and its resistance to rifampicin, isoniazid, quinolone and second line injectable drugs from cultivated samples and from direct patient sample.
Genotype MTBDR*plus* and MTBDR*sl* are the two commonly used LPA techniques for diagnosis of drug resistant tuberculosis. The Genotype MTBDRplus line probe assay was designed for the rapid detection of resistance to rifampicin and isoniazide by detecting mutation in *rpoB, katG* and *inhA* gene and MTBDR*sl* detect resistance to quinolone (ciprofloxacin, ofloxacin, moxifloxacin) dy detecting mutation in quinolone associated gene *gyrA* and second line injectable drugs (kanamycin, capreomycin, amikacine) by detecting mutation in *rrs* gene. MDR-TB and XDR-TB can be diagnosed within 1 or 2 days by this method from direct sputum sample.

DNA amplification



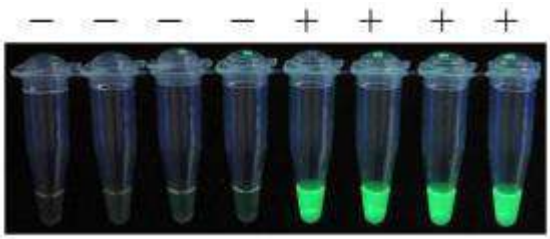
Hybridyzation



Result evaluation

3.7.2.5 Loop mediated isothermal amplification (LAMP):

LAMP is a single tube technique for the amplification of DNA. It is an isothermal nucleic acid amplification technique. In LAMP PCR, isothermal amplification is carried out at a constant temperature, and does not require a thermal cycler.
In LAMP, the target sequence is amplified at a constant temperature of 60 - 65 °C using either two or three sets of primers and a polymerase with high strand displacement activity in addition to a replication activity. Typically, 4 different primers are used to identify 6 distinct regions on the target gene, which adds highly to the specificity. An additional pair of "loop primers" can further accelerate the reaction. Amplification of DNA takes one hour.



3.7.2.6 Lipoarabinomannan (LAM):

LAM test is based on the detection of mycobacterial lipoarabinomannan (LAM) antigen in urine have emerged as potential point-of-care tests for tuberculosis (TB). LAM antigen is a lipopolysaccharide present in mycobacterial cell walls, which is released from metabolically active or degenerating bacterial cells and appears to be present only in people with active TB disease. Urine-based testing would have advantages over sputum-based testing because urine is easy to collect and store, and lacks the infection control risks associated with sputum collection.

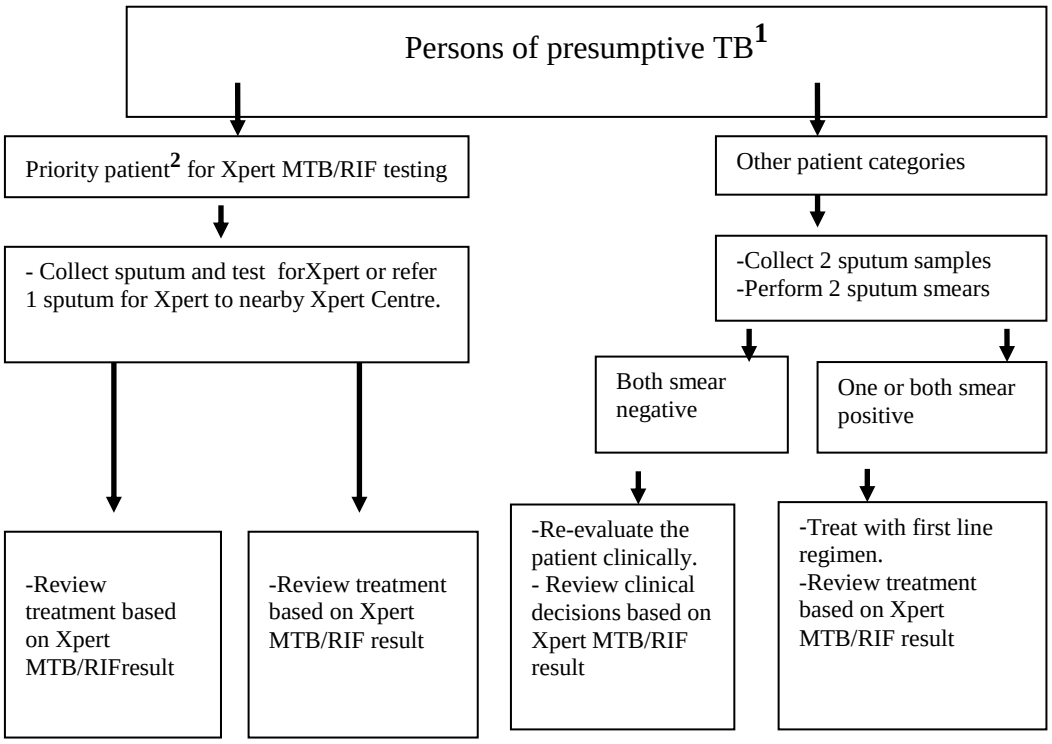
3.7.2.7 Interferon-gamma assay (IGRA):

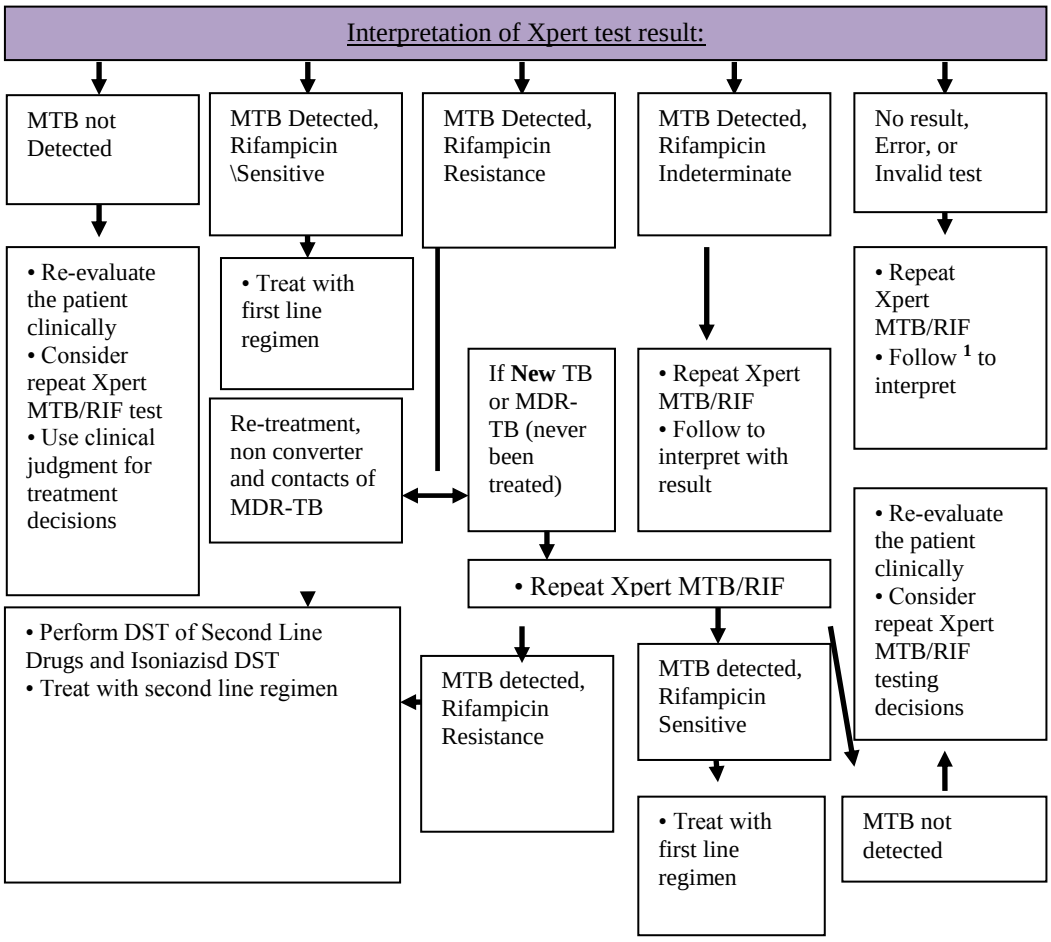
Interferon-gamma release assays (IGRAs) is an important advance in the diagnosis of latent tuberculosis infection (LTBI). IGRA is in vitro blood tests of cell-mediated immune response; they measure T cell release of interferon (IFN)-gamma following stimulation by antigens unique to *Mycobacterium tuberculosis*.

The goal of testing for latent TB infection is to identify individuals who are at increased risk for the development of tuberculosis (TB) and therefore who would benefit from treatment of latent TB infection. Only those who would benefit from treatment should be tested, so a decision to test presupposes a decision to treat if the test is positive.

3.8 Diagnostic Algorithm for General TB:

Algorithm 1:



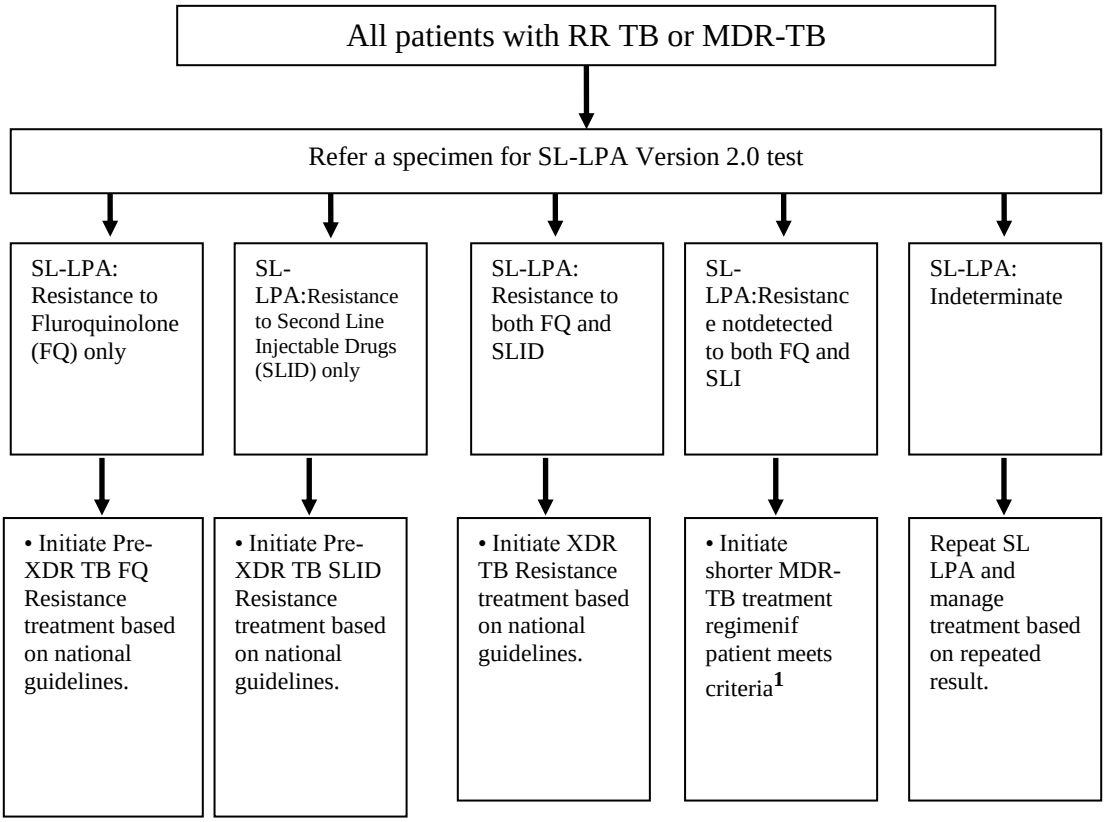


[1]Persons being evaluated for TB include all persons with signs or symptoms suggestive of TB or persons with a chest X-ray with abnormalities suggestive with TB. This algorithm may also be used for persons being evaluated for extrapulmonary TB.

[2] Priority patients include PLHIV, contacts of RR/MDR-TB, lost to follow-up, relapse, failure, non converters (smear positive at end of the intensive phase of treatment), children, patient live in close (congregate)setting, sample collected through courier system, diabetic, patient live near to Xpert Centre.

3.9

Algorithm 2: Algorithm for testing for second-line drugresistance among rifampicin-resistant (RR) TB or MDR-TB patients.



3.10 How does TB Spread?

TB bacilli spread from the lungs of people with TB when they cough, sneeze, speak or spit. Another person inhales the droplets containing TB bacilli through respiration and may become infected with TB. It is actually not spread from food, smoking or drinking alcohol. Neither is it spread by insects and parasites nor by heredity.

Not everyone who is infected with TB bacilli gets TB. If the infected person has good immunity, the disease may not develop. However if their immunity is weak (e.g., due to malnutrition, HIV infection) then TB can develop soon after infection.

By cough
About 1.5 m

By sneeze
About 3 m

Not by food

Fig:-by close contact with tuberculosis patient

3.11 What are the symptoms of pulmonary TB?

- Cough for the duration of 2 week or more *(the commonest symptom of pulmonary TB)*
- Fever *(Often in the evening or at night)*
- Shortness of breath
- Chest pain
- Haemoptysis (blood stain sputum)
- Loss of appetite
- Weight loss
- Night sweats
- Fatigue (feeling tired)
- Sputum production

3.12 How many types of TB are there?

The type of tuberculosis is defined by the site of the disease in the body. 80 % of tuberculosis occurs in the lungs and is called *pulmonary TB*. The occurrence of TB on the sites other than lungs is called *extra-pulmonary tuberculosis*.

Type of Tuberculosis on the basis of organs involves:

1. Pulmonary Tuberculosis:

a. Pulmonary bacteriological confirmed (PBC)

b. Pulmonary clinically diagnosed (PCD)

2**. Extra pulmonary Tuberculosis**

a. Extra Pulmonary bacteriological confirmed

b. Extra Pulmonary clinically diagnosed

3.13 What are the common extra-pulmonary TBs?

Gland TB	Intestinal TB
Bone and Joint TB	Urogenital TB
TB Meningitis	Skin TB
Abdominal TB	Eye TB etc.
Miliary TB	Genitourinary TB
Laryngeal TB	Pleurisy TB

3.14 Registration categories of TB patient

After diagnosis, the patient must be registered and should be given the treatment. The registration categories of TB patients designed by the NTP will help the health workers to decide which category of treatment is appropriate to the patient.

3.14.1. New:

A patients who has received no or less than one month of anti-tuberculosis treatment

3.14.2. Previously treated

3.14.2.1 Relapse:

A patient whose most recent treatment outcome was “cured” or “treatment completed”, subsequently diagnosed with bacteriologically positive TB by sputum smear microscopy or culture

3.14.2.2 Treatment after failure:

A patient who has received treatment for TB and in whom treatment has failed. Failure is defined as sputum smear positive at five or end of treatment.

3.14.2.3 Treatment after lost to follow up:

A patient who returns to treatment, bacteriologically positive by sputum smear microscopy or culture, following interruption of treatment for two or more consecutive months.

3.14.2.4 Other previously treated patient:

Patients who have completed TB treatment but not evaluated or have no evaluation document available. The TB patient registered in NTP and transferred from any treatment centre to another centre.

3.14.3 Previous Treatment history unknown

Case that does not fulfil the above criteria. This is usually a patient who has been taking anti-tuberculosis drugs for more than 4 weeks but has not been registered within the NTP.

3.15 What are the drug regimens and how do we use them in Nepal ?

Treatment Regimen

TB Treatment Regimen	Regimen	Follow up Examination
Category I - New pulmonary TB cases (PBC+PCD)	2 HRZ E + 4 HR	Months 2, 5, and End of Treatment → Sputum Smear Test ➔ If still +ve at 2 months • go for Rapid DST while continuing the treatment • if Xpert +veRiF sensitive • extend intensive phase for 1 more month. ➔ If smear is still +ve at 5 months or end of Treatment • declare as Treatment Failure • switch to category II and screen for DR TB
- New EP-TB cases (BC+CD)	2 HRZ E + 4 HRE	For EP TB → 2 months follow up with sputum smear regardless of chest symptoms. ➔ If smear +ve • label as Treatment Failure and manage accordingly. ➔ At the end of continuation phase, based on the clinical judgement (Improving) by treating physician, the continuation phase can be extended up-to 3 more months.
Category II - All Pulmonary Retreatment Cases At-least RIF sensitive - All (Mild form) EP TB Retreatment cases **For exceptional (severe form) Pulmonary TB cases (New or Relapse) - Milliary TB	3 HRZ E + 6 HRE	Months 3, 5 and End of Treatment →Sputum Smear Test ➔ If Smear +ve at 3 months: • Go for Rapid DST while continuing the treatment • Extend intensive phase for 1 more month. ➔ If Smear +ve at 5 months • Repeat Rapid DST while continuing the treatment If RiF Sensitive • continue ATT while waiting for report of Culture DST and manage accordingly. ➔ Culture, FLDST and SLDST If Smear +ve at end of Treatment ➔ Repeat Rapid DST while continuing the treatment → if RiF Sensitive →continue ATT for 3 more months ➔ At the same time send for Culture, FLDST and SLDST → manage based on the result.
**For exceptional (severe form) EP TB cases (New or Relapse) Milliary TB CNS, Musculo & Skeletal TB	3 HRZ E + 6 HRE	➔ At the end of continuation phase, based on the clinical judgement (Improving) by treating physician, the continuation phase can be extended up-to 3 more months.
Use of Streptomycin: • Streptomycin is in the process of being phased out. • Streptomycin can still be used when: o Other drugs have to be replaced cause of toxicity, especially Ethambutol. o Can be used in CNS TB based on the judgement by treating physician.		

(Continuation phase can go up to additional of 3 more months at 5 months follow up with sputum smear regardless of chest symptoms).

*Use of Streptomycin:

- Streptomycin from previously known category II is now phased out.
- Streptomycin can still be used when:
- Other drugs have to be replaced cause of toxicity, especially Ethambutol. For exceptional complicated EP TB cases
- CNS TB
- Miliary TB (pulmonary TB)
- Musculo-skeletal TB **3 HRZE + 6 HRE**

3.16 INTRODUCTION TO HIV AND AIDS

HIV stands for "Human Immunodeficiency Virus", which is the causative organism of the HIV infection. HIV virus infects the human immune cells called CD4 cells and weakens the immune system of the body leading to a condition called "Acquired Immunodeficiency Syndrome (AIDS)". During the course of infection HIV destroys the CD4 cells making human body prone to many infections which are not common in the absence of HIV. Such infections are termed as opportunistic infections (OI).

3.16.1 Why is tuberculosis so common in people with AIDS?

The ability of a person to restrict and contain tuberculosis infection depends on its cellular immunity. Because HIV weakens cellular immunity, TB bacilli can grow more easily, and tuberculosis disease develops. HIV infection can therefore cause latent tuberculosis infection to progress to tuberculosis disease.

The aim of the tuberculosis control programme, in countries where HIV infection occurs, must be to quickly diagnose and cure the largest possible number of newly occurring tuberculosis cases, especially those who are smear-positive. This will reduce the risk of increased transmission of tuberculosis infection to the general population.

3.16.2 Epidemiology of HIV in Nepal

The human immunodeficiency virus (HIV) pandemic presents a massive challenge to the control of tuberculosis (TB). According to UNAIDS estimates 33 million people in the world are living with HIV and every year 2.5 million new people get infected with the virus (UNAIDS 2007). In Nepal according to recent estimates about 70,000 people are living with HIV making 0.49% prevalence in the general adult population. Nepal is categorized as a country with the concentrated HIV epidemic with less than one percent in general adult population and over 5% in certain risk groups, mainly Intravenous Drug Users (IDU). The recent prevalence of HIV in IDU is 20.7% in Kathmandu, 3.4% in Pokhara, 8.1% in Eastern Terai districts and 8.0 in Western Terai districts (IBBS 2009).In other groups HIV prevalence is lower than 5% except in the group of male sex workers (a sub group of “Men who have Sex with Men”) prevalence of HIV is 5.2%(IBBS 2009). According to sentential Surveillance of Nepal 2011/12, the prevalence of HIV positive among TB patient is 2.4%. Moreover, the progression of TB among HIV positive individuals, decreased by about 5-15%.

3.16.3 Modes of transmission

HIV is found in all body secretion of the HIV infected person. The amount of HIV is adequate enough for transmission only in blood, semen, vaginal secretion and breast milk. Any contact with HIV infected blood, semen, vaginal secretions and breast milk with broken skin or broken mucous membrane of previously not infected person causes infection. Any way which facilitates such contact is the mode of transmission of HIV. The modes of HIV transmission are:

- Sexual contact without using condom-facilitating the mixing of infected semen with blood (through micro trauma of the vaginal mucosa) or infected vaginal secretion with blood (through micro trauma of penile skin) or infected blood with blood (micro trauma of penile skin and mucous membrane of anal mucosa- in case of anal sexual contact). Among the sexual contact anal sex has got highest risk of HIV transmission as chances of trauma are comparatively higher during this sexual act.
- Needle sharing between HIV infected persons and not infected with HIV-
- Transfusion of HIV infected blood or transplant of HIV infected organ
- Mother to child transmission during pregnancy, labor and breast feeding: highest risk is during labor.
- During medical procedures if universal precautions are not followed

3.16.4 Window period

The window period represents the stage immediately after becoming infected but before body creates antibodies. In most people, it takes the body 3-4 weeks to make enough antibodies to

be detected by laboratory tests. It may take up to 3 months for laboratory tests to detect HIV antibodies in a person's body.

If people are tested during the window period, they test negative even though they are infected because the body does not produce enough antibodies to trigger a positive test result. The virus can pass from one person to another during the window period.

3.16.5 Diagnosing HIV

There are two ways of diagnosing HIV – by identification of the viral particles in the body of infected person or by identification of the antibodies developed by the body against the viral particles. The first method is called virological method and is not commonly available in Nepal. DNA PCR is the nucleic acid based qualitative test for identification of viral particles. The diagnosis of HIV has traditionally been based on the detection of antibodies against HIV. Wide ranges of different HIV antibody tests are available including ELISA (Enzyme linked Immunosorbent Assays), rapid HIV tests and Western Blot. Rapid HIV tests are widely used in Nepal and are supplied through National HIV logistic system of the country. More than one rapid test kits should be used in order to come with a definitive diagnosis. This order is known as testing algorithm or testing strategy. Serial testing strategy for HIV testing is used in Nepal. Three test kits are used in order following this strategy. Using the rapid test kits, the result of HIV test is provided within same day. A system of quality assurance of the rapid test is necessary for using rapid tests.

