REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Module for Laboratory Technicians

Central TB Division
Directorate General of Health Services
Ministry of Health and Family Welfare, Nirman Bhavan,
New Delhi 110011

Module for Laboratory Technicians

October 2005

Central TB Division, Directorate General of Health Services,
Ministry of Health and Family Welfare, Nirman Bhavan,
New Delhi 110011

First edition, December 1997
Second Edition, October 2005

CONTENTS

AIM OF MODULAR TRAINING

This module contains information on tuberculosis and sputum microscopy. The module includes exercises on the activities and skills which the Laboratory Technician (LT) has to perform to implement the Revised National Tuberculosis Control Programme (RNTCP).

On successful completion of training, including hands-on training in a microscopy laboratory, the LT will be able to understand and perform all the job requirements related to the RNTCP.

WHAT IS TUBERCULOSIS?

Tuberculosis (TB) is an infectious disease caused by the bacterium, Mycobacterium tuberculosis. Tubercle bacilli mainly affect the lungs, causing lung tuberculosis (pulmonary tuberculosis). However, in some cases, other parts of the body may also be affected, leading to extra-pulmonary tuberculosis.

HOW DOES TUBERCULOSIS SPREAD?

TB germs usually spread through the air. When a patient with pulmonary tuberculosis cough, sneezes, or talks he throws TB germs into the air the form of tiny droplets. These tiny droplets when inhaled by another person may spread TB. When patients with tuberculosis begin taking effective treatment, they stop spreading the germs within a few weeks. But unless they take treatment regularly and complete it, they are likely to develop more dangerous forms of tuberculosis, known as drug-resistant tuberculosis, which they can then spread to others.

MAGITUDE OF TUBERCULOSIS IN INDIA

Tuberculosis remains a major public health problem in the country. Every year, approximately 18 