3.16.6 Care of HIV positive

As soon as the person is identified positive for HIV, he should be enrolled into the system of Continuum of Care for HIV positive. The first task of the HIV clinician is to do the clinical staging or CD4 testing. Depending on the result of the clinical staging or CD4 test the person is entitled for Cotrimoxazole prophylaxis, or Anti-retroviral therapy (ART). All HIV positive clients are investigated for Tuberculosis immediately after diagnosis and Isoniazid preventive therapy (IPT) is started if indicated.

3.16.7 Antiretroviral Therapy

Anti-retroviral drugs are the drugs used against HIV. These drugs inhibit the replication of HIV. When antiretroviral drugs are given in combination, HIV replication and immune deterioration can be delayed, and survival and quality of life improved. There are five classes of antiretroviral drugs available in use: Fusion inhibitors, Nucleoside reverse transcriptase inhibitors, Nucleotide reverse transcriptase inhibitors, Non-nucleoside reverse transcriptase inhibitor and protease inhibitors. Fusion inhibitors are not currently available in Nepal. The drugs available in Nepal for the first line regimen through Government supply are- Zidovudine (AZT)/ Stavudine (d4T) ; Lamivudine (3TC), Nevirapine (NVP) /Efavirenz(EFV). At least three ARV should be used in combination for effective treatment. Among these ARV, Nevirapine is hepato-toxic and should be used with caution in patient taking Rifampicin. Reduction of HIV related morbidity and mortality, maximal and durable suppression of viral load, restoration and/or preservation of immunologic function and Improvement of quality of life are the goals of Anti-Retroviral Therapy (ART)

3.16.8 Monitoring Antiretroviral Therapy

CD4 and Viral loads test are done for monitoring ART. CD4 cells are the cells of the immune system, which are targeted by HIV in the body. Number of CD4 decrease as HIV progresses in the body. Normal range of CD4 in human body ranges from 500 to 1500/microliter. If the CD4 count decreases even with ART, this is considered as immunological failure of antiretroviral therapy.

The viral load test is a quantitative measurement of HIV nucleic acid (RNA) that provides important information that is used in conjunction with the CD4 cell count:

- to monitor the status of HIV disease,
- to guide recommendations for therapy, and
- to predict the future course of HIV.

Routine blood test for Hb and liver function are indicated in regular interval for all taking ART.

3.16.9 TB-HIV Co-infection in Nepal

National TB program has conducted periodic sentinel site HIV prevalence among TB patient since 1993/94. According to these surveys the prevalence of HIV among TB patients is rising.

Table 1 : Results of HIV prevalence among TB patients 1993/94 – 2006/07

Year of sentinel survey	% of HIV among TB patients
1993/94	0.00
1995/96	0.60
1998/99	1.88
1999/2000	1.39
2001/2002	2.44
2006/2007	2.40
2011/2012	2.40

Source: NTP report 2012

3.16.10 Recommended collaborative TB/HIV activities

Collaborative activities should address the interface of the Tuberculosis and the HIV/AIDS epidemics for which joint programs should be carried out as part of health sector responding to the intersecting Tuberculosis and HIV epidemics.

Chapter IV LABORATORY PREPARATION FOR SPUTUM SMEAR EXAMINATION

4. LABORATORY PREPARATION FOR SPUTUM SMEAR EXAMINATION

4.1 ROLE OF LABORATORY PERSONNEL

NTP Laboratory personnel should persuade the following tasks to keep well - managed laboratory;

Safety
- Keep the laboratory neat and clean.
- Forbid smoking, eating and drinking in the laboratory.
- Destroy examined sputum and contaminated materials appropriately.
- Regularly disinfect working bench, towels and laboratory gowns.
- Proper hand washing.

Facility
- Keep the laboratory neat and clean.
- Keep the microscope in good condition.
- Order equipment, reagent and other materials on time.

Sputum collection
- Explain the reason for the sputum examination to the patients.
- Explain the importance of follow-up sputum examination to the patients.
- Give instructions to patients for quality sputum collection.
- Label the patient's serial number on the side of sputum container, not on the cap.
- Check the quality of the sputum.

Examination
- Register the patient's vital information.
- Make the smear and fix it.
- Stain the smear by Ziehl-Neelsen method.
- Examine the smear by microscopy.

Recording & Reporting
- Maintain the NTP laboratory register daily.
- Send the results of examination to the treatment centre or give to the patient.
- Submit a monthly report to the DHO/DPHO.
- Fill up the LQAS 1 form well.

Quality control
- Keep all examined slides in serial order for LQAS so that supervisor can select the sample slides easily.
- Send the collected slides with LQAS-1 forms to Regional TB Quality Control Centre/Assessor.
- Receive and file the LQAS-3 feedback report.
- Maintain a high quality of examination.
- Planning.

For the health workers of Non-Microscopy Centre
The worker should send the sputum smear slides after fixation with the examination request form to the DHO laboratory or the nearest microscopy centre for sputum smear examination.

4.2 SAFETY PRECAUTIONS

Safety
Tuberculosis is transmitted through air; therefore laboratory workers must avoid the occurrence of aerosols from sputum samples to keep themselves safe from TB infection.
The laboratory worker should keep the following rules:

- Keep the laboratory environment as clean as possible and minimise the movement of people.
- Wear an apron, gloves and masks in the laboratory.
- Wash hands with soap and water after making each smears and before leaving the laboratory.
- Sterilise all contaminated materials by burning, boiling or soaking in disinfectant.
- Do not eat, drink and smoke in the laboratory.
- Do not use the same desk for smear making and microscopy work.
- Do not flame the wet smear. , it can create aerosols.
- Do not handle sputum specimen roughly to avoid production of aerosols.
- Do not mouth pipette in the laboratory

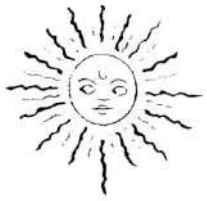
Handle specimens carefully especially,
- When you open or close the specimen container
- When you pick up the sputum
- When you smear the sputum

4.3 STERILISATION AND DISINFECTION

- **Sterilisation is to make an article free of any micro-organisms.**
- **Disinfection is to make an article free from vegetative form micro-organisms.**

TB bacilli can be killed by the following methods:

Method	**Duration**	**Materials which can be sterilised in this way.**
Burning 	Few Seconds	Bamboo sticks Cottons Papers Containers Sputum
Boiling 	10-20 minutes	Forceps
Sunlight	2-7 hours	Clothes

		
5 % Lysol 	Few seconds - 12 hours	Hand Clothes Working bench Floor
5 % Phenol 	1-24 ours	Floor Working bench Clothes
By autoclaving 	121°Cfor 15 minutes at 15 lbs pressure	Sputum container Test tube, Forceps etc.

4.4 USE OF DISINFECTANTS

The high lipid content of cell wall of mycobacteria confers resistance to classical disinfectants. Quaternary ammonium compounds inhibit tubercle bacilli but do not kill them and they are also resistant to acid and alkali. The efficient disinfectants suitable for use in tuberculosis laboratories are those containing phenols, hypochlorite, alcohol, formaldehyde, iodophor or glutaraldehyde. These are usually selected according to the material to be disinfected. Sweet-smelling “antiseptics” should not be used. Disinfectant solutions should be prepared fresh every day and should not be stored in diluted form because their activity will diminish. Work surfaces should be decontaminated at least once a day with an appropriate disinfectant ad immediately after any accidental contamination with infectious materials. Laboratory personnel should disinfect their hands after manipulating the infectious materials and after removing the gloves and before leaving the laboratory.

Phenol should be used at a concentration of 0.5% and contact time should be 15-30 minutes, depending on the type and volume of material to be disinfected.

Hypochlorite should be used at a concentration of 5%, with a contact time of 15-30 minutes, depending on the type or volume of material to be disinfected. Hypochlorite solutions are useful for the disinfecting of material containing organic debris because of their digestive action.

Glutaraldehyde does not require dilution but an activator (provided separately by the manufacturer) must be added. Glutaraldehyde is usually supplied as a 2% solution, while the activator is a bicarbonate compound. Glutaraldehyde is useful for decontaminating bench surfaces and glassware. The activated solution should be used within two weeks and discarded if turbid.

Alcohols, usually 70% ethanol (methylated spirits) or propanol is used for decontaminating benches and surfaces. It should also be used instead of water to balance centrifuge tubes. When hands become contaminated, a rinse with 70% isopropanol followed by thorough washing with soap and water is effective.

Iodine and Iodophores preparations should be used at a concentration of 3% to 5% and contact time should be 15-30 minutes. Iodophors are useful for mopping up spills and for hand washing.

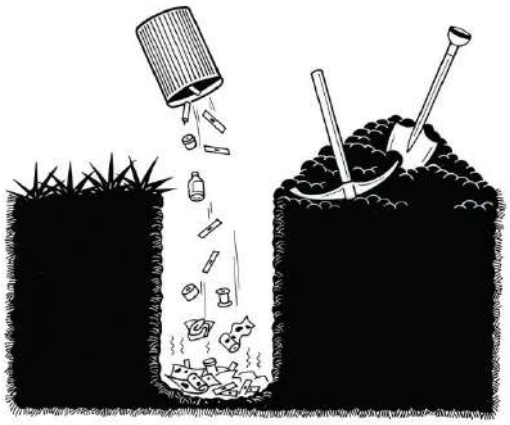
Formaldehyde vapours can be used to disinfect biosafety cabinets and laboratory.

All of the above disinfectants are toxic and undue exposure may result in respiratory distress, skin rashes or conjunctivitis. However, used normally and according to the manufacturers' instructions, they are safe and effective.
Source: SAARC Training Module For AFB Smear Microscopy and Quality Assurance in AFB Smear Microscopy, 2011

4.5 Disposal system

Do not leave any contaminated material without sterilisation after examination. It may be a source of disease transmission to people. Therefore, all the contaminated materials must be disposed of properly.

By burying



Dig a deep hole (depth about one meter). Cover the hole with wood or a plate.

Fill the hole when contaminated material have filled half of the hole.

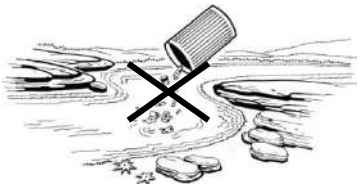
By burning

Incinerator

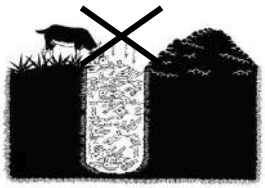
Autoclaving

X Contaminated materials should not be discarded in the following places or ways;

X Not on the ground



X Not in to the river or pond

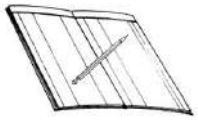


X Do not leave the hole unfilled

X Not in the municipality containers

4.5 LABORATORY MANAGEMENT

Materials needed


Laboratory register book

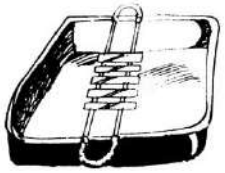

Lab result form


Sputum container *1

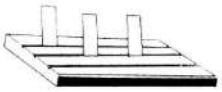

Glass slide


Diamond pencil *3

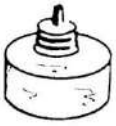
Staining solutions


Staining pan & bridge


Bamboo stick *2

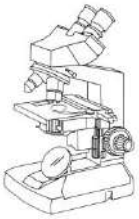

Slide stand


Anisole *4


Spirit Lamp

Steel rod with cotton


Forceps


Microscope


Slide Box

Waste disposal container

Note:

*1. Transparent and leak proof plastic container

*2. Wooden or bamboo sticks can be used. Length of stick should be approximately 10 cm

*3. Ampoule cutter can be used instead of diamond pen for writing the slide number on the glass slide. A marking pen should never be used. Frosted slide can be used instead.

*4. Anisole can be used instead of immersion oil. Anisole can be removed by water therefore it is no need to use xylene

Register Book and Forms

It will be according to HMIS forms. (HMIS-6.1, 6.2)

4.6 PREPARATION OF REAGENTS

High quality reagents should be prepared

- Staining solutions must be prepared in a proper way
- Make sure the balance is in proper level before chemicals are weighed
- Read the bottom of meniscus when you measure liquid
- Use clean equipment

x Never let any chemical mix with other chemicals

- **Label the name of the reagent, concentration and date of preparation on the bottle**
- Store the chemicals in a safe place and keep for the specified period
- Store the reagents in a dark place to avoid exposure to light.
- Store the reagent in dark amber colour bottle.

Formulas to prepare the reagents for Ziehl-Neelsen staining method:

1) Stock carbol fuchsine

Fuchsine (basic)	10 g
Ethanol (95 %)	100 ml

Dissolve the basic fuchsine in ethanol.

2) 5 % Phenol solution

Phenol melted	5 ml
Distilled water	95 ml

Add the melted phenol slowly to distilled water while stirring.

** Gently Warm the bottle with pure phenol crystals to liquefy and measure it with a warm pipette.*

*Be careful that the phenol is corrosive so **Avoid mouth pipetting.***

3) Ziehl's solution (Working carbol fuchsine solution)

Stock alcoholic fuchsine	10 ml
5 % phenol solution	90 ml

Mix the stock alcoholic fuchsine with 5 % phenol while stirring.

Filter solution before use for remove fuchsine crystals or particles.

Preparation of working solution (carbol fuchsine solution) directly

Basic fuchsine powder	1gm
Ethanol (95 %)	10 ml
Phenol melted	5 ml
Distilled water	85 ml

Dissolve the basic fuchsine powder in ethanol

Add distilled water in alcoholic fuchsine with melted phenol while stirring

Filter solution before use to remove fuchsine crystals or particles.

4) 25% Sulphuric acid solution

Sulphuric acid (conc. H_2SO_4)	25ml
Distilled water	75 ml

Add concentrate sulphuric acid slowly to distilled water using a safety pipette. ***Never add water to sulphuric acid.***

Avoid mouth pipetting.

5) 0.1 % Methylene blue solution

Methylene blue	0.1 g
Distilled water	100 ml

Dissolve the Methylene blue in distilled water.

Filter solution before use.

4.7 MICROSCOPE (BRIGHT FIELD AND FLUORESCENCE)

4.7.1 Introduction

Bright field microscopy is the simplest of all the optical microscopy illumination techniques. Sample illumination is transmitted (i.e., illuminated from below and observed from above) white light and contrast in the sample is caused by absorbance of some of the transmitted light in dense areas of the sample. Bright field microscopy is the simplest of a range of techniques used for illumination of samples in light microscopes and its simplicity makes it a popular technique. The typical appearance of a bright field microscopy image is a dark sample on a bright background, hence the name.

A **fluorescence microscope** is an optical microscope used to study properties of organic or inorganic substances using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption. The term "fluorescence microscope" is colloquially synonymous with *epifluorescence microscope,* but also refers to microscope designs such as the confocal microscope which also use fluorescence to generate the image.

4.7.2 Basic principle of fluorescence staining

Mycobacteria retain the primary stain even after exposure to decolorizing with acid alcohol, hence term AFB. A counter stain is employed to highlight the stained organisms for easier recognition. Potassium permanganate is used as counter stain and it helps prevent non-specific fluorescence. With auramine staining, the bacilli appear as slender bright yellow luminous rods. The identification of the mycbacteria with auramine O is due to the affinity of the mycolic acid in the cell walls for the fluorochromes. In fluorescent microscopy, light rays of shorter wave length pass through smear stained with fluorescent dye, such as auramine O, which have the property of absorbing light rays of shorter wave length and emitting light rays of longer wave length. A mercury vapour lamp is used as a source of light and by means of suitable filter only light rays of shorter wave lengths are allowed to emerge and these rays are used for microscopy. The condenser of the microscope is made of quartz which will not absorb UV rays.

Auramine O, also called Basic yellow 2, aizen auramine, Pyoktanin Yellow, Canary Yellow, is a diarylmethane dye used as a fluorescent stain. In its pure form, Auramine O appears as yellow needle crystals. It is very soluble in water and soluble in ethanol. Auramine O can be used to stain acid-fast bacteria (e.g. *Mycobacterium*, where it binds to the mycolic acid in its cell wall) in a way similar to Ziehl-Neelsen stain.

4.7.3 Preparation of fluorescence staining solution (auramine method)

1. Primary stain:

Auramine O
……..……1.0 g
Ethano……..100
.0 ml
Phenol
(melted)…….30.0 ml
Distilled water
……..870.0 ml
Dissolve auramine O powder in ethanol, mix liquid phenol in distilled water and mix both solutions. Keep in a dark bottle with label.

2. Decolourizer:
Hydrochloric acid (conc.)
……10 ml
Ethanol 95%
……..990 ml
Add acid slowly in ethanol.

3. Counter stain:
Potassium permanganate ($KMnO_4$)
…….0.5 g
Distilled water
……...100 ml
Dissolve $KMnO_4$ in distilled water. $KMnO_4$ is explosive, therefore, avoid from combustible materials.

4.7.4 Staining procedure:

1. Place the smeared slide on the staining rack over a sink, leaving some distance between individual slides.
2. Pour auramine O solution (freshly prepared) over the slides so that the smears are completely covered.
3. Leave it for 15 to 20 minutes.
4. Wash with clean water and cover the slides well with acid alcohol solution and leave for 2-3 minutes.
5. Rinse with clean water
6. Flood the slides with counter stain and wait for about 1 minute.
7. Dry it in room temperature.

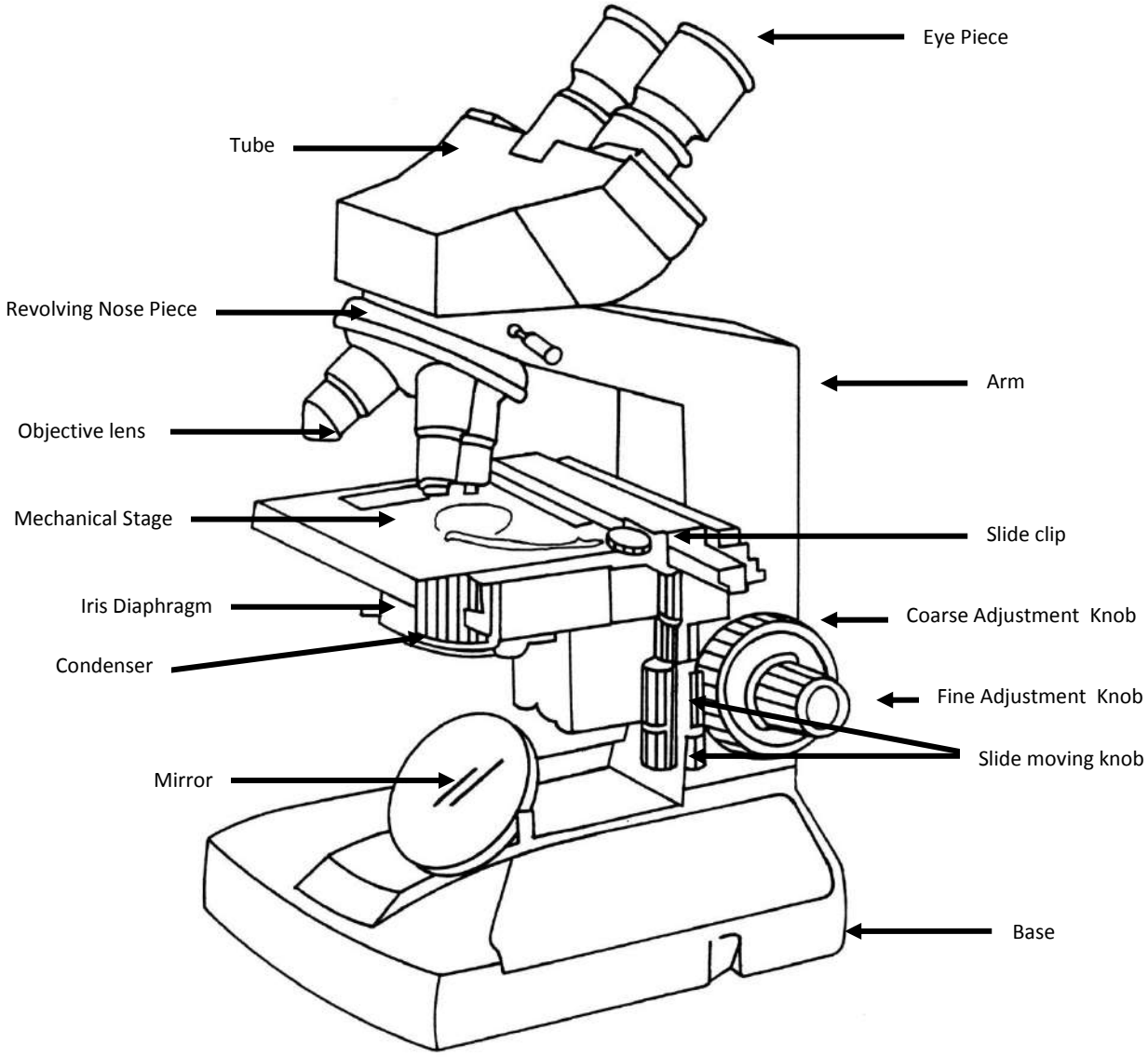
***Note**: In practice; decolourizer and counter stain same to Z-N can be used satisfactorily*

Table 2 : Fluorescence Microscopy AFB Quantification Scales

No. of AFB (200 x)	Result	No. of AFB (400 x)	Result
0	Negative	0	Negative
1-29 / 100 F	Scanty (exact number)	1-19 / 100 F	Scanty (exact number)
30-299 / 100 F	1+	20-199 / 100 F	1+
10-100 / F	2+	5-50 / F	2+
> 100 / F	3+	>50 / F	3+

Source: The Union. Priorities for TB bacteriology Services in Low-Income Countries. Second edition, 2007.

PARTS OF BINOCULAR MICROSCOPE



USE OF BINOCULAR MICROSCOPE

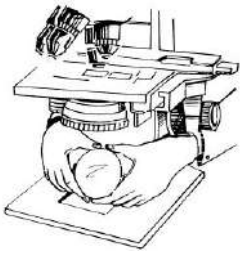


Fig. 1

Fig. 2

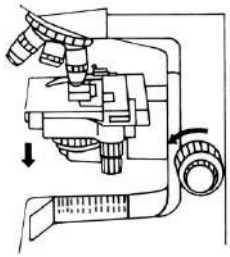


Fig. 3

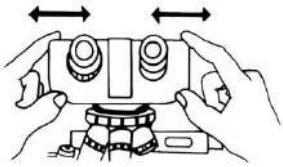


Fig. 4

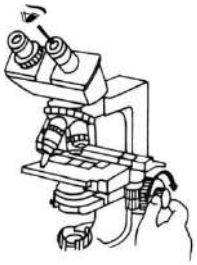


Fig. 5

Fig. 6

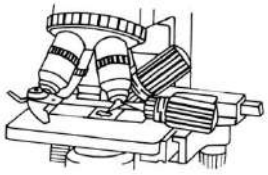
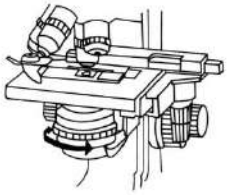


Fig. 7



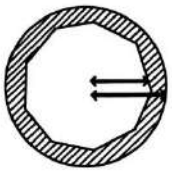


Fig. 8

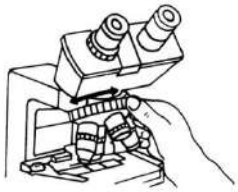


Fig. 9

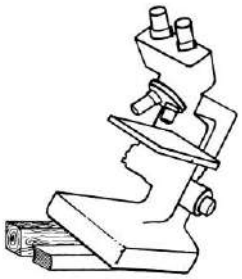
Fig. 1 : Adjust the mirror.
Fig. 2 : Place the specimen slide on the stage.
Fig. 3 : Raise the condenser as high as possible.
Focus the specimen with x 10 objective by turning the coarse focusing knob.
Fig. 4 : Adjust the distance between the eyepieces until both right and left images Become one.
Fig. 5 : Focus the image with the right eye looking into the right eyepiece by turning the focusing knob.
Fig. 6 : Focus the image with the left eye looking into the left eyepiece by turning the diopter ring.
Fig. 7 : Put the Anisole on the smear slide.
Fig. 8 : Remove the eyepiece, and looking at the exit pupil of the objective through the empty eyepiece tube. Open the condenser diaphragm iris to 70 - 80 % of the aperture angle of an objective.
Fig. 9 : Change the objective to x100 objective and focus it using fine adjustment knob.

4.8 MAINTENANCE OF MICROSCOPE

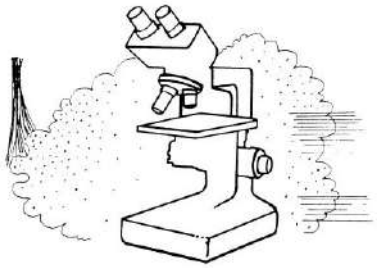
A microscope is a precision instrument therefore it should be maintained carefully.

- Handle the microscope with care
- Store in a cabinet or box
  - In a dry place
  - Dust free place
  - Vibration free place
- Keep lenses clean
  - Use a soft cloth or lens paper moistened with pure alcohol
  - Do not rub the surface of a lens roughly or hard
  - Wipe away immersion oil or Anisole from the surface of an objective every day after use

X Never disassemble the microscope for repairing or for cleaning by yourself

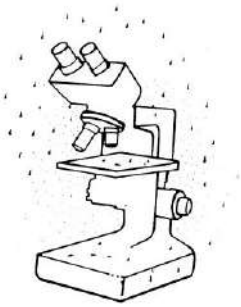


Avoid unstable positions

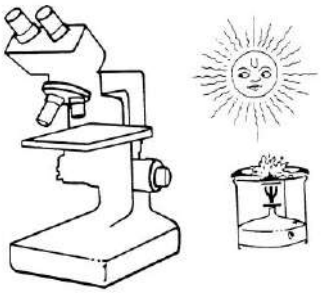


Protect from dust

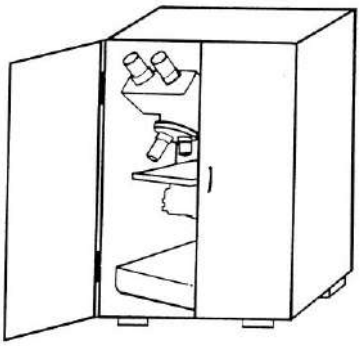
Handle with care



Protect from moisture or direct



Keep away from a heater or direct sunlight

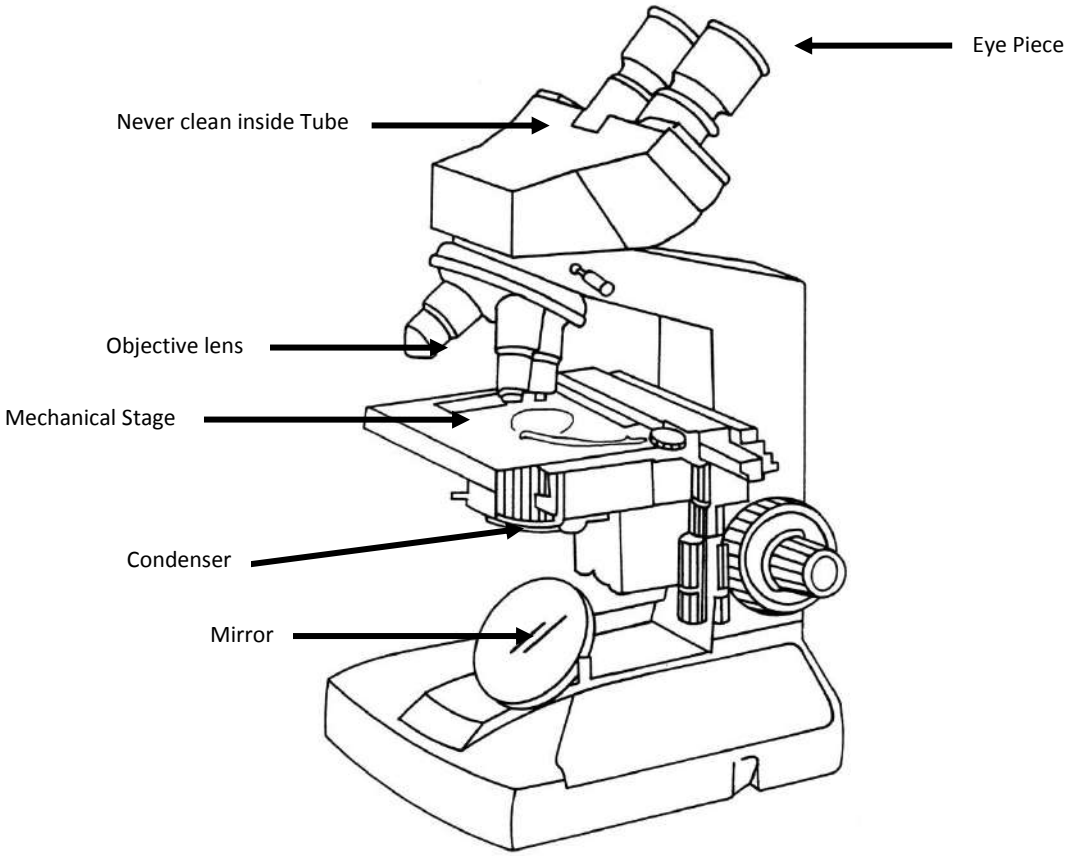


Put vinyl cover on or keep in a box

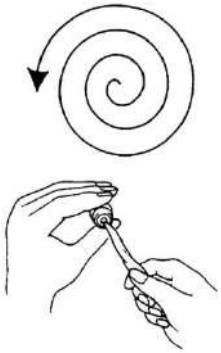
4.9 MICROSCOPE CLEANING

When using a microscope, always start with removing dust in the optical system.
It is recommended that, as a rule, laboratory staff always clean the points indicated below before and after using a microscope.

Dust and Grease cleaning points



Note: for wiping lenses and filters, the basic procedure is to wipe the object from the centre, winding a spiral to the periphery.



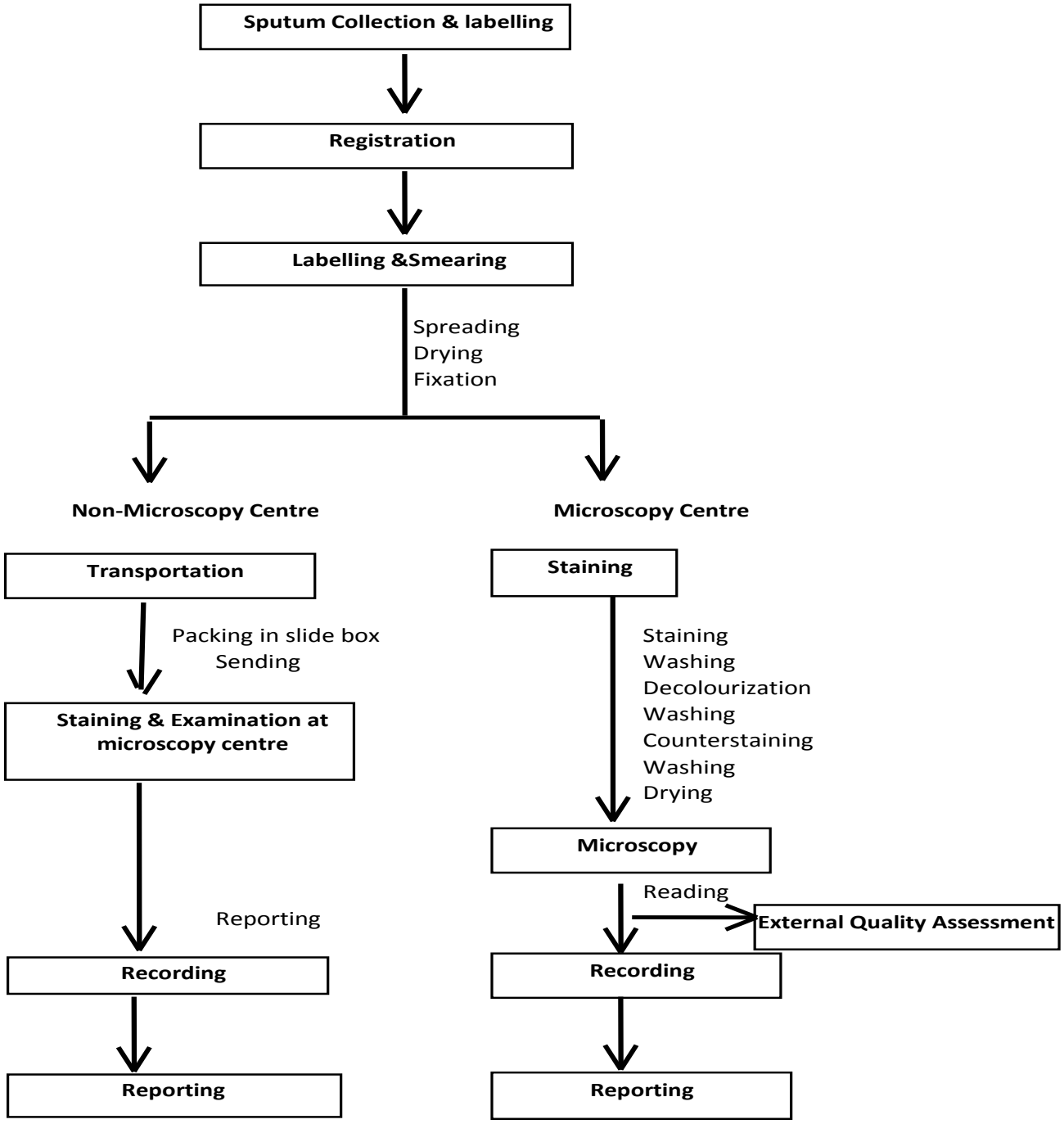
COMMON TROUBLES WITH MICROSCOPE AND POSSIBLE SOLUTIONS

Trouble/Problem	Possible Cause	! Action/ Solution!
• **The brightness of view field is poor or illuminated irregularly.**	* Condenser is too low.	**!** Raise the condenser to correct its position.
	* The mirror is not properly adjusted.	**!** Adjust the mirror correctly or use the concave side.
	* Low light source	! Replace the light bulb
	* Condenser iris diaphragm is Closed.	**!** Open the diaphragm properly.
• **There are dark shadows in the field which move, as you turn around the eyepiece.**	* The surface of the eyepiece has scratches.	**!** A new eyepiece may be needed.
	* The eyepiece is dirty.	**!** Clean the eyepiece.
• **The image is not as clear as it should be.**	* The slide is upside down.	**!** Turn over the slide.
• **Nothing can be seen through the oil immersion objectives.**	* There is an air bubble in the oil.	**!** Move the 100x lens quickly from side to side to remove bubbles.
	* There is dirt on the objective.	**!** Clean the lens.
	* The oil is too sticky.	**!** Use thinner immersion oil or specified immersion oil.
• **The view field through the <u>low power objective</u> is not clear as it should be.**	* There is a layer of dust on the upper surface of the objective.	**!** Clean the lens.
	* There is oil on the lens.	**!** Clean the lens.
	* Objective has been broken or is out of order.	**!** A new objective may be needed.

Chapter V 5. PROCEDURE FOR SPUTUM SMEAR EXAMINATION

5. PROCEDURE FOR SPUTUM SMEAR EXAMINATION

Flow chart 2 : Sputum smear examination Procedure



5.1 SPUTUM COLLECTION, STORAGE AND TRANSPORTATION

Sputum collection:

Sputum should be collected properly. Good quality and quantity of sputum are required as a specimen; otherwise it might give a false result and lead to wrong diagnosis.

Good sputum sample:

- The thick sputum that comes from deep inside the lung
- Includes purulent (yellowish) parts and mucoid (sticky) parts
- Collect sufficient amount of sputum sample (3 ml - 5 ml)

Not only saliva, nasal mucus or blood stained parts.

Good container:

- Disposable, clean, unbreakable, leak proof, wide mouthed plastic container.
- Plastic container SHOULD BE TRANSPARENT
- Newspaper and a used vial of streptomycin must not be used for the sample of culture and drug susceptibility test
- Every specimen container must be labelled with a serial number

Good sputum collection place:

- Well-ventilated room or outside in an open place
- As far from other people as possible

Sputum storage:

- Sputum sample must be kept in the cool place to be protected from insects or mice
- The sputum should not be left at room temperature
- If not kept under cool & dry conditions, the sputum sample has limited validity, and can be stored no longer.
- If the sample is for culture and DST store it in 2-8°c as soon as possible.

Collect 2 Sputum Samples as follows:

1. Supervised **spot sputum** specimen at the first visit.
2. Early **morning sputum** specimen on the next day.

5.2 INSTRUCTIONS TO THE PATIENT FOR SPUTUM COLLECTION

- Explain to the suspects (respiratory symptomatic patient) about the reason for sputum examination
- Explain how many sputum samples are needed
- Give instructions on how to collect the sputum

Instructions should be as follows to get sufficient sputum:

- Gargle to remove any thing remained inside mouth
- Continue to take breaths as deep as possible.

(Fig. a)

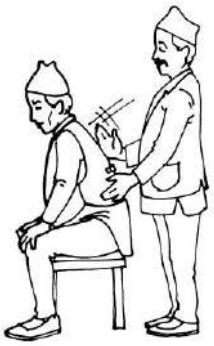
- Drink a glass of warm water, or black tea with pepper or ginger. (Fig. b)
- Let the head down over the side of the bed and cough up.
- Get someone else to pat gently on the back of the patient with his fists. (Fig. c)
- Cough should come from as deep down in the chest as possible. (Fig. d)

x Do not give saliva or nasal mucus.

- The early morning sputum must be collected before eating anything.
- The patients who are taking anti-tuberculosis drugs do not need to stop taking drugs before collection, but they must gargle well to remove the food and anti-tuberculosis drugs remaining in the mouth just before collection of sputum for culture and susceptibility test.

a) drink warm salt water

b) take deep breaths



c) pat gently on back

d) cough up

5.3 SPUTUM TRANSPORTATION

Sputum sample for culture and drug susceptibility test:

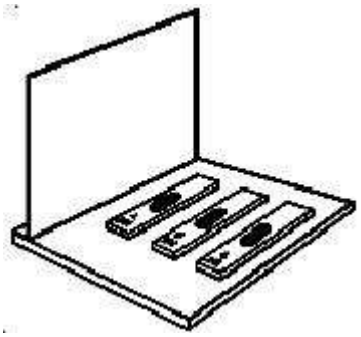
1. Sputum should be collected in a sterile container. It should be kept in a refrigerator until transported, and should be reached to the laboratory within 48 hours of collection.
2. Sputum should be protected from excessive heat and direct sun light.
3. Send sputum to the NTC or the nearest facility where these tests are performed.

Smear slide transportation:

Sputum must be smeared as soon as possible. If unavoidable, a sputum specimen can be kept for a few days, preferably in the refrigerator. Slides must be sent to a Microscopy Centre within two weeks of preparing the smear.

Send fixed smear slides to the Microscopy Centre as follows:

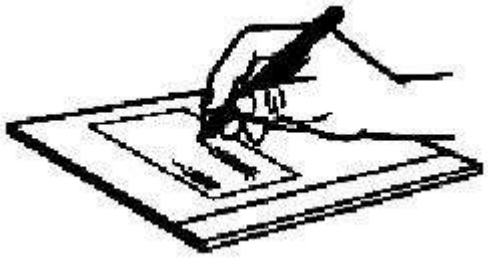
Slide-Box/Mailer

1. 

Put the smears into a slide box/mailer.
Note: Three slides can be put in one mailer. If one suspect has submitted 2 sputum samples, put 2 smear slides from same suspect in one slide box/mailer.

↓

Fill in the sputum request form.

2. 

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Send the smeared slides and request form to the Microscopy Centre.

Registration

The following items should be registered in the laboratory register. (Form HMIS -6.1,6.2)

- Date when first slide was made
- Slide serial number
- Treatment centre code
- Name in full
- Age
- Sex
- Reason for examination (diagnosis or follow-up)
  **** If it is for a follow up examination, record TB number.***

5.4 NUMBERING FOR EXAMINATION SLIDE

Importance:

It is essential to maintain the Laboratory serial number for recording and reporting. One way to make sure the results were initially recorded correctly on the Treatment Card is to compare the result in the TB Register to those in the Laboratory Register. This also gives valuable information when sending slides for Quality Control.

Note:

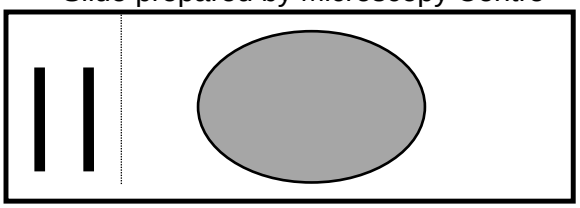
1. It is necessary to write the slide number on the each slide by non-microscopy centre (NMC) and microscopy centre (MC), to differentiate the slides prepared at NMC or MC.

2. When microscopy centres write the slide No. on each slide, either only Nepali or English should be used.
3. For follow up slides, write the slide No. and then mention 'F' and the month of treatment. (eg. Slide No.-F- treatment month, HI Code, date)

The preferable size of the Microscopic glass slide is 75mm X 25mm X 1.3mm approximately.

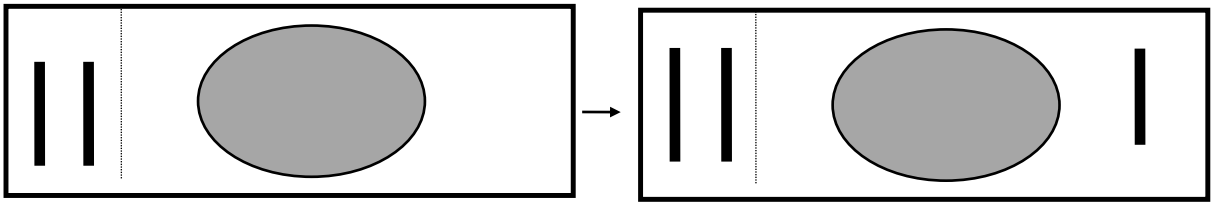
5.5 Instruction for slide numbering:

1. Slide prepared by Microscopy Centre



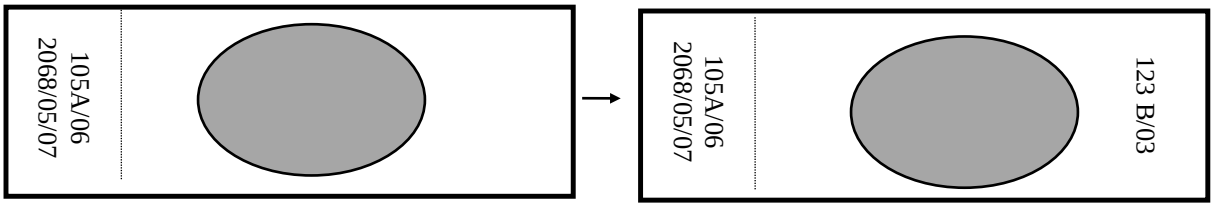
MC Lab. serial No./HI code No. and Date

2. Slide prepared by Non-Microscopy Centre → Slide received at Microscopy Centre



NMC slide No./HI code No./ Date + MC Lab. serial No./HI code No.

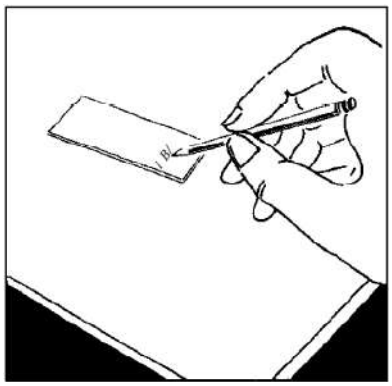
EXAMPLE:



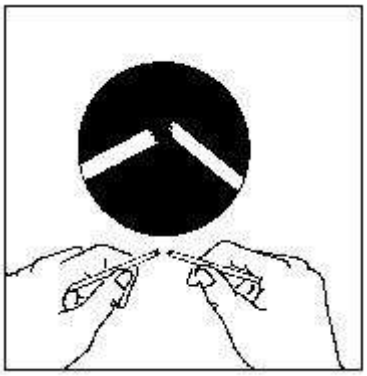
This is the slide sent by NMC

After getting the slide to the MC

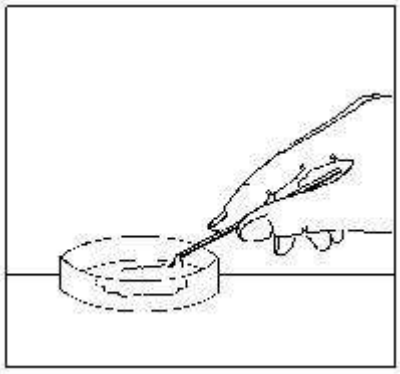
INSTRUCTION FOR SMEARING



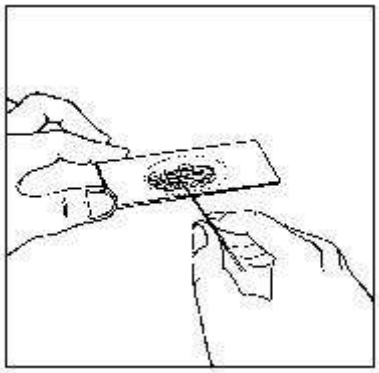
1. Put the serial number one edge of the glass slides.



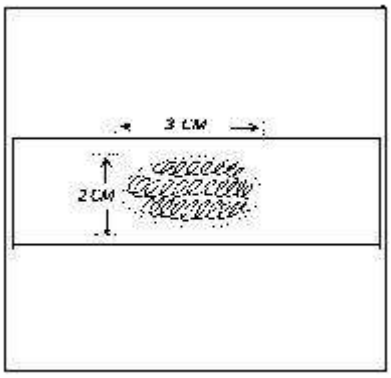
2. Break a bamboo stick in two pieces to make one end rough.



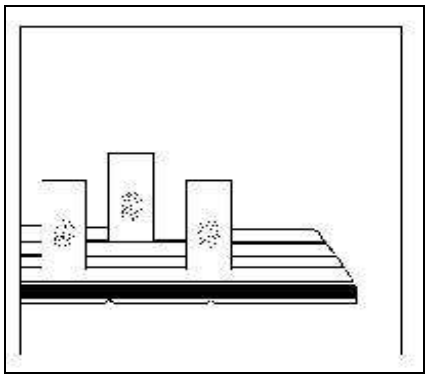
3. Take the yellowish purulent part of the sputum with the stick using the rough end.



4. Spread the sputum evenly on the glass slide.



5. Make a smear approximately 2cm X 3cm size on middle part of the slide.



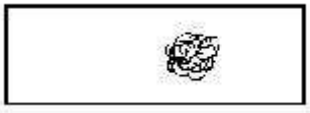
6. Dry the smear at room temperature completely.

Good Smear:

Take a piece of sputum the size of a match stick head

- Spread evenly
- Not too thick and not too thin
- Approximately smear size 1 cm x 2 cm

Bad smear:

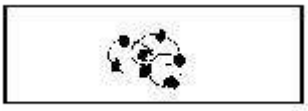


Too small

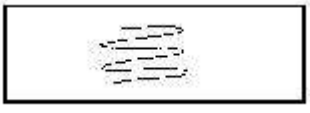
Too big

Too thick

Not centre



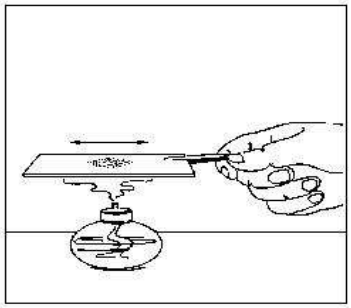
Uneven



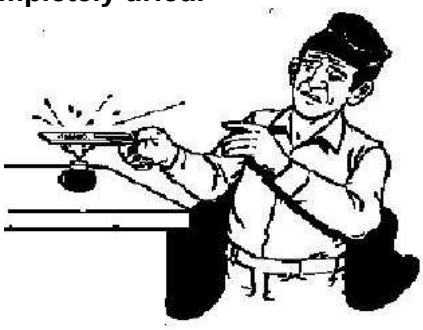
Too thin

Fixation

x Do not put slide over the flame before smear is completely dried.

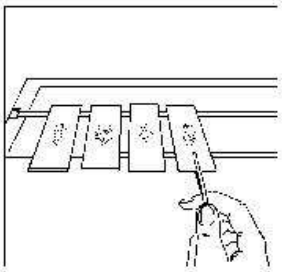


Fix the completely dried smear by passing it through the flame 2-3 times, about 5 seconds each time.

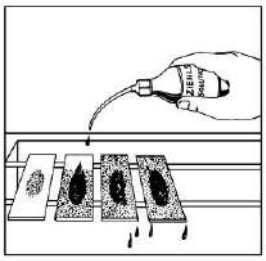


If the slide is put in the flame before smear is completely dried, aerosols of infectious bacteria might be formed.

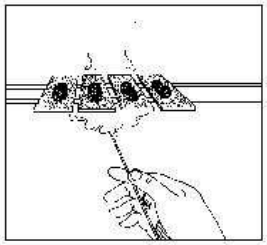
STAINING (ZIEHL - NEELSEN METHOD)



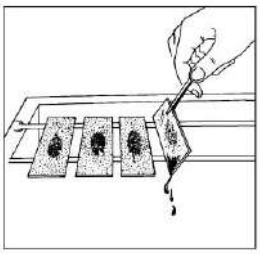
1) Place the slide on the staining bridge.



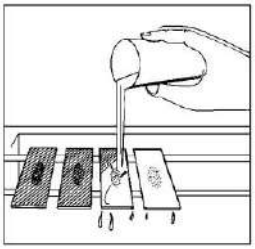
2) Cover the whole surface of the slide with Ziehl's solution (Carbol fuchsin solution).



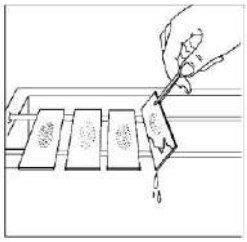
3) Heat the slide until vapour rises from the stain. Leave it for 5 minutes. **Do not boil.**



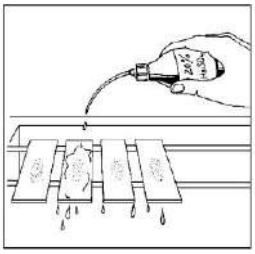
4) Tip off excess staining solution.



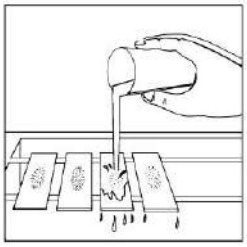
5) Wash off the slide with water.



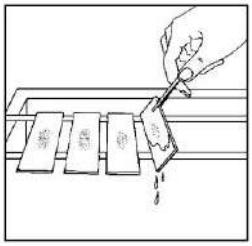
6) Tip off excess water.



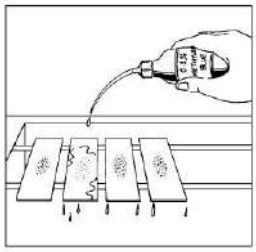
7) Decolorize with 25% Sulphuric Acid until no more stain remains. (about 5 - 10 minutes).



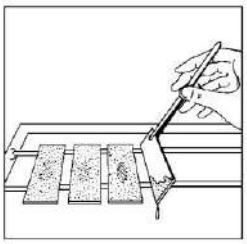
8) Wash off the slide with water. If stain still comes off, repeat No. 7) again.



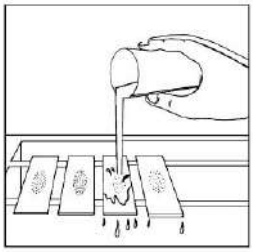
9) Tip off excess water.



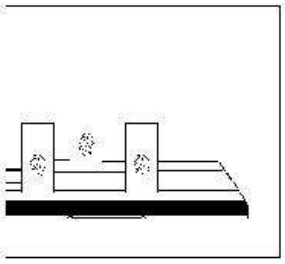
10) Counter-stain with 0.1 % Methylene Blue on the slide for 10 - 20 seconds.



11) Tip off excess staining solution.

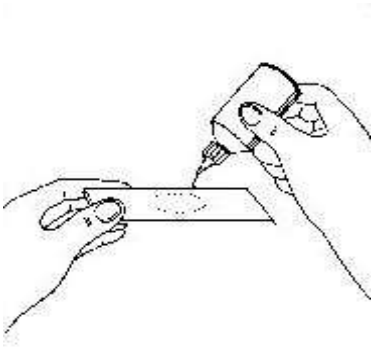


12) Wash the slide with water.

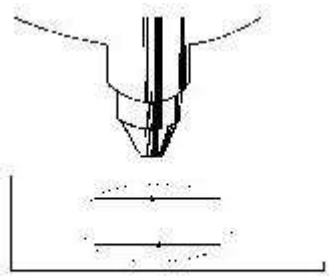


13) Dry the stained slide at room temperature.

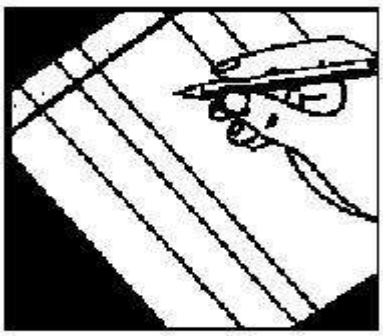
MICROSCOPY EXAMINATION



1) Put one drop of Anisole on the stained smear. Do not touch the smear with the Dropper.

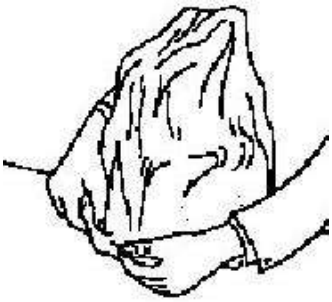


2) Screen the smear with x 10 eyepiece and x 100 objective. Examine 100 visual fields.

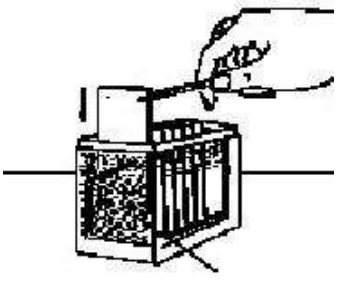


3) Record the result on register book and report form.

4) Clean the objective with Lens/tissue paper or soft cloth.



5) Cover the microscope with vinyl dust cover or soft cloth and put in the box.



6) Wash Anisole off the smear by water and let it dry then keep it in slide box.

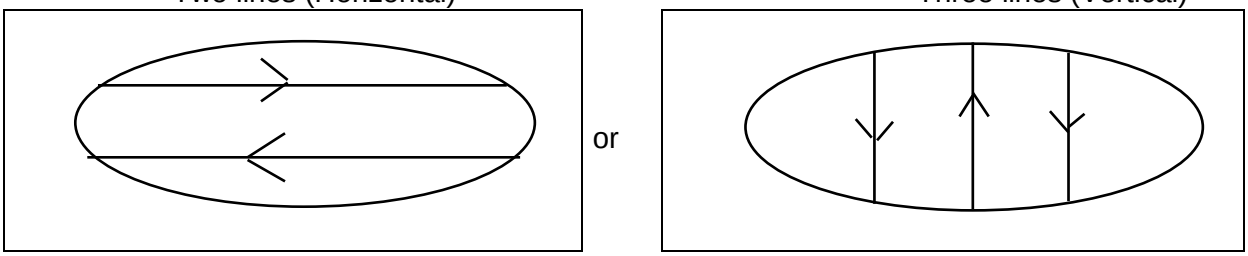
Smear screening

- For **NEGATIVE** result read at least **100 F (fields)**
- For **1-9 AFB,** read at least **100 F (fields)**
- For **1+ result,** read at least **100 F (fields)**
- For **2+ result,** read at least 5**0 F (fields)**
- For **3+ result AFB,** read at least 2**0 F (fields)**

Note: If you screen the smear with the size of 1 cm x 2 cm, two horizontal lines or three vertical lines give 100 V F.

**** F (One Field): the area which is seen at one time through the eyepiece***

Two lines (Horizontal) Three lines (Vertical)



Note: keep the examined slides on serial order then send it according to the LQAS system for EQA

REPORTING SCALE

The number of AFB (Acid fast bacillus) found in an examination of 100 fields, according to the WHO/IUATLD grading scale, is very important information because it is related to the degree of infectivity and clinical severity of the patient.

x Do not report it as "*Mycobacterium tuberculosis*" instead of "AFB"
AFB means any bacteria which stain red or pink by Ziehl-Neelsen staining. Several kinds of AFB are present in nature.

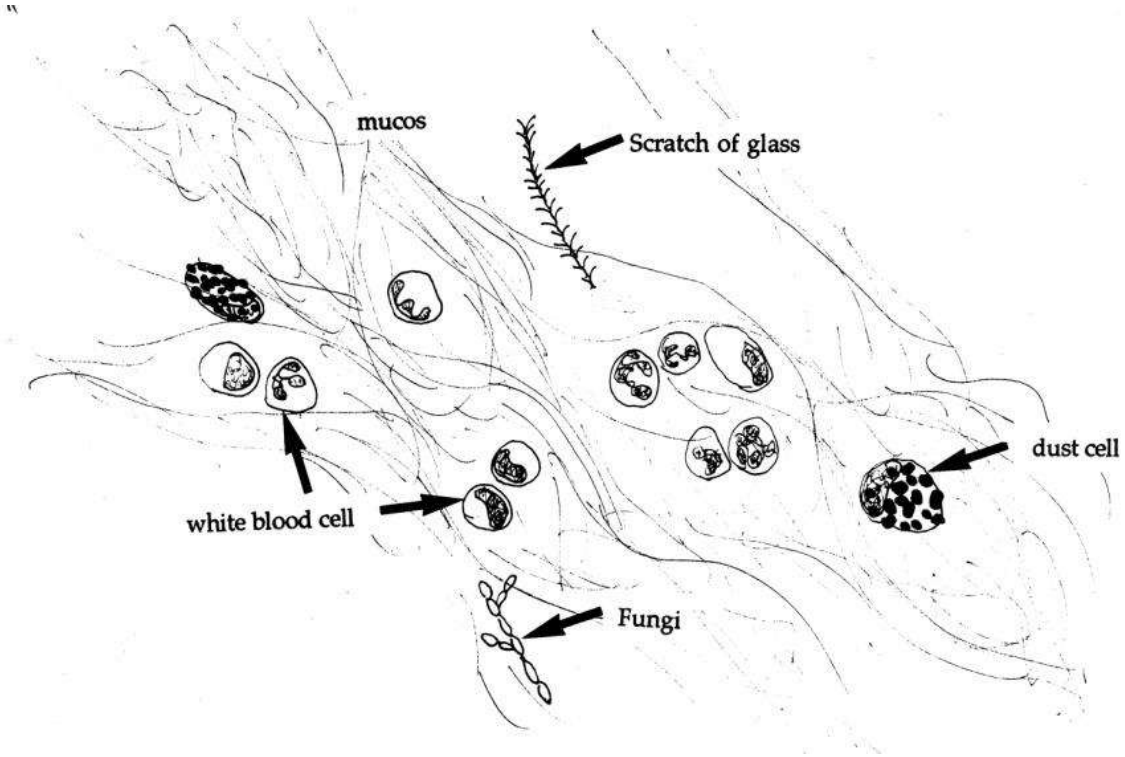
Table 3: Scale (Based on WHO/IUATLD grading scale)

No. of AFB	***Reporting scale***
No AFB found per 100 VF*	**Negative**
1 to 9 AFB found per 100 VF	**Record exact no. of AFB per 100 VF**
10 to 99 AFB found per 100 VF	**1+**
1 to 10 AFB found per VF	**2+**
More than 10 AFB found per VF	**3+**

*VF – Visual field (*the area which is seen at one time through the eyepiece)*

Note: *WHO (World Health Organization)*
IUATLD (International Union Against TB and Lung Diseases/ The Union)

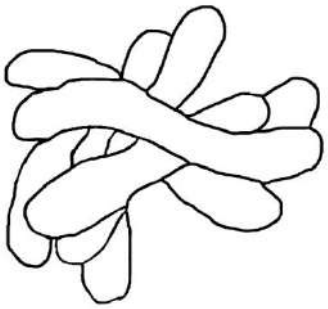
OBJECTS WHICH CAN BE SEEN ON SPUTUM SMEAR SLIDE



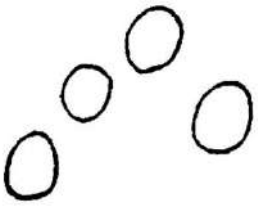
Various shapes of AFB

Rod type

Thin and long type



Clumped type



Coccus type

5.6 MAIN CAUSES OF FALSE POSITIVE/FALSE NEGATIVE SPUTUM SMEAR RESULTS

5.6.1 False Positive Result:
Although the result is **Positive,** the sample itself is actually **AFB negative**

5.6.2 False Negative Result:
Although the result is **Negative,** the sample itself is actually **AFB positive**

Step	Causes	False (-)	False (+)
Specimen collection	- Poor sputum in quality & quantity (Improper collection of sputum)	*	
	- Inadequate volume of sputum	*	
	- Using reusable container		*
	- Artefact such as Nocardia species, spores of bacillus species, pine pollen, food particles		*
Smear preparation	- Using scratched slides		*
	- Poor selection of sputum for smear making	*	
	- Improper preparation of the smear *too thin / too thick / uneven / too little material	*	*
	- Insufficient fixation	*	
	- Contamination from another positive sputum		*
Smear staining	- Over-heated staining	*	*
	- Precipitated stain (Fuchsin crystal)		*
	- Too short staining time with Ziehl's solution	*	
	- Improper decolourization	*	*
	- Intensive counter staining	*	
	- Using low quality of chemicals	*	
	- Using expired solution	*	
	- Transfer of positive smear particle to another		*
Microscopic reading	- Insufficient scanning	*	
	- Physical problem (colour blindness, other visual disturbances) of examiners.	*	
	- Erratic attitude	*	*
Recording Reporting	- Misidentification of patient and specimen	*	*
	- Mis-transcription of the recording & reporting	*	*
	- Mislabelling of specimen	*	*

Note:

- ***False positive (F+)*** results can cause not only unnecessary treatment for non-TB patients but also incorrect change of regimen to category II treatment after 5 months of drug taking.

- ***False negative (F-)*** results can cause not only no treatment for smear positive patients but also incorrect continuation of the first regimen to failure cases.

Chapter VI RECORDING AND REPORTING

6. RECORDING AND REPORTING

Recording: The practice of capturing data on patients' management over time and across clinical sites and writing information either directly on paper forms and/or entering into a computer.

Reporting: The routine tracking (monitoring) of priority program management information and its intended aggregated patient outcome data (evaluation) at the facility, County, State and National Level.

Forms and reports:
The laboratory workers should fill out the following forms and reports on time as needed.

6.1 Sputum sample examination request and report HMIS 6.1

This ***request form*** is used for sending the sputum to be examined from OPD or treatment centre and also this is the sputum examination ***result form*** that is sent from the laboratory or microscopy centre to treatment centre or OPD.

6.2 Laboratory registration book HMIS 6.2 or NTP Lab Register):

This registration book is kept at the microscopy centre.
This is the register, which contains details of all the sputum smear examinations carried out at the microscopy centre.

- The laboratory serial number comes from the laboratory register. It starts from 01 at the beginning of every reporting year (1st Shrawan). The Laboratory staffs write this number on the Smear Request Form, on the Sputum Container, and on the Smeared Slide.
- Every tuberculosis suspect has a separate line in the Laboratory Register. Remember to collect three specimens from each tuberculosis suspect and record the result in the register. Remember to write the suspect's address in full, so that you can trace him if the result is positive and he does not come back.
- Result of follow up smear examinations are also recorded in the register.

For General Information:

6.3 TB Treatment Card (HMIS 6.3)

This card is the record of TB patient under treatment. We make one for each patient and keep it at the treatment centre.

6.4 Patient's Card (HMIS 6.4)

This card contains similar information to the TB Treatment Card. This card belongs to the patient and is filled in by the treatment supervisor.

6.5 TB Treatment Register (HMIS 6.5)

This register contains details of every TB patient registered at the treatment centre. The DTLO/A transfers information from the Tuberculosis Treatment Cards into this register when he visits the treatment centre each month. Four-monthly case finding, sputum conversion and treatment outcome reports are prepared by consulting this register.

* *HMIS- Health Management Information System*

HMIS 6.1: क्षयरोग खकार परिक्षण अनुरोध फाराम
(Tuberculosis Specimen Collection Form)

क्षयरोगको सम्भावित बिरामीको निदान तथा उपचारमा रहेका क्षयरोगका बिरामीको अनुगमन खकार परिक्षण गर्नु पर्ने हुँदा खकार परिक्षणका लागि बिरामीलाई अनुरोध गरी प्रयोगशालामा परिक्षणका लागि पठाऊन यस फारमको प्रयोग गरिन्छ।

यस फारममा दुइ भागहरु छन, पहिलो भागमा खकार परिक्षण अनुरोध गर्नका लागि आवश्यक बिवरण स्वास्थ्यकर्मीले भरि सो सेवा उपलब्ध हुने प्रयोगशालामा पठाइन्छ, दोस्रो भागमा बिरामीको खकार परिक्षण (स्पुटम माइक्रोस्कोपी वा जिनेएक्सपर्ट) गरी सो को नतिजा नतिजा फारममा उल्लेख गरी अनुरोध गर्ने स्वास्थ्य संस्थामा पठाऊनु पर्दछ।

HMIS 6.1: Specimen Collection Form

नेपाल सरकार

स्वास्थ्य तथा जनसंख्या मन्त्रालय

स्वास्थ्य सेवा विभाग

स्वास्थ्य व्यवस्थापन सूचना प्रणाली

खकार परिक्षण अनुरोध फाराम

१. क्षयरोग उपचार केन्द्रः ... ... २. ओपिडि नं. ... ... ३. क्षयरोगी दर्ता नं. ... ... ४. मितिः ... .../ ... ... /... ...

५. बिरामीको नाम र थरः ... ... ६. उमेरः ... ... ७. लिङ्गः ... ...

८. ठेगानाः जिल्लाः ... ... गा.वि.स./न.पाः ... ... वार्ड नं. ... ... टोलः ... ...

९. अभिभावकको नाम ... ... १०. सम्पर्क नं. ... ...

११. परिक्षण गराउनुको कारणः रोग निदान ☐ RR TB/MDR हो ☐ होइन ☐

अनुगमन ☐ महिनाः ... ...

१२. एचआइभि संक्रमणः ☐

१३. क्षयरोगको उपचारः पहिला लिएको ☐ नलिएको ☐ थाहा नभएको ☐

१४. अनुरोध गरिएको परिक्षणः माइक्रोस्कोपी ☐ जिनएक्सपर्ट ☐ अन्य ☐ (उल्लेख गर्ने) ... ...

१५. अनुरोध गर्ने ब्यक्तिको नाम, थर तथा हस्ताक्षरः .....

नतिजा फाराम

स्मेयर माइक्रोस्कोपिक परीक्षण **ल्याव सि.नं. ... ...**

नमुना संकलन मिति (ग.म.सा.)	नमूना	किसिम	नतिजा (कुनै एकमा √ चिन्ह लगाउनुहोस्)					प्रमाणित गर्ने व्यक्तिको दस्तखत	नतिजा आएको मिति (ग.म.सा.)
			नेगेटिभ	१-९/१०० फिल्ड	१+	२+	३+		
	A	B/M/S*							
	B	B/M/S*							

* दृष्टिगत खकारको किसिमः रगत मिसिएको (B), पहेलो र ढिका (M), रयाल मात्र (S)

जिनएक्सपर्ट परीक्षण नतिजा **ल्याव सि.नं. ... ...**

नमुना संकलन मिति (ग.म.सा.)	खकारमा क्षयरोग ब्याक्टेरिया		अन्य (Invalid/ No result/ Error) (I)	खकारमा ब्याक्टेरिया भएको भए यसको प्रकार (कुनै एक महलमा गोलो लगाउनुहोस)			नतिजा आएको मिति (ग.म.सा.)
	Yes	No		रिफाम्पिसिन सेन्सिटिभ (T)	रिफाम्पिसिन रेसिस्टान्स RR)	Indeterminate (I)	
	1	2		1	2	3	

परिक्षण गर्नेको नाम र थरः	प्रमाणित गर्नेको नाम र थरः ... ...
हस्ताक्षरः	हस्ताक्षरः ... ...
पदः	पदः ... ...

कार्ड भर्ने तरिका:

सि.नं.	शीर्षक		निर्देशन
१	क्षयरोग उपचार केन्द्र		उपचार गर्ने स्वास्थ्य संस्थाको नाम यस महलमा लेख्नु पर्दछ।
२	ओ.पी.डी. नं.		क्षयरोगका सम्भावित बिरामीलाई खकार परिक्षणको लागि पठाउंदा दैनिक बिरामी सेवा रजिष्टरको ओपीडी दर्ता नं. लेख्नु पर्दछ।
३	क्षयरोग दर्ता नं.		उपचारमा रहेका क्षयरोगका बिरामीलाई अनुगमन(Follow-up) खकार परिक्षणको लागि पठाउँदा क्षयरोग उपचार रजिष्टरबाट क्षयरोग दर्ता नम्बर लेख्नु पर्दछ।
४	मिति		खकार परिक्षनका लागि पठाइएको मिति (गते,महिना र सालमा) लेख्नु पर्दछ।
५	बिरामीको नाम थर		सेवा लिन आएका व्यक्तिको नाम थर लेख्नु पर्दछ।
६	उमेर		सेवा लिन आउने व्यक्तिको पुरा गरेको उमेर (वर्षमा) लेख्नु पर्दछ।
७	लिंग		सेवा लिने व्यक्ति महिला भए महिला र पुरुष भए पुरुष लेख्नु पर्दछ।
८	ठेगानाः जिल्ला, गा.बि.स., न.पा., वार्ड नं., टोल		बिरामी बसोबास गर्ने ठेगाना जिल्ला, गा.बि.स.रन.पा.,वडा नं.र टोल समेत खुल्ने गरी लेख्नु पर्दछ।
९	अभिभावकको नाम		बिरामीको अभिभावकको नाम लेख्नु पर्दछ।
१०	सम्पर्क नं.:		बिरामीको वा बिरामीको परिवारको घरको फोन नं. वा मोबाइल नं. लेख्नु पर्दछ।
११	परिक्षण गराउनु को कारण	रोग निदान	बिरामीको रोग निदानका लागि खकार परिक्षण गरेको भए रोग निदानमा (√) चिन्ह लगाउनु पर्दछ र RR TB/MDR TB निदानको लागि परिक्षण गरिने हो भने सो समेत उल्लेख गर्नुपर्दछ।
		अनुगमन	उपचारमा रहेका क्षयरोगको बिरामी को अनुभवमा खकार परिक्षण गरिने भए अनुगमनमा (√) लगाई उपचारको कुन महिना (२/३,५ र उपचारको अन्त्यमा) के हो सो महिना लेख्नु पर्दछ
१२	एच.आई.भी. संक्रमण		बिरामी संग भएको प्रमाण को आधारमा एच.आई.भी. संक्रमण भए नभएको एकिन गरी एचआईभी संक्रमण सम्बन्धि छ छैन वा थाहा छैन मध्ये संक्रमण भएको भए कोड १, नभए कोड २ र थाहा नभएको कोड ३ लेख्नु पर्छ *(एचआईभी संक्रमणको अवस्था सम्बन्धि सोध खोज वा अन्य कुनै पनि क्रियाकलाप गर्दा सेवाग्राहीको गोपनीयतालाइ मध्यनजर गर्दै राष्ट्रिय एड्स तथा यौन रोग नियन्त्रण केन्द्रले जारि गरेका नीति नियम तथा निर्देशिकाको पालना अनिवार्य रुपमा गर्नु पर्छ।)*
१३	क्षयरोगको उपचार (पहिले लिएको, नलिएको, थाहा नभएको)		बिरामी संग एकिन गरेर क्षयरोग उपचार पहिला लिएको नलिएको एकिन गरी उपयुक्त एक कोठामा (√) चिन्ह लगाउनु पर्दछ।

१४	अनुरोध गरिएको परिक्षणस(माइक्रोस्कोपी, जिनएक्सपर्ट, अन्य)	परिक्षणको लागि अनुरोध गरिएको माइक्रोस्कोपी वा जिनएक्सपर्ट जुन हो सो को कोठामा (√) चिन्ह लगाउनु पर्छ अन्य परिक्षण (कल्चर,डी.एस.टि वा एल.पी.ए.) भए अन्यमा (√) चिन्ह लगाई विवरण खुलाउनु पर्दछ
१५	अनुरोध गर्ने व्यक्तिको नाम,थर तथा हस्तक्षर	खकार परिक्षण को लागि अनुरोध गरी पठाउने स्वास्थ्यकर्मिको नाम र थर स्पष्ट खुलाई हस्ताक्षर गर्नुपर्दछ

नतिजा फारम:

माइक्रोस्कोपी परिक्षण नतिजा (ल्याबोरेटरीमा भर्नु पर्ने)

स्वास्थ्य संस्थाबाट प्राप्त अनुरोध फारम अनुसार खकार परिक्षण गरि सो को नतिजा यस फारममा भरी जुन स्वास्थ्य संस्थाबाट अनुरोध फारम भरिएको हो सोहि संस्थामा माइक्रोस्कोपी नतिजा पठाउनु पर्दछ ।

ल्या.सि.नं.: त्यस संस्थाको ल्याब रजिस्टरमा उल्लेखित क्रम संख्या अनुसार लेख्नु पर्दछ . हरेक आ.व.मा. ज्ञ देखि नयाँ क्रमसंख्या लेख्नु पर्दछ ।

नमुना संकलन मिति: यस महलमा प्रयोगशालामा खकार नमुना संकलन गरिएको मिति (गते,महिना र सालमा) उल्लेख गर्नुपर्दछ

नमुना: ल्याब परिक्षणको लागि दिएको नमुना पहिलो भए A दोश्रो भए B मा (√) चिन्ह लगाउनु पर्दछ .

किसिम: कस्तो प्रकारको खकार नमुना संकलन भएको हो सो निर्धारण गरी रगत मिसिएको भए B, पहेंलो र ढिक्का परेको भए M र र्याल मात्रै भए S मा गोलो घेरा लगाउनु पर्दछ ।

स्मेयर माइक्रोस्कोपिक नतिजा

माइक्रोस्कोपी परिक्षण पछी प्राप्त नतिजा अनुसार ५ वटा नतिजा महल मध्ये कुनै एउटा महलमा मात्र (√) चिन्ह लगाउनु पर्दछ ।

Scale	Bright Field Ziehl Neelseen stain 1000×Magnification (1 line =2cm=100 field)	Florescence (Auramine stain) 200×magnification (1line=2cm=20 field)	Florescence (Auramine Stain) 400×mmagnification (1line=2cm=40 field)
Negative	No AFB	No AFB	No AFB
Scanty (actual number)	1-9 AFB/line	1-29/ 100F	1-19/ 100 F
1+	10-99/ field	30-299/ 100 F	20-199/ 100 F
2+	1-10 AFB/ field	10-100/ F	5-50/ F
3+	>10 AFB/ field	>100/ F	>50 AFB/ field

Bright Field Ziehl Neelseen stain को लागि तल दिईएको अनुसार गर्नु पर्दछ ।

- AFB नदेखिएमा (Neg) को कोठामा (√) चिन्ह लगाउनु पर्दछ ।
- 1-9 AFB/100 HPF (Scanty) देखिएमा Extra number लेख्नु पर्दछ ।
- 10-99 AFB/100 HPF देखिएमा १ को कोठामा (√) चिन्ह लगाउनु पर्दछ ।
- 1-10 AFB/HPF देखिएमा २ को कोठामा (√)चिन्ह लगाउनु पर्दछ ।
- 10AFB/HPF देखिएमा ३**+** को कोठामा (√) चिन्ह लगाउनु पर्दछ ।

यसैगरी **Florescence Auramine stain** को पनि सम्बन्धित column को नतिजा अनुसार (Neg) मा (√) चिन्ह लगाउने लेख्ने 1+ ,2+ वा 3+ को महल मध्ये एकमा (√) चिन्ह लगाउनु पर्दछ ।

परिक्षण गर्ने व्यक्तिको दस्तखत: यस कोठामा खकार परिक्षण गर्ने व्यक्तिले दस्तखत गर्नु पर्दछ ।

नतिजा आएको मिति: माइक्रोस्कोपी नतिजा निस्केको दिनको मिति (गते, महिना र सालमा) यस कोठामा उल्लेख गर्नुपर्दछ ।

जिनएक्सपर्ट (Gene Xpert) **नतिजा:**

नमुना संकलन गरेको मिति: यस महलमा खकार नमुना संकलन गरिएको मिति (गते,महिना,र सालमा)उल्लेख गर्नु पर्दछ ।

खकार क्षयरोग को ब्याक्टेरिया भए/नभएका](M. Tuberculosis Detected/not Detected-Y/N)**:** खकार परिक्षणमा Tuberculosis Bacteria देखिएमा कोड नं. १ मा र Bacteria नदेखिएमा कोड नं. २ मा गोलो घेरा लगाउनु पर्छ

अन्य(Invalid/No result/Error-I): खकार नमुनाको गुणस्तरजन्य नभएको कारणले परिक्षणको नतिजामा एकिन गर्न नसकेको अवस्थामा अन्य(Invalid/No result/Error-I) मा (√) चिन्ह लगाउनु पर्दछ ।

खकारमा क्षयरोग Bacteria पत्ता लागेमा(M. Tuberculosis Detected): खकारमा क्षयरोग को Bacteria भएमा तीन किसिमको नतिजा मध्ये कुन नतिजा आएको छ सो नतिजा को कोड नं. मा गोलो चिन्ह लगाउनु पर्दछ । जस्तै रिफाम्पिसिन सेन्सीटिभ(Rifampicin Sensitive-T) भएमा कोड नं.१ मा, रिफाम्पिसिन सेन्सीटिभ(Rifampicin Sensitive-RR) भएमा कोड नं. २ मा र इनडीटरमिनेट (Interminatr-TI_ भएमा कोड नं. ३ मा गोलो घेरा लगाउनु पर्दछ ।

नतिजा आएको मिति: जिनएक्सपर्ट परिक्षण पश्चात नतिजा प्राप्त भएको मिति (गते,महिना र सालमा) खकार परिक्षण गर्ने ल्याबकका कर्मचारीले भर्नु पर्दछ ।

परिक्षण गर्नेको नाम तथा हस्ताक्षर: जिनएक्सपर्ट परिक्षण गर्ने ल्याब कर्मचारीको नाम र थर स्पष्ट खुलाई हस्तक्षर गर्नु पर्दछ ।

प्रमाणित गरेको नाम तथा हस्ताक्षर: जिनएक्सपर्ट परिक्षण गर्ने ल्याब कर्मचारीको सुपरभाइजर वा ल्याब प्रमुखको नाम, थर र हस्ताक्षर गर्नु पर्दछ ।

HMIS 6.2: क्षयरोग प्रयोगशाला रजिष्टर
(Tuberculosis Laboratory Register Specimen)

क्षयरोग प्रयोगशाला रजिष्टर														
ल्याब सि.नं.	नमुना संकलन मिति			विरामीको		जाति कोड	अभिभावक को नाम थर	सम्पर्क नं.	उमेर		विरामीको ठेगाना			अनुरोध गर्ने उपचार केन्द्रको नाम
	ग	म	सा	नाम	थर				म	पु	जिल्ला	गा.वि.स./न.पा.	वडा नं.	
१	२	३	४	५	६	७	८	९	१०	११	१२	१३	१४	१५

क्षयरोग प्रयोगशाला रजिष्टर											
क्षयरोग निदानका लागि प्रेषण			क्षयरोग ओपी डी दर्ता नं.	एचआईभी संक्रमण			क्षयरोग को पहिला उपचार भए नभएको			परिक्षणको प्रकार	
निजी स्वास्थ्य संस्था (P)	समुदाय (c)	सम्पर्क परिक्षण(T)		भएको	नभएको	थाहा नभएको	भएको	नभएको	थाहा नभएको	निदान	अनुगमन (महिना लेख्ने)
१६	१७	१८	१९	२०	२१	२२	२३	२४	२५	२६	२७

परिक्षण नतिजा मिति												कैफियत
जिन एक्सपर्ट				स्मेयर माइक्रोस्कोपी								
				A				B				
नतिजा *	ग	म	सा	नतिजा*	ग	म	सा	नतिजा *	ग	म	सा	
२८	२९	३०	३१	३२	३३	३४	३५	३६	३७	३८	३९	४०

Chapter VII QUALITY Assurance FOR SPUTUM SMEAR Microscopy

7. QUALITY ASSURANCE FOR SPUTUM SMEAR MICROSCOPY

7.1 Introduction

Quality assurance of sputum microscopy is an indispensable part of an effective TB control program. It encompasses the whole process of sputum collection, smear preparation, smear staining, microscopy, recording and reporting.
The purpose of quality assurance programs is the improvement of the efficiency and reliability of smear microscopy services.

In many countries with a high prevalence of tuberculosis (TB, direct sputum smear microscopy remains them most cost effective tool for diagnosing patients with infectious tuberculosis and for monitoring their progress on treatment. The World Health Organization strategy for tuberculosis control (DOTS) relies on a network of laboratories that provide acid fast bacilli (AFB) sputum smear microscopy. The establishment of a broad network of well-Functioning peripheral laboratories within the context of the health system and readily accessible to the population is a high priority for any tuberculosis control program. If the laboratory diagnosis is unreliable, all other activities will be affected. However, the quality of laboratory services often may not be considered a high priority of the National Tuberculosis Program (NTP). Microscopy errors are likely to result in failure to detect persons with infectious TB who will then continue to spread infection in the community, or unnecessary treatment for "non-cases". Errors in reading follow up smears can result in patients being placed on prolonged treatment or re-treatment, or in treatment discontinued prematurely. Therefore, quality assurance of laboratory services, including AFB sputum smear microscopy, is essential. Both the availability and quality of AFB smear microscopy are dependent on national programs that support, train, and monitor the testing performance of individual laboratories.

7.2 A quality assurance program has three main components:

a. **Quality Control (QC):** Quality control is a process of effective and systematic internal monitoring which aims to detect the frequency of errors against established limits of acceptable test performance. Although it is not usually feasible to determine error frequencies accurately, it is nevertheless a mechanism by which tuberculosis laboratories can at least validate the competency of their diagnostic services

b. Proficiency testing: Also known as External Quality Assessment (EQA), this is a program designed to allow participant laboratories to assess their capabilities by comparing their results with those obtained with the same specimens in other laboratories of the network, e.g., Regional and National Reference Laboratories. EQA might be on-site evaluation of the laboratory for review QC and on-site rereading of smears.

c. Quality Improvement (QI): A process by which the components of smear microscopy diagnostic services are analyzed with the aim of looking for ways to permanently remove obstacles to success. Data collection, data analysis, and creative problem solving are the key components of this process. It involves continued monitoring, identifying defects, follows by remedial action including retraining when needed, to prevent recurrence of problems. QI often relies on effective on-site evaluation visits.

7.3 INTERNAL QUALITY CONTROL

1. Poor quality sample gives false result. Always ask for the good quality sample. Mucopurulent sample is recommended.
2. Too thick or too thin smear can give false result. The thickness of smear should be such that we can partly read newspaper kept back of the slide.
3. Too old reagent may give false result. Carbol fuschin and methylene blue should be filtered before use.
4. All newly prepared staining reagents should be checked for its quality by staining a known positive and negative slide. If the known positive slide gives the negative result, that staining reagent must not be used.
5. All the steps of staining procedure should be strictly followed according to SOP (Standard operating procedure). Any alteration in the procedure can give false result.
6. Distilled water should be used in staining instead of Tap water. (tap water may give false positive result.)
7. High rate of positivity among the suspected patient may be due to false positive and low rate of positivity may be due to error in reagent, microscope and staining procedure.

7.4 LABORATORY NETWORK:

It is very important to know the level of various laboratories network:

7.4.1 Peripheral

Laboratories located at primary health centers or district hospitals. Staff have technical proficiency to perform sputum smear microscopy utilizing Ziehl-Neelsen (ZN) staining. Peripheral laboratories must be visited on a regular basis by a district supervisor, who has been adequately trained to evaluate the basic functions of the microscopy.

7.4.2 Intermediate

Regional or provincial laboratories existing in larger hospitals or cities. Staff have technical proficiency to perform ZN microscopy, and may have capacity to perform fluorescence microscopy if volume is high. Intermediate laboratories should be capable of providing supervision, monitoring, training, and quality assurance to peripheral laboratories, including rechecking of smears.

7.4.3 National Reference or Central

May exist as part of the central public health laboratory, a research laboratory, or as an upgraded laboratory in the country's principal tuberculosis institution. Serves as the national reference laboratory for the TB program, with competence in direct ZN microscopy and, where appropriate, fluorescence microscopy. The national TB reference laboratory plays an essential role in the organization and maintenance of the network in terms of developing guidelines, ensuring high quality and standardized smear microscopy, and therefore must have the capacity to provide training and External Quality Assessment, including providing panel testing and rechecking to intermediate and peripheral laboratories.

What is an External Quality Assessment System (EQAS) for sputum microscopy?

External Quality Assessment (EQA) system is defined as a system to ensure high quality laboratory services to detect AFB in sputum smear microscopic examinations. It consists of adequate sputum collection and handling, proper sputum smear preparation, proper staining, correct microscopic examinations of the slide and appropriate recording & reporting.

EQA or Proficiency testing is a system of retrospectively and objectively compared results from different laboratories by external agency or Reference laboratory. There are three methods of EQA:

- On-site Evaluation
- Panel Testing
- Blinded Rechecking

On-site Evaluation

A field visit is the best method to obtain a realistic picture of the conditions and practices in the laboratory; therefore, on-site evaluation of peripheral laboratories is an essential component of a meaningful EQA programme.

Panel Testing

Panel testing is a method of EQA that is used to determine whether a laboratory technician can adequately perform AFB smear microscopy
A panel of 10 sputum slides (5 stained & 5 unstained) with known result form NRL to IRL, IRL to PL

Random Blinded Rechecking (RBR)

Blinded rechecking or rereading a sample of routine smears from the peripheral sites and intermediate labs by controllers at a higher level laboratory is considered the best method for evaluating performance and providing motivation to staff for improvement. **A countrywide program for blinded rechecking of slides at regular intervals should be the long-term goal for optimal EQA.**

Till the date Random Blinded Rechecking is implemented as a method of EQA for sputum smear microscopy throughout the country.

Blinded rechecking or rereading a sample of routine smears from the peripheral sites and intermediate labs by controllers at a higher level laboratory is considered the best method for evaluating performance and providing motivation to staff for improvement. ***A countrywide program for blinded rechecking of slides at regular intervals should be the long-term goal for optimal EQA.***

Function of Regional TB Quality Control Centre
NTP has established Regional TB Quality Control Centres (RTQCC) in all the five regions in July 1996 (2053/54).

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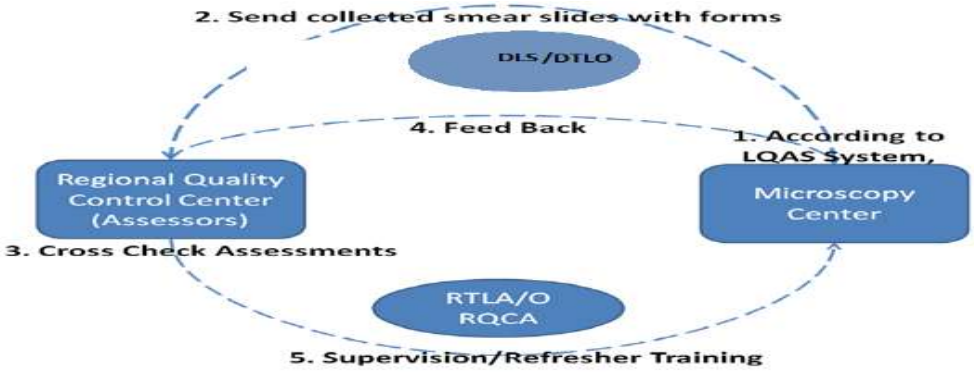
The functions of regional quality control centre are as follows;

1. ***Quality check centre for examined slides,*** and feedback those results to the microscopy centres.
2. Regional ***training centre*** for TB microscopy.
3. ***Reagent preparation*** and ***distribution centre*** in the region.
4. Regional quality control assessors, who are working at the regional TB quality control centre, are mainly t***echnical supervisor*** for the microscopy centres.

7.5 Method of External Quality Assessment (EQA)

EQA is carried out on a four month basis.



1. **Microscopy Centre keeps all** examined sputum slides serially as per lab register, so that supervisor {District Laboratory Supervisor (DLS)/DTLA (O)} can selects the slides according to LQAS System for EQA.
2. Microscopy Centre sends those selected slides with results filled in LQAS-1 to Regional TB Quality Control Centre/Assessor through DLS or DTLA/O.
3. Assessor re-exams those smear slide using LQAS – 2A, If discrepancy arise LQAS-2B will be filled and send to NQCC.
4. Assessor sends Quality Check /Feedback Report (LQAS- 3) to Microscopy Centre through {District Laboratory Supervisor (DLS)/DTLA (O)}
5. If the quality of TB slide is not satisfactory, or any technical problem identified as per DLS or DTLA/O's supervision report, Regional Quality Control Assessor (RQCA) visits the Microscopy Centre and supervises as soon as possible. Also the RQCA, in co-ordination with RTLA/O (Regional TB/Leprosy Assistant/Officer), reports to the training centre about the necessity of refresher training to that Microscopy Centre, so that the training could be organized by central or regional level.

LOT QUALITY ASSURANCE SYSTEM (LQAS): LQAS is a method to determine an optimum sample size which when applied properly, yields statistically acceptable samples to assess quality of work, in this case, the work of the laboratory technicians. This method was originally designed for manufacturing processes where an efficient statistical model was necessary in order to keep sampling costs to a minimum. This method has been applied in health care systems to determine whether a population meets a certain standard. A number of variables are used to determine sample size using LQAS.

Lot (N) : Total number of negative slides prepared in a specified period of time (one months, one quarter, one year). It is an operational quantity used to determine the sample size. Example: Lot = 5000/yr, 1250/quarter, 417/month. It is important to choose an interval of time that produces a Lot size that results in an economical and statistically valid sample. If the Lot size is too small, this may not be possible.

Acceptance Number (d): The maximum number of false negative errors allowed in the sample after which the NTP/NRL can no longer be certain that the expected performance has been achieved. The value chosen for "d" has a direct impact on sample size, the larger the acceptance number, the larger the sample size required. In order to achieve the smallest, most efficient sample size, a value of d=0 is recommended.

Slide Positivity Rate (SPR): This is the proportion of positive smears among all slides (diagnostic and monitoring) in the laboratory from which the sample is being taken. This number is estimated using the laboratory registers from the previous year or the preceding four quarters. Sample sizes can be set using the average positivity rate for a laboratory, region or country.

Sensitivity: This is the expected performance in detecting positives, as compared to the controllers. Acceptable sensitivity should be determined by the NTP and NRL. The sensitivity, as defined here, is the detection of all positives, including low positives (1-9 AFB/100). Therefore, an overall sensitivity of 75-85% is recommended.

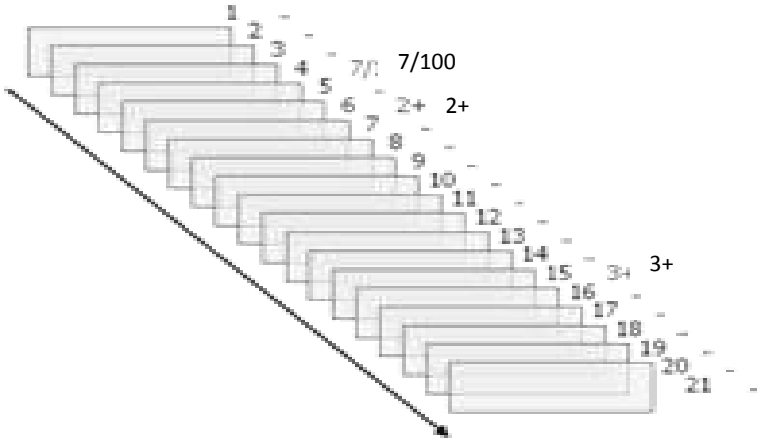
Specificity: This is the expected performance in detecting negatives as compared to the controllers, which is set at 100%.

Confidence Interval (CI): All of the sample sizes have been developed to determine if the laboratory has met the expected sensitivity within a 95% confidence level. Therefore, if the d=0 and there are no false negatives detected within the sample then the NRL can determine with a 95% confidence level that the peripheral laboratory is performing at or above the acceptable sensitivity.

Slides collection/sampling for EQA

To make the slide rechecking programme valid representation of routine laboratory performance, the sample collected should be random and representative of all the smears examined by lab staff. The technical requirements for sampling are outlined here;

Slide storage: store all the cleaned slides in the slide boxes in the same order as they are listed in lab register. (Shown in the figure)



Slide collection/selection: DTLA/O in coordination with Lab staff will collect/select the slides during quarterly visit to MC. The selected slides will be collected by DTLA/O using the lab register for EQA.

* Method of selecting slides for Blinded Rechecking from TB Lab Register. Circles indicate slides to be collected:

Lab. S. No	Date	Name	Sex M/F	Name of Treatment Unit	Address New Patients	Reason for Examination		Result		Sign	Remark
						Diagnosis	Follow up	A	B		
								Neg			
								Neg	Neg		
								Neg	Neg		
								Neg			
								Neg	Neg		
								Neg	Neg		
								Neg	Neg		
								Neg	Neg		
								Neg			
								~~Neg~~	Neg		
								Neg	Neg		
								Neg	Neg		
								Neg	~~Neg~~		
								Neg	Neg		
								Neg	Neg		
								Neg	(2+)		
								Neg			
								~~Neg~~	Neg		
								Neg	Neg		
								Neg			
								Neg	Neg		
								Neg	**Neg**		

7.6 SAMPLE SIZE DETERMINATION WITH EXAMPLES

There are some important steps for sample size determination;

Step 1

From the lab register of the previous year:
Calculate the total slides examined/year = 1250
Total No. of positive slides/year = 125
Total No. of negative slides/year =1125

Step 2

Calculate slide positivity rate (SPR) = total No. of positive slides per year / total No. of slide *100 (round off to the nearest % i.e. 10 %. (*SPR is valid for one yr only or new SPR should be calculated for each year)*

Step 3

Decide on acceptable limits of performance in your lab
Relative sensitivity (ability of lab staff to detect AFB relative to the controller) = 75%
Acceptance No. {(Maximum number of errors (d)} = 0

Step 4

Select appropriate sample size table
If a sensitivity of 75 % and d = 0 is chosen, look at the left side of the table to find the annual negative slide volume = 1000 and SPR =7.5%, then sample size for this year = 96

Step 5

Decide on a convenient interval to select the slide
Recommended 3 times per year i.e. quarterly. Therefore, 96/3 = 32 slides to be collected every quarter (for current fiscal year)

Step 6

Systematically collect the slides using the laboratory register
Suppose 250 slides have been processed during last quarter (the quarter participating in EQA), 32 slides need to be collected for the current quarter, therefore: 250/32 = 7.8
Collect every 7^{th} slide.

Step 7

For the first or starting slide; use the last digit of a bank note (repeat for digit 0 or 1)

The annual sample size should be fixed beforehand by the Reference Level using LQAS Table 4 and 5.

Recommended annual sample sizes: Table 4 and 5 shows the number of slides recommended for blinded rechecking at various workloads and slide positivity rates. The table has been compiled on the basis of a sensitivity of 75% and specificity of 100% (for negative slides); acceptance number for errors equal to zero (d=0); and confidence level of 95%. The number of slides to be selected (sample size) should be fixed beforehand by the NTP.

Determining sample size should not be left to the supervisor collecting the slides or to the technicians.

Table 4: Sensitivity Relative to the Controllers at 75%

Negatives Examined Annually	Acceptance Number	Slide Positivity Rate (SPR) Total Sample Required						
		2.5%	5.0%	7.5%	10.0%	13.0%	15.0%	18.0%
100	d=0	78	63	54	47	40	36	32
200	d=0	123	91	71	59	48	42	37
300	d=0	154	105	80	64	52	45	38
400	d=0	175	115	85	68	53	47	39
500	d=0	192	121	89	69	54	47	40
700	d=0	215	129	93	72	55	48	40
1000	d=0	236	136	96	73	56	49	41
2000	d=0	267	145	102	77	59	51	41
5000	d=0	289	152	104	78	59	51	43
10000	d=0	297	154	105	78	60	51	43
20000	d=0	302	155	106	79	60	52	43
50000	d=0	305	156	106	79	60	52	43
100	d=1	103	96	85	74	64	59	52
200	d=1	187	143	116	96	79	71	61
300	d=1	241	169	132	106	85	75	65
400	d=1	280	186	141	111	89	78	66
500	d=1	309	198	147	114	91	80	67
700	d=1	349	213	155	119	93	81	68
1000	d=1	386	224	160	123	95	82	70
2000	d=1	439	241	169	128	98	85	71
5000	d=1	478	252	173	130	99	86	72
10000	d=1	493	256	175	131	100	86	72
20000	d=1	501	258	176	131	100	86	72
50000	d=1	506	259	176	132	100	87	72

Table 5: Sensitivity Relative to the Controllers at 75%

Negatives Examined Annually	Acceptance Number	Slide Positivity Rate (SPR)						
		Total Sample Required						
		20.0 %	23.0 %	25.0%	28.0%	30.0%	33.0%	35.0%
100	d=0	30	26	24	22	21	19	18
200	d=0	34	29	27	24	23	19	18
300	d=0	35	30	28	25	23	21	18
400	d=0	35	31	28	25	23	21	18
500	d=0	36	31	28	25	23	21	20
700	d=0	36	31	29	25	23	21	20
1000	d=0	38	31	29	25	23	21	20
2000	d=0	38	32	29	25	24	21	20
5000	d=0	38	32	29	26	24	21	20
10000	d=0	38	32	29	26	24	21	20
20000	d=0	38	32	29	26	24	21	20
50000	d=0	38	32	29	26	24	21	20
100	d=1	49	44	41	38	36	33	31
200	d=1	56	49	45	40	39	34	32
300	d=1	59	51	47	42	39	36	34
400	d=1	60	52	48	43	40	36	34
500	d=1	61	52	48	43	40	36	34
700	d=1	61	53	49	43	40	36	34
1000	d=1	63	53	49	44	41	37	34
2000	d=1	64	55	51	44	41	37	34
5000	d=1	64	55	51	44	41	37	34
10000	d=1	64	55	51	44	41	37	35
20000	d=1	65	55	51	44	41	37	35
50000	d=1	65	55	51	44	41	37	35

The volume of annual sample size is calculated for sensitivity relative to the controllers at 75%; specificity 100% and acceptance number = 0

Note :

- MCs < 5% SPR should analyze the reasons

- ANSV(Annual Negative Sample Volume) < the indicated Annual Sample Size;
  - MC should submit all the slides

7.7 ASSESSMENT POINTS OF STAINED SMEAR SLIDES

- **Sputum quality: by microscopic observation**

1) The presence of dust cells (macrophages) and leukocytes in the sputum smear is considered as sputum, which is evidence that it is not saliva. The presence of more than 25 leukocytes and less than10 epithelial cells per field at the total magnification of x 100 is considered proper sputum specimen.

- **Smear making technique : by macroscopic observation**

2) **Size of smear**
Acceptable size of smear should be approximately 1 x 2 cm. Two horizontal lines or three vertical lines of reading would cover about 100 fields.

3) **Thickness of smear**
Acceptable thickness of smear can be checked by looking at printed letters thorough the smear holding the smeared glass slide 4 - 5 cm over a printed paper. If letters cannot be read, it is too thick.

4) **Evenness of smear**
Sputum should be spread evenly on the glass slide. Not too thick and not too thin.

- **Staining technique: by microscopic observation**

5) **Condition of decolourization of the smear stained by Ziehl-Neelsen method**

AFB and background of the smear must be clearly distinguished by decolourization. AFB in faint red colour is over-decolourization. Under-decolourization is observed by remaining of Fuchsine colour on the background or remaining of Methylene blue on the unsmeared parts.

6) **Smear cleanness**
Stained smear must be free from stain deposits, dirt, crystals produced by overheating of staining, debris, etc.

Microscopic reading technique

Assessor reads smear slides and compares his/her results with the result of Microscopy Centre. Analysis and calculation of overall agreement rate and false (+)/ (-) result can be done by using correlation table.

7) **Agreement:**

Number of Negative (-ve) and positive (+ve) result consistencies within range by grading.

8) **Disagreement:**

False (+) ve : Although the result of microscopy centre is ***Positive,*** the sample itself (result of assessor) is ***AFB negative***

False (-) ve : Although the result of microscopy centre is ***Negative,*** the sample itself (result of assessor) is ***AFB positive***

Table 6: Correlation table

Sputum Smear Results →		***Result of Test Laboratory (Microscopy Centre)***					
		Negative	*1-9/100VF*	*1+*	*2+*	*3+*	***Total***
Result of Controlling Laboratory (RQCA/O)	*Negative*	**Negative consistency**	*False (+ve)*				***Assessor's total results***
	1-9 /100VFf	*False (-ve)*	**Positive**				
	1+			**Consis**	**Tency**		
	2+						
	3+				**within**	**Range**	
	Total	***MC's total results***					***Grand total***

Example:

Result by Assessor	**Report from Microscopy Centre**					
	Neg	**1-9/100F**	**1+**	**2+**	**3+**	**Total**
Neg	**10**					**10**
1-9/100F	**2**					**2**
1+	**3**	**1**				**4**
2+			**1**	**1**		**2**
3+					**2**	**2**
Total	**15**	**1**	**1**	**1**	**2**	**20**

$$\textbf{Overall Agreement Rate} = \frac{\text{Number of (-ve) and (+ve) consistencies Within range by grading}}{\text{Grand Total}} \times 100\ (\%)$$

$$\textbf{Sensitivity} = \frac{\text{Total No. of +ve result agreed between MC and QC}}{\text{Total +ve result of RQCA}} \times 100$$

Example; $\frac{5}{10} \times 100 = 50\%$ (Se. 50%)

$$\textbf{Specificity} = \frac{\text{Total No. of –ve result agreed between MC and QC}}{\text{Total –ve result of RQCA}} \times 100$$

Example; $\frac{10}{10} \times 100 = 100\%$ (Sp. 100%)

$$\textbf{False negative} = \frac{\text{Total F -ve results}}{\text{Total –ve results of MC}} \times 100$$

Example; $\frac{5}{15} \times 100 = 33\%$ (F –ve = 33%)

$$\textbf{False positive} = \frac{\text{Total F +ve results}}{\text{Total +ve results of MC}} \times 100$$

Example; $\frac{0}{10} \times 100 = 0\%$ (F –ve = 0%)

"Good Quality Smear Examination makes A Good Quality TB Control Programme "

Instructions for filling in the Smear result sheet for EQA [LQAS 1] for sputum smear examination

1. Introduction:- This form is used to collect the examined slide with their results and to collect the activity information from Microscopy Centres.

2. Necessity:- This form is important because the result obtained by Quality Control Assessor after re-checking the slide of Microscopy Centres should be compared with their original results to prepare the Quality Check Feedback Report [LQAS 3] which should be sent to each Microscopy Centres.

3. How to fill in this form:-

Microscopy Centre:-	Write the name of Microscopy Centre.
District:-	Write the name of District.
Month:-	Write the month of slide collection for EQA purpose. e.g. from Shrawan to Kartik, 2054
Quarter/Year:-	Mention the quarter (4 monthly) and the year. e.g. 1st quarter of 2054/55
Date of record:-	Write the date when the results have been recorded in the form.
Lab. In-charge:-	Write the name of Laboratory In-charge.
Recorded by:-	Write the name of staff, who has recorded the information in this form.
MC Report of Previous Year (20 /20)	**Report of Microscopy Centre from the Lab Register of Previous Year**
Total Examined Slides	Write down from the Lab Register of Previous Year
Total Negative Slides	Only Negative Slides from the Lab Register of Previous Year
Total Positive Slides	Only Positive Slides from the Lab Register of Previous Year
Slide Positivity Rate	Calculate from the Lab Register of Previous Year (SPR = Total +ve/Grand Total*100)
Process of Slide Selection	A process of determination sample size of LQAS System
Annual Sample Size	Sample Size to be chosen for current Fiscal Year (According to prescribed LQAS table)
Quarterly Sample Size	Sample size to be chosen for participating quarter in EQA (Annual sample size/3)
Total Slides Examined During last Quarter	**Total slides examined during the Quarter participating in EQA (Total (-ve & +ve)**
Slide interval……th	Interval of Selected slide (collect every ……th slide)
MC Report of current F/Y (,..Qtr 20 / 20)	Details of MC for participating quarter in current Fiscal Year (,,,,Qtr /Year……)
S. No. of First slide selected	Serial Number of the First Slide selected as per the last digit of a Bank Note
Total Neg. Slides sent for EQA	Total Number of Negative Slides sent for EQA
Total Pos. Slides sent for EQA	Total Number of Positive Slides sent for EQA
Total Slides Sent for EQA	Total Number of selected Slides (Positive and Negative) sent for EQA
Slide No.:-	Write the Slide No. (given by the Laboratory) in a serial order as per Lab Register.
Result:-	Write the result of individual slide separately in the result column. e.g. Neg., 1 – 9 /100VF, 1+, 2+, 3+

- Suspected person: Total no of suspected person, screening during defined Quarter/ year
- Slide A: No of slide from spot sputum sample.
- Slide B: No of slide from Early morning sputum sample

- Follow up slide: Fill the No of follow up slide.

Comments from Microscopy Centre:-
In this space, Microscopy Centre can mention their problem or saying to the Regional TB Quality Control Centre or Assessor.
LQAS – 1 (MC to RTQCC)

National Tuberculosis Programme
राष्ट्रिय क्षयरोग कार्यक्रम
Smear Result Sheet for Lot Quality Assurance System
खकार लेप परीक्षणको गुणस्तर जाँच नतीजा फारम

Microscopy Center सूक्ष्मदर्शकीय केन्द**:…… District** जिल्ला**:……**

Month: From ……..To…… Quarter/Year……quarter of 20…../20...
महिना::.....देखि..... सम्म चौमासिक/साल:.....चौमासिक
२०...../२०.....

Date of Record रेकर्ड मिति**:…… Lab Incharge** प्रयोगशाला प्रमुख**:……**

Recorded दथ रेकर्ड गर्ने**:…… Signature** सहीछाप**:……**

Name of LQAS Supervisor:-..... Signature:- .....

MC Report of Previous Year ***20 / 20*** अघिल्लो आ.ब. २०.../२० को प्रतिवेदन		**Process of Slide Selection** स्लाइड छनौट गर्ने तरीका		**MC Report of Current Fiscal Year ……Qtr. 20 / 20** चाल¬ आ. ब. को प्रतिवेदन:चौमासिक..२०.../२०.....	
Total Slides Examined कूल स्लाइड परीक्षण		Annual Sample Size वार्षिक नमूना संकलन संख्या		S. No. of First Slide Selected छनौट गरिएको पहिलो स्लाइड कम संख्या	
Total Negative Slides कूल नेगेटिभ स्लाइड परीक्षण		Quarterly Sample Size चौमासिकनमूना संकलन संख्या		Total Negative Slides Sent for EQA गुणस्तर जा¤चका लागि पठाइएको जम्मा नेगेटिभ स्लाइड संख्या	
Total Positive Slides कूल पोजिटिभ स्लाइड परीक्षण		Total Slides Examined during last Quarter गत चौमासिकको कूल स्लाइड परीक्षण संख्या		Total Positive Slides Sent for EQA गुणस्तर जा¤चका लागि पठाइएको जम्मा पोजिटिभ स्लाइड संख्या	
Slide Positivity Rate % स्लाइड पोजिटिभिटी प्रतिशत		Slide Interval ……th slide स्लाइड अन्तर‘‘‘ औ स्लाइड		Total Slides Sent for EQA गुणस्तर जा¤चका लागि पठाइएको क ल स्लाइड संख्या	

Suspect Person		**F**	**M**	**Total**	**Smear A Slide A**		**F**	**M**	**Total**	**Smear B Slide B**		**F**	**M**	**Total**	**Follow-up Slide**		**F**	**M**	**Total**
	+ve					**+ve**					**+ve**					**+ve**			
	-ve					**-ve**					**-ve**					**-ve**			
	Total					**Total**					**Total**					**Total**			

Note: Fill the information of current Quarter properly in this table (Suspected person, Smear A, B and Follow-up Smear)

S.N. क. सं.	**Slide No.** स्लाइड नं.	**Result** नतीजा	**S.N.** क. सं.	**Slide No.** स्लाइड नं.	**Result** नतीजा	**S.N.** क. सं.	**Slide No.** स्लाइड नं.	**Result** नतीजा
1			11			21		
2			12			22		
3			13			23		
4			14			24		

5			15			25		
6			16			26		
7			17			27		
8			18			28		
9			19			29		
10			20			30		

Instructions for filling in the Smear result sheet for EQA [LQAS 2] for sputum smear examination

1. Introduction:- This form is used by Regional TB Laboratory Coordinator (RTLC) to provide the examined slides without their results to the RTQCC.

2. Necessity:- This form is important because the result obtained by RTQCAs after **re-checking** the slides of Microscopy Centres in a **Blinded** manner, which should be compared with their original results to prepare the Quality Check Feedback Report [LQAS 3], which should be sent to each Microscopy Centre.

3. How to fill in this form:-

Microscopy Centre:- Write the name of Microscopy Centre.

District:- Write the name of District.

Month:- Write the month of slide collection for EQA purpose.
e.g. from Shrawan to Kartik, 2054

Quarter/Year:- Mention the quarter (4 monthly) and the year.
e.g. 1st quarter of 2054/55

Slide No.:- Write the Slide No. in a serial order as per LQAS 1.

Result:- Write the result of RTQCA for individual slide in the Assessor's result column.
e.g. Neg., 1 – 9 /100VF, 1+, 2+, 3+

Specimen quality Mention the quality of sputum specimen (good or poor) after observing slide under Microscope

Size B/S Mention the size of the smear whether it is **B**igger or **S**maller than the recommended size

Thickness K/N Mention the thickness of the smear whether it is Thick **(K)** or Thin **(N)** than usual thickness

Evenness Mention the evenness of the smear whether it is evenly spread **(good)** or not **(poor)**

Staining U/O Mention the staining quality of the smear whether stained properly **(good)** or **U**nder- decolorized / **O**ver decolorized **(poor)**

Cleanness Mention the cleanness of the smear whether it is clean **(good)** or dirty **(poor)**

Memo In this space, RTQCA can make a note, if found anything remarkable during slide rechecking

LQAS -2A (RTQCC)

National Tuberculosis Programme

Quality Control for Sputum Smear Examination (worksheet /)

Microscopy Centre: …….District: ……

Month: …… to ……Quarter/Year: …Qtr……..20…./20……..

SN	Slide No.	AFB Result by		Specimen quality		Size B/S		Thickness K/N		Evenness		Staining U/O		Cleanness	
		MC	Assessor	Good	Poor	Good	Poor	Good	Poor	Good	Poor	Good	Poor	Good	Poor
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															
11															
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20															
21															
22															
23															
24															
25															
26															
27															
28															
29															
30															
Total															

Memo:

Date of Exam……

Assessed by……..

QC 2B

National Tuberculosis Programme

राष्ट्रिय क्षयरोग कार्यक्रम

Smear Result Sheet for Discrepant QC Slides

Month: From ……………………………..To……………………………… Quarter/Year……………..quarter of 20…../20

Regional Quality Control Center:………………………………………… Region…………………………………………

Total Mcs in the Region ……………………. Total MCs Participated in the EQA………………………..
Total Slide screened in the Region … …… (Total Negative slide ………. Total Positive slide …………)
Total Slide send for QC…………………….. (Total Negative slide ……….Total Positive slide …………)
Total slide found False Report ………….. (False Negative ……………. False Positive ………………)

S.No.	Districts	Microscopy Centre	MC's Report	QC 1		QC 2		Final Report	Remarks
				Report	Name of QCA	Report	Name of QCA		

Instructions for filling in the Smear result sheet for EQA [LQAS 3] for sputum smear examination

1. Introduction:- This form is used by RTQCC to prepare the final report of Quality Check Report of Smear Microscopy.

2. Necessity:- This form is important because the final results of RTQCAs and original results of Microscopy Centres will be compared and the summarized data with appropriate suggestions computed/prepared as Quality Check Feedback Report, which will be sent back to each Microscopy Centre. This report must be available at each Microscopy Centre.

3. How to fill in this form:-

Microscopy Centre:- Write the name of Microscopy Centre.

District:- Write the name of District.

Month:- Write the months of slide collection for EQA purpose.
e.g. from Shrawan to Kartik, 2054

Quarter/Year:- Mention the quarter (4 monthly) and the year.
e.g. 1st quarter of 2054/55

Sputum Smear Result:- Write the compared results of RTQCA and MC in the result columns of the correlation table appropriately, which gives a clear picture of RTQCA's and MC's total results.
e.g. Neg., 1 – 9 /100VF, 1+, 2+, 3+ along with their individual additional total.

Assessment for Reading Ability This portion is used to calculate overall agreement rate (the compared results lying inside the highlighted area of the correlation table), MC's **Se**nsitivity relative to RTQCA, MC's **Sp**ecificity relative to RTQCA and disagreement between RTQCA and MC (Total Minor and/or Major errors with the correct Slide No.).

Specimen quality Mention the quality of total sputum specimens adequate (good) or inadequate (poor) after observing slide under microscope as per recommendation.

Size B/S Mention the size of the total smears whether it is adequate (the recommended size) or inadequate (**B**igger or **S**maller)

Thickness K/N Mention the thickness of the total smears whether it is adequate usual (thickness) or inadequate (Thick or Thin)

Evenness Mention the evenness of the total smears whether it is adequate **(good)** or uneven **(poor)**

Staining U/O Mention the staining quality of the total smears whether stained properly **(good)** or **U**nder- decolorized / **O**ver decolorized **(poor)**

Cleanness Mention the cleanness of the total smears whether it is adequate or dirty

Comments: Overall assessment for total sputum smears examination; in this space, RTQCA can write important suggestion to MC regarding quality improvement

Reporting Date: Date of form completion

Signature of Assessor Signature of the RTQCA

LQAS-3 (RTQCC to MC)

National Tuberculosis Programme

राष्ट्रिय क्षयरोग कार्यक्रम

Quality Check Report on Sputum Smear Examination

खकार लेप परीक्षणको गुणस्तर जाँच प्रतिवेदन

Microscopy Center सूक्ष्मदर्शकीय केन्द्र**: …… District** जिल्ला**: ……..**

Month: ……..... to …… Quarter/Year: …Qtr……..20…./20……..

महिना:: ……देखि…… सम्म चौमासिक/साल:……चौमासिक ……२०…../२०……..

Sputum Smear Results खकार लेप नतीजा		**Report from Microscopy Center** सूक्ष्मदर्शकीय केन्द्रको नतीजा					
		Negative नेगेटिभ	1-9/100 VF १ - ९ एएफबी	1+ 1+	2+ २+	3+ 3+	Total जम्मा
Result by Assessor जाँचकीको नतीजा	Negative						
	1-9/100 VF १ - ९ एएफबी						
	1+ 1 +						
	2+ 2+						
	3+ 3+						
	Total जम्मा						

Assessment for Reading Ability लेप परीक्षण क्षमताको मूल्यांकन**स**

Overall agreement Rate एकमुष्ट समानता दर: ……% (/) Sensitivity सेन्सिटिभिटि: ……....%, Specificity स्पेसिफिसिटि:……%

Disagreement: असमानता

No. of False Result गलत नतीजाको संख्या	**Total** जम्मा	**Slide No.** स्लाइड नं.				
False (-ve) फल्स नेगेटिभ		**LFN**				
		HFN				
False (+ve) फल्स पोजिटिभ		**LFP**				
		HFP				

Errors गल्ती कमजोरी: Minor Errors साधारण गल्ती (LFN+LFP+QE) =

Major Errors असाधारण गल्ती (HFN+HFP) =

Assessment for Smear Preparation Ability लेप बनाउने क्षमताको मूल्यांकन**:**

Specimen Quality नमूना स्तर: Adequate उपयुक्त ( ) Inadequate अनुपयुक्त ( )

Size आकार: Adequate उपयुक्त ( ) Small सानो ( ) Big ठूला ( )

Thickness बाक्लोपन: Adequate उपयुक्त ( ) Thin पातलो ( ) Thick बाक्लौ ( )

Evenness Psgf;kg: Adequate उपयुक्त ( ) Uneven एकनास नभएकौ ( )

Staining रंगाउनेकार्य: Adequate उपयुक्त ( ) Under decolourization कर्माडिकलराइजेशन ( )

Over decolourization बढी डिकलराइजेशन ( )

Cleanness सफापन: Adequate उपयुक्त ( ) Dirty फोहरी ( )

Comments टिका टिप्पणी**:** Overall assessment for sputum smears examination खकार परीक्षणको एकमुष्ठ मूल्याङ्कन:

Reporting Date प्रतिवेदन तयारी मिति: …… Signature of Assessor एसेसरको सही: …

Note: RTQCC should prepare five copies of this record. Report to MC, DHO (DTLA/O), RHD (RTLA/O/RTLC), NTC & one copy at RTQCC.

CLASSIFICATION OF ERRORS

Choosing '0' error means that one can be 95% certain that a laboratory has met the performance goal if no error is reported. For the purpose of EQA, the types of errors are classified on the basis of expected laboratory performances, not on the potential impact of patient management. The terms of errors widely used in LQAS are briefly mentioned here;

Minor error: For the purpose of evaluating laboratory performance, this type of error is less serious and the frequency of minor errors may indicate technical deficiencies. But in clinical practice, these errors may have some impact on patient management. The types of minor error are given below;

- Quantification error (QE): Difference of more than one grade in reading a positive slide between examinee and controller.
- Low False Negative (LFN): Previously called a scanty false negative. A low positive (1-9AFB/100VF) smear that is misread as negative.
- Low False Positive (LFP): Previously called a scanty false positive. A negative smear that is misread as low positive (1-9AFB/100VF).

Major error: For the purpose of evaluating laboratory performance, this type of error is more serious and the frequency of major errors may indicate technical deficiencies and indicat both HFP and HFN errors. In clinical practice, these errors may have serious impact on patient management. The types of major error are given below;

- High False Negative (HFN): A 1+ to 3+ positive smear that is misread as negative.
- High False Positive (HFP): A negative smear that is misread as 1+ to 3+ positive.

Table 7: Rechecking is not a method for validating individual patient diagnosis.

Result of Controlling Laboratory(RQCA)	***Result of Test Laboratory (Microscopy Centre)***				
	Negative	*1-9 AFB/100f*	*1+*	*2+*	*3+*
Negative	*Correct*	*LFP*	*HFP*	*HFP*	*HFP*
1-9 AFB/100f	*LFN*	*Correct*	*Correct*	*QE*	*QE*
1+	*HFN*	*Correct*	*Correct*	*Correct*	*QE*
2+	*HFN*	*QE*	*Correct*	*Correct*	*Correct*
3+	*HFN*	*QE*	*QE*	*Correct*	*Correct*

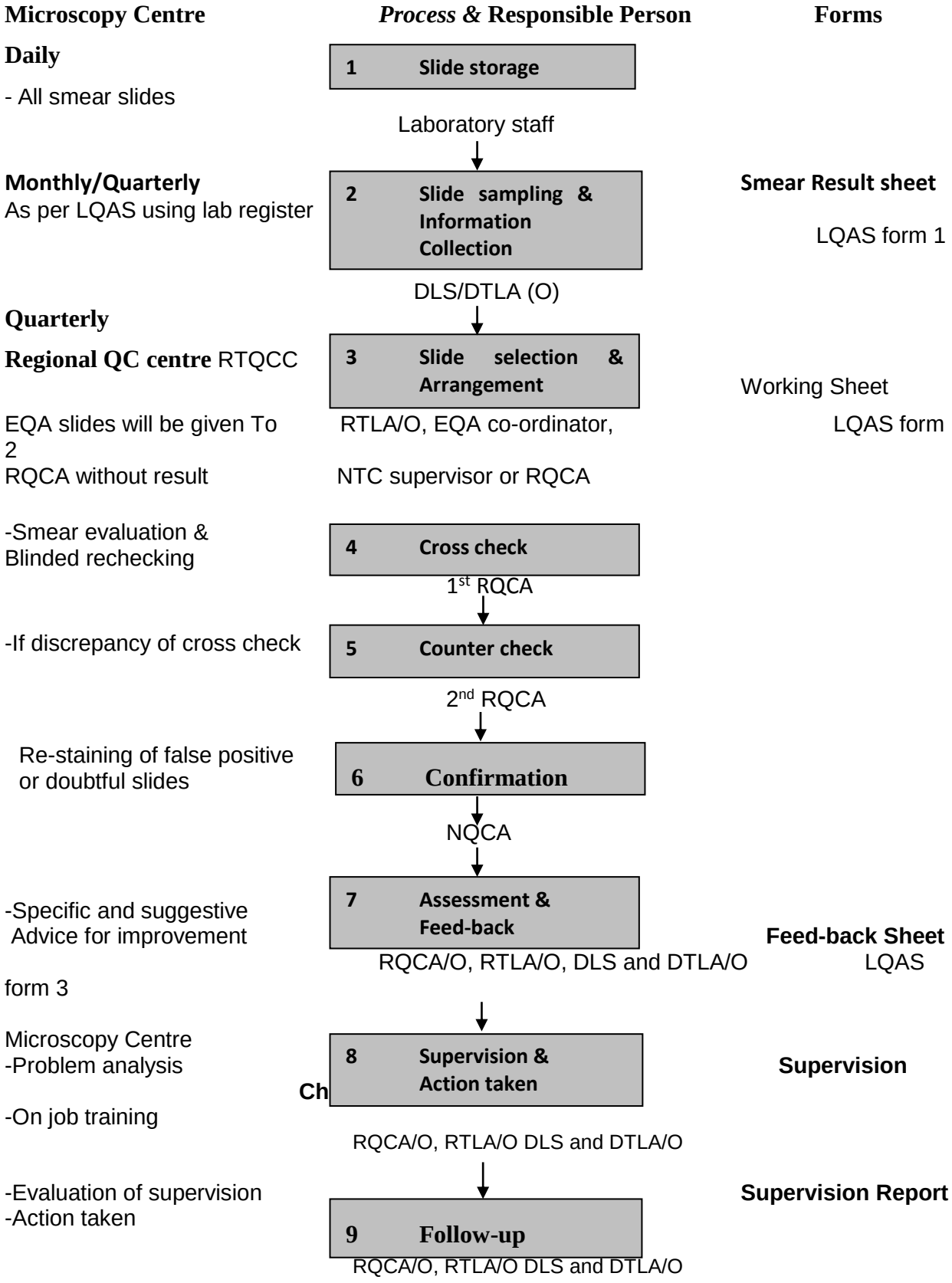
Correct: *No errors*
Minor error: *Quantification error (QE)*
Low False Negative (LFN)
Low False Positive (LFP)

Major error: *High False Negative (HFN)*
High False Positive (HFP)

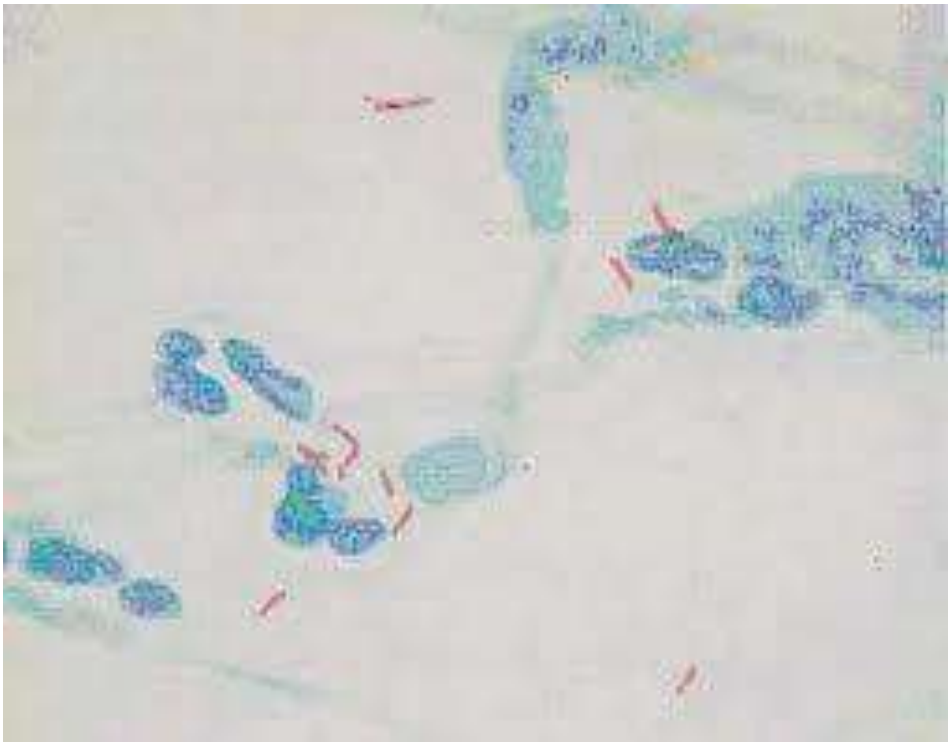
Classification of Microscopy Centre according to Performance

1. Excellent = No. False Result (+ve/-ve) and more than 95% overall agreement rate.
2. Satisfactory= Less than 5% false report(+ve/-ve) and more than 95% overall agreement rate.
3. Poor = More than 5% false report (+ve/-ve) with less than 95% over all agreement rate.

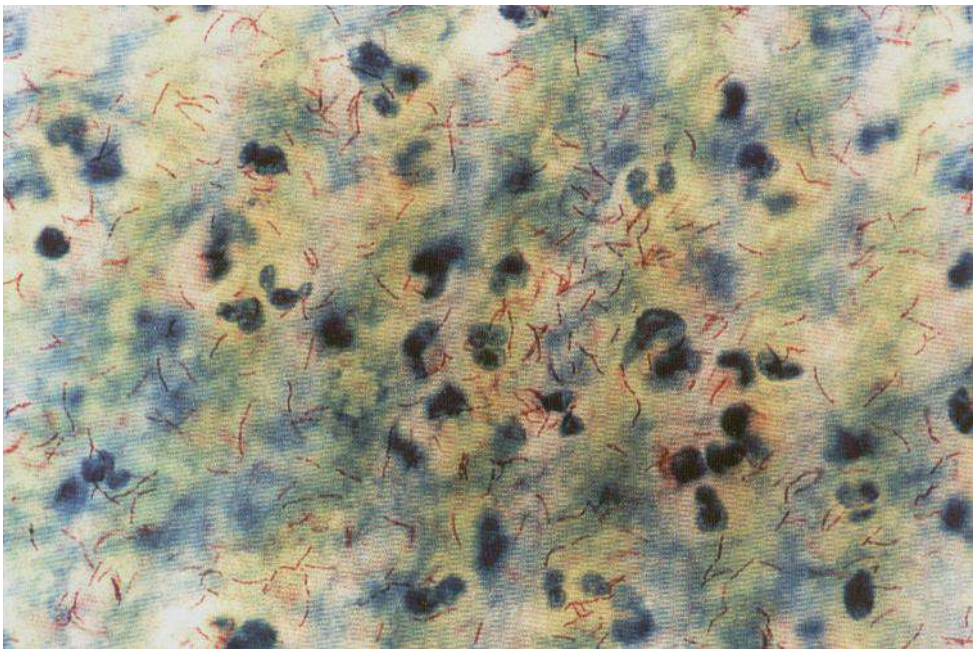
Flow Chart 3: External Quality Assessment System (EQAS) in Nepal



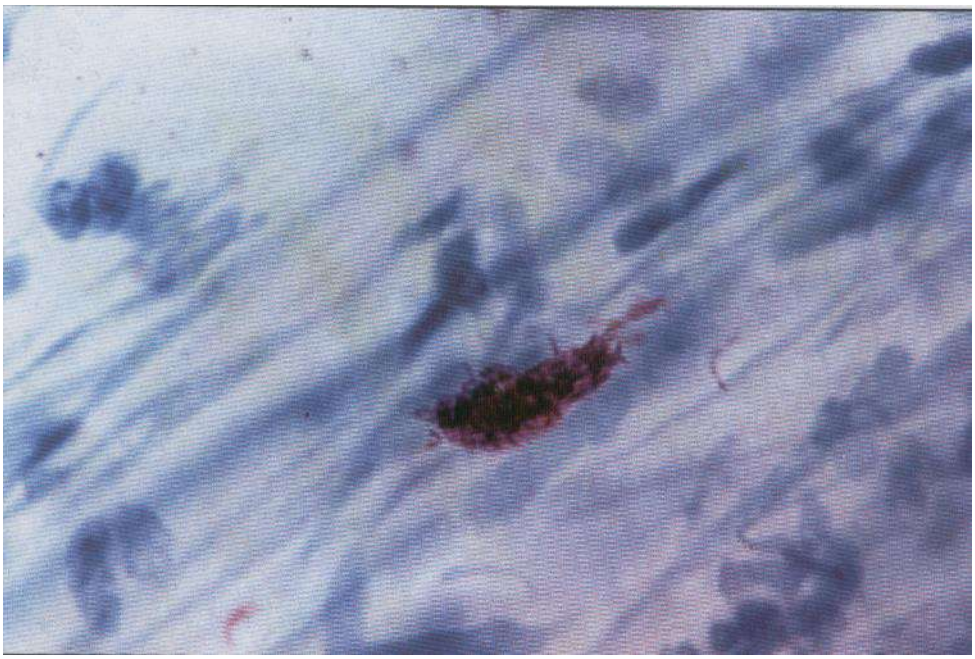
Attachment (संलग्न)



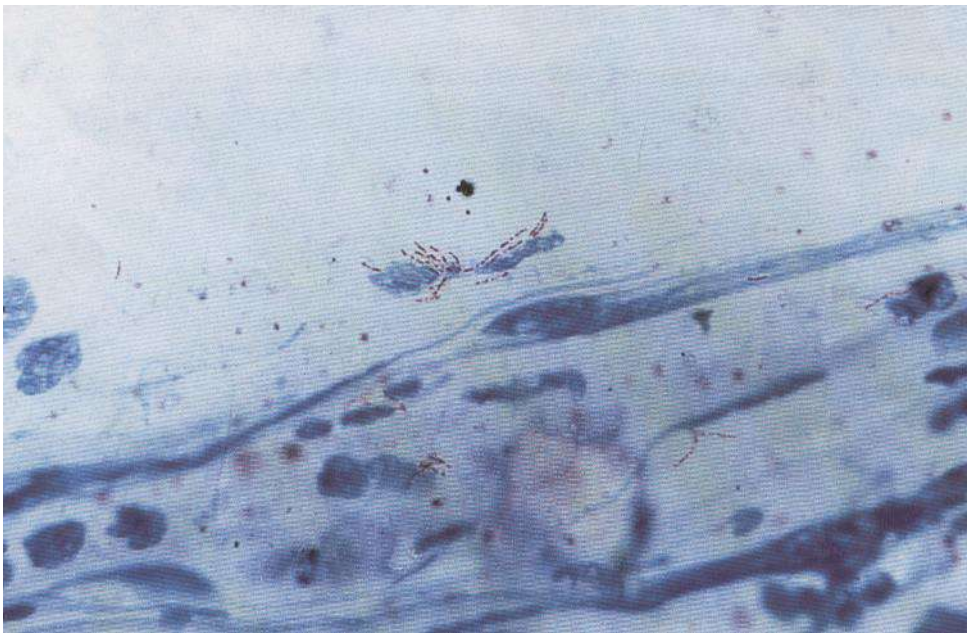
Mycobacterium tuberculosis in sputum stained by
Ziehl-Neelsen method at magnification x 1000
जील-नील्सन तरीकाद्वारा रंगाइएको खकारमा १००० गुणा म्याग्नीफिकेसनबाट हेर्दा
देखिने एसिड फास्ट ब्यासिलाइ



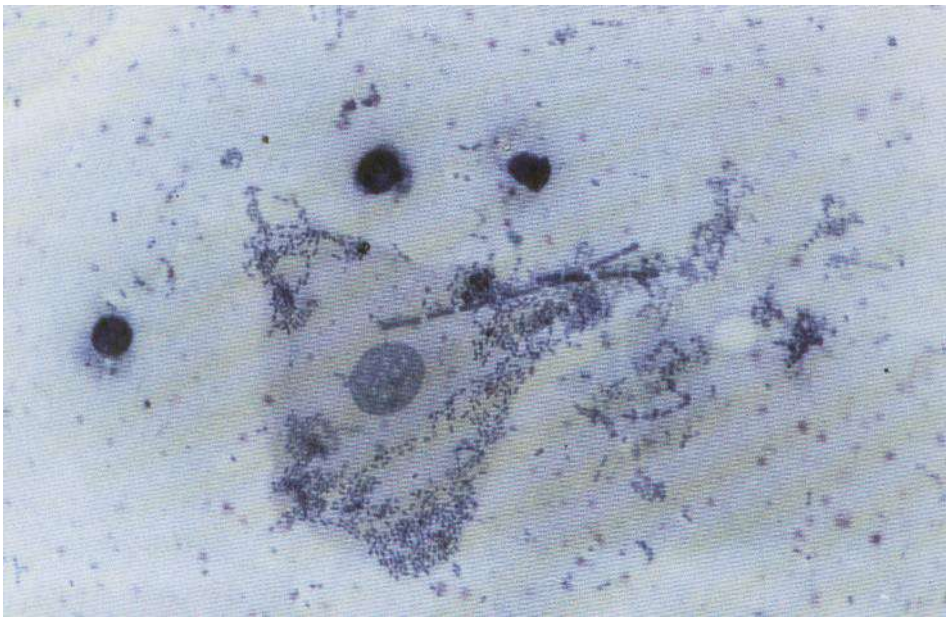
Severe Positive (यथेष्ठ पोजिटिभ)



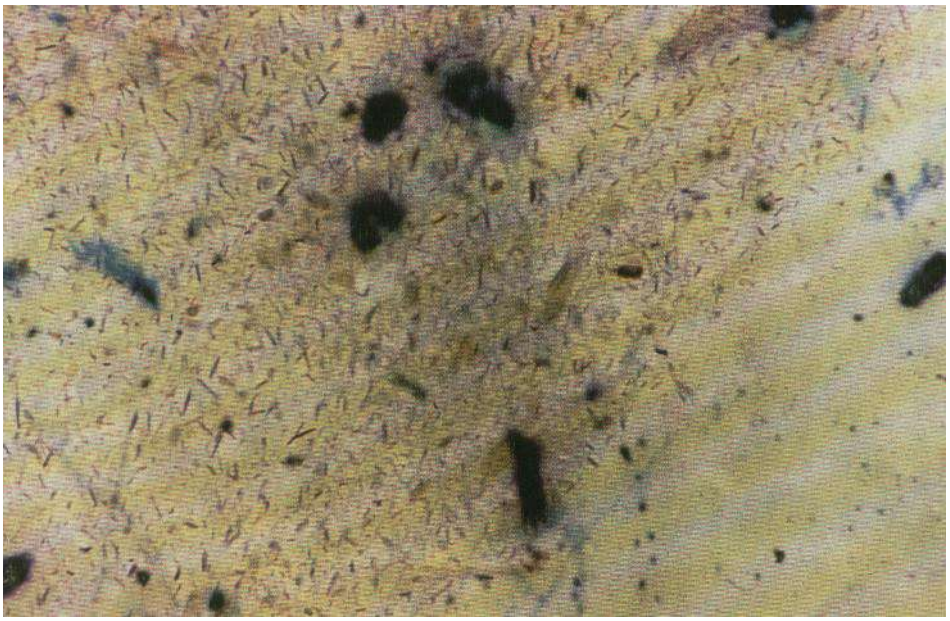
Clump of AFB (थुप्राकार ए एफ बी)



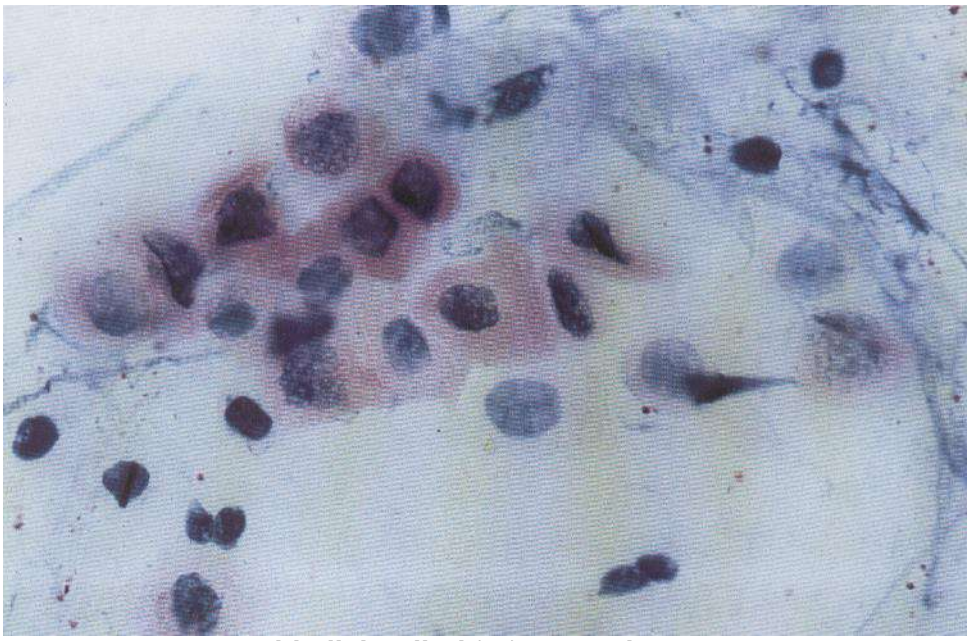
Coccus type of AFB (कोकस जस्तै ए एफ बी)



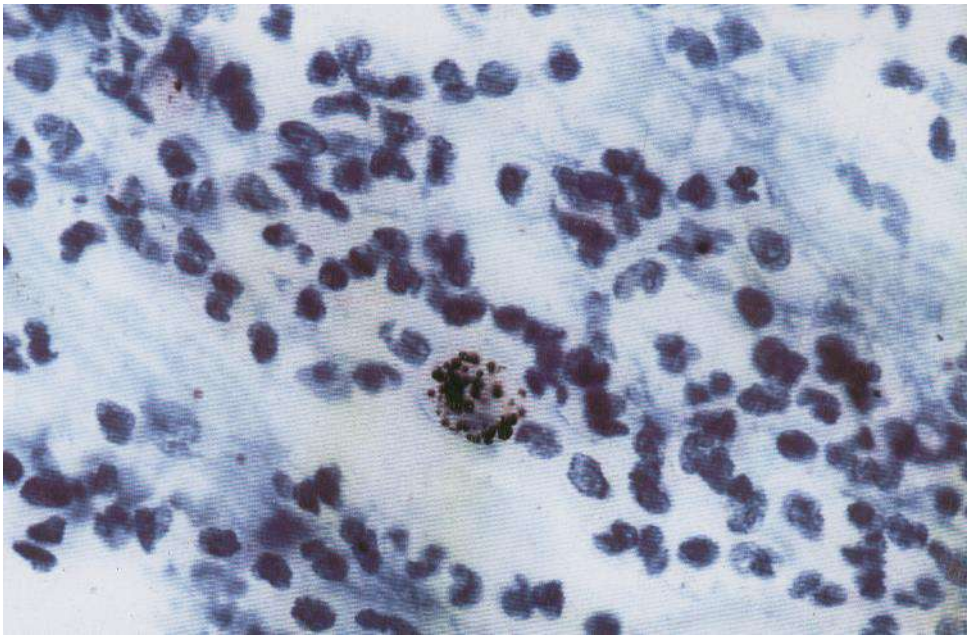
Bacteria other than AFB (ए एफ बी बाहेक अन्य ब्याक्टेरिया)



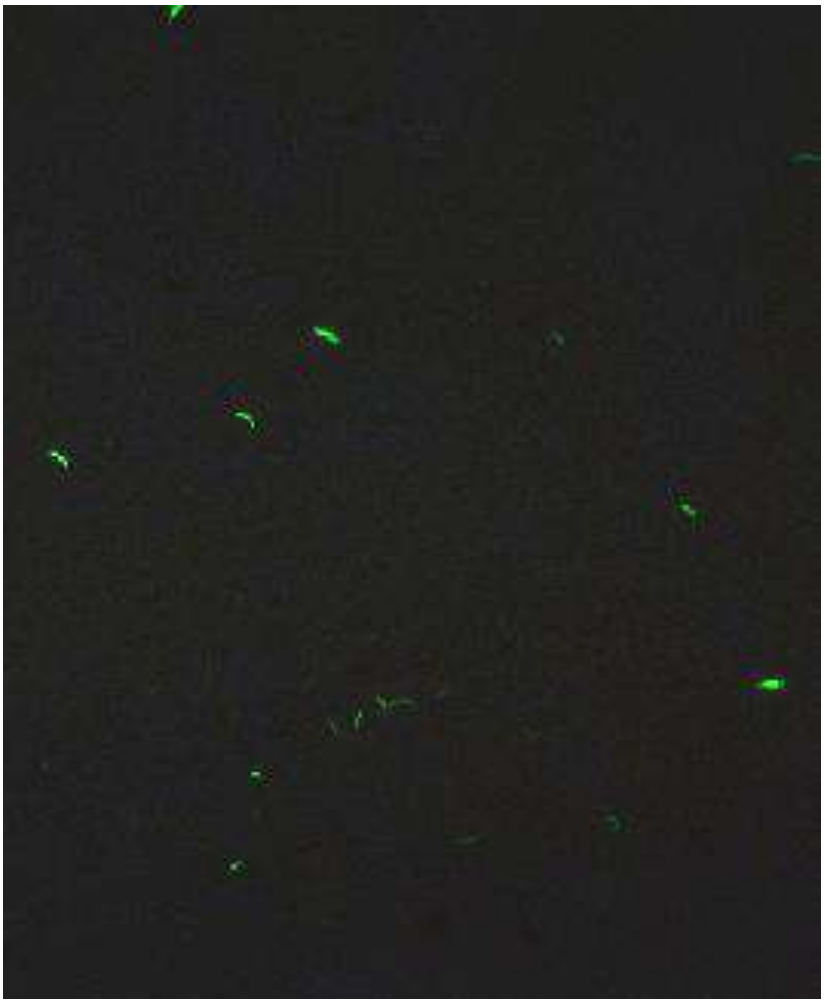
Crystals (क्रीष्टलहरु)



Epithelial Cells (एपिथेलिएल कोषहरु)



Dust cell (फोक्सोमा पाइने डस्ट कोष)



Auramine O stained AFB at x 250 magnification
(औरामिन ओ बाट रंगाई २५० गुणा म्याग्निफिकेसन गरी देखिएको ए एफ बी)

ANNEX

List of Lab reagents

EQA Guidelines standard for reagents and equipment

1) Standards for Reagents
   a. Specifications:
      i. Basic fuchsin
         1. The chemical name : Pararosaniline hydrochloride
         2. The chemical structure : $C_{19}H_{18}N_3C1$
         3. Molecular Wt : 323.8
         4. Colour : Metallic green
         5. Dye content: Should be available on the container. Approximately 85% - 88% (to calculate the required amount of Basic fuchsin, divide the actual amount by dye content. For example: Dye content = 85%, actual amount = 10 gms, required amount = 10/0.85 = 11.76 gms.)
      ii. Carbolic acid :
         1. The chemical name : Phenol
         2. The chemical structure : C_6H_5OH
         3. Molecular Wt : 94.11
         4. Melting point : 40°C±2
         5. Purity : 99.5%
         6. Please note : the critical concentration of Phenol in Carbol fuchsin is 5%.
         7. Phenol is highly corrosive, handle with extreme care.
      iii. Methylated Spirit
         1. Chemical name : Ethanol denatured – 5% Isopropyl alcohol – 5% Methanol
         2. Molecular structure : C_2H_5OH
         3. Molecular wt : 46.07
         4. Purity : 90%
      iv Sulphuric acid :
         1. Chemical structure : H_2SO_4
         2. Molecular wt : 98.08
         3. Purity : 95-97%
         4. Colour : Clear
      v. Methylene blue:
         1. The chemical name: Methylthionine chloride
         2. The chemical structure: $C_{16}H_{18}C1N_3S$
         3. Molecular Wt: 319.9
         4. Dye content: Should be available on the container. Approximately 82% (to calculate the required amount of Methyl blude, divide the actual amount by dye content. For example: Dye content = 82%, actual amount = 1 gms, required amount = 1/0.82 = 1.22 gms.)

   b. Immersion oil :
      i. Immersion oil supplied by the manufacturer of microscope with refractive index closer to that of Glass or 1.515
      ii. Liquid paraffin (heavy), refractive index of 1.48, a colourless, odourless, transparent, free from fluorescence in day light with relative density of 0.827 to 0.890, viscosity of 110 to 230 mPa 5, specific gravity of 0.6-0.78 at 15.5°C.

2) Shelf life of prepared reagents: Carbol fuchsin, sulphuric acid, methylene blue reagents may be kept for a maximum period of 4 months.
3) Identification: All reagents should have a label with name of the reagent, name of the TU, name of MC, the date of preparation and the expiry date. The containers of Carbol fuchsin, Sulphuric acid, Methylene blue reagents should in addition have the name of the person preparing the reagent. Freshly prepared reagents should not be mixed with old stock.
4) Equipments :
   a. Slides :
      i. Size : 75 mm x 25 mm, (approx.)
      ii. Thickness: 1.3 mm
      iii. Edges : Polished
      iv. Sealed in a moisture absorbing desiccant pack
   b. Balance :
      i. Type of Electronic or Analytical balance
         1. Electronic balance :
            a. General purpose table top laboratory balance, 220-230 V, stainless steel platform, keypad auto calibration function, auto off, prolonged battery life, overload and under load, low battery LCD indicator.
            b. Range : Wide range, 0.01 – 120 gms, (two digit decimal)
            c. Resolution : 0.01 gm
         2. Analytical balance :
            a. Enclosed in a glass box with shutters, dimensions of the box in cms : 46 x 34 x 20
            b. Oscillator type of balance, with leveling screws, two aluminium pans, plumb line for adjusting horizontal level
            c. Weighing capacity: 1 mg to 200 gms, with fractional weight and regular weight in boxes including rider and forceps to handle weights.
   c. Binocular microscopes:
      i. Specifications: As per Expert Committee recommendations.

Specification for Auramine O and $KMnO_4$

Auramine O:

Auramine hydrochloride;
(1,1 –bis(p-dimethylaminophenyl) methylenimine hydrocholoride)
Formula : $C_{17}H_{21}N_3HCL, H_2O$)
Mol Wt. 321.85
Appearance: Yellow to brown powder
Potency (Dye content): approximately 85.0%
Absorbance: 435nm
Auramine O is a yellow fluorescent dye; very soluble in water, soluble in ethanol use to stain acid-fast bacteria in sputum or in paraffin sections of infected tissue.

Potassium Permanganate :

Formula : $KMnO_4$
Mol Wt : 158.04
Potency: $\geq$ 99%
Appearance: Purple solid, dissolves in water to give deep purple solutions.

Source: RNTCP Lab Network Guidelines, India

अनुसूची

पाठक्रम बिकास तथा परिमार्जनमा योगदान पुऱ्याउने महानुभावहरुको नामावली :

क्रास	नामथर	पद	कार्यरत संस्था
१	डा. बिकाश लामिछाने	निर्देशक	रा.क्ष.के.
२	डा.शरद चन्द्र वर्मा	निर्देशक	शार्क टि.बी. सेन्टर
३	अनिल थापा	प्रमुख यो.अ.तथा मू. शाखा	रा.क्ष.के.
४	डा.मोहन कुमार प्रसाई	ब.छाती रोग बिशेषज्ञ	रा.क्ष.के.
५	सागर घिमिरे	बरिष्ट ज.स्वा.प्र.	स्वा.से.वि.
६	नारायण प्रसाद दाहाल	लेखा (उपसचिव)	रा.क्ष.के.
७	डा.पुष्पा मल्ल	छाती रोग बिशेषज्ञ	अल्का अस्पताल
८	डा.रमेश कान्त अधिकारी	बाल रोग बिशेषज्ञ	कृष्ट अस्पताल
९	डा.ब्रजेश श्रीवास्तब	छाती रोग बिशेषज्ञ	बिरेन्द्र सैनिक अस्पताल
१०	डा.भावना श्रेष्ठ	प्रोग्राम मेनेजर	जेनेटप कालिमाटि
११	डा.अषिम श्रेष्ठ	लेक्चरल	धुलिखेल अस्पताल
१२	डा.नबिन प्रकाश साह	छाती रोग बिशेषज्ञ	रा.क्ष.के.
१३	डा.निहाल सिंह	मेडिकल अधिकृत	बिश्व स्वास्थ्य सघं
१४	डा.आशिष श्रेष्ठ	कन्सल्टेट	बिश्व स्वास्थ्य सघं
१५	डा.शुभेश कुमार श्रेष्ठ	टीबी एच.आई.भि बिज्ञ	सेभ द चिल्डेन
१६	डा.प्रमोद भटराई	डी आर टीबी मे अ	डामिन फाउन्डेसन
१७	डा.राजेन्द्र खडका	मे.अ.	रा.क्ष.के.
१८	डा.राधे श्याम के सी	एन.डब्लु.हेल्थ अफिसर	आई.ओ एम.
१९	ज्योती आचार्य	माईक्रोबायोलोजिष्ट	एन पी एच एल
२०	गोकर्ण घिमीरे	माईक्रोबायोलोजिष्ट	रा.क्ष.के.
२१	यशोदा राजभण्डारी	तालिम संयोजक (प.हे.न.अ.सातौं	रा.क्ष.के.
२२	भगवान महर्जन	माईक्रोबायोलोजिष्ट	जेनेटप कालीमाटी
२३	बिधान आचार्य	भाषा बिज्ञ	टियू किर्तिपुर
२४	जानु धिताल	एम.डी.	SAIPZ Sanepa, Lalitpur.
२५	संजय दहाल	ज. स्वा. अ.	रा.स्वा.शि.सू.तथा संचार केन्द्र, टेकु
२६	चित्र जगं शाही	क्षयकुष्ट अधिकृत	जि स्वा का काठमाण्डौं
२७	बिश्व नाथ न्यौपाने	क्षयकुष्ट अधिकृत	जि स्वा का पाल्पा
२८	राम गुलाम कर्ण	क्षयकुष्ट अधिकृत	जि स्वा का धनुषा
२९	पुष्पा क्षेत्री	प.हे.न.अ.	रा.क्ष.के.
३०	पुष्प राज जोशी	त.अ.	रा.क्ष.के.
३१	गोकुल मिश्र	एल.एच.एल. फोकल पर्सन	रा.क्ष.के./ एल.एच.एल.आई
३२	कमला वाग्ले	प.हे.न.अ.	रा.क्ष.के.
३३	राजेश कुमार मिश्र	कम्पुटर अधिकृत	रा.क्ष.के.
३४	डि एन शर्मा	कल्सलन्टेन्ट	रा.क्ष.के.
३५	ठाकुर प्रसाद अधिकारी	नासु	रा.क्ष.के.
३६	कमल ढुगेंल	नासु	रा.क्ष.के.
३७	बिष्णु श्रेष्ठ	लाईब्रेरियनअधिकृत	रा.क्ष.के.
३८	राजेन्द्र बस्नेत	प्रोग्राम मेनेजर	रा.क्ष.के./एस.सी.आई.
३९	राम बाबु श्रेष्ठ	ल्या टे अधिकृत छैठौं	रा.क्ष.के.
४०	कृष्ण अधिकारी	ल्या. टे.	रा.क्ष.के.
४१	समिस्ठा सिहं श्रेष्ठ	प्रोग्राम असिस्टेण्ट	डब्लु एच ओ

४२	हरी बहादुर कुवंर	तालिम संयोजक	रा.क्ष.के./एस.सी.आई.
४३	रत्न श्रेष्ठ	लेखापाल	रा.क्ष.के.
४४	रत्न भटराई	एम.एण्ड ई स्पेसिलीस्ट	रा.क्ष.के. सेभ
४५	अभय श्रेष्ठ	आईटी अधिकृत	रा.क्ष.के./एस.सी.आई.
४६	नेत्रा बुढा क्षेत्री	प्रिभ्यालेन्स सर्भे सेक्रेटरी	रा.क्ष.के./एस.सी.आई.
४७	कृतिका तिमल्सीना	अफिस सेक्रेटरी	रा.क्ष.के./एस.सी.आई.
४८	पार्वती थापा	फिल्ड सुपरभाईजर	JANTRA
४९	रविन श्रेष्ठ	एम एण्ड ई अधिकृत	HERD
५०	अज्यूदेयश्रेष्ठ	पि.एस.एम. संयोजक	रा.क्ष.के./एस.सी.आई.
५१	तुसार कान्ती राय	एडभाईजर	रा.क्ष.के./के.एन.सी.भी.
५२	सजना श्रेष्ठ	जिल्ला संयोजक	बि. एन. एम. टि
५३	लाल मनी अधिकारी	एफ सि	आई ओ एम.
५४	रबिन खतिवडा	खरिदार	रा.क्ष.के.
५५	भरत खतिवडा	का स	रा.क्ष.के.
५६	मुकुन्द सुबेदी	स चालक	रा.क्ष.के.
५७	बिश्व हरी खडका	स चालक	रा.क्ष.के.
५८	श्री प्रसाद आचार्य	स चालक	रा.क्ष.के.
५९	सिता राम सापकोटा	स चालक	रा.क्ष.के.
६०	टिका राउत	स चालक	रा.क्ष.के.
६१	कृष्ण बस्नेत	का स	रा.क्ष.के.
६३	शरण गोपाली	एक्ज्यूटिभ डाइरेक्टर	JANTRA
६४	ओम आचार्य	ल्या टे अधिकृत	सुर्खेत क्षे. स्वा. नि.
६५	नवल किशोर श्रेष्ठ	आईटी अधिकृत	रा.क्ष.के./एस.सी.आई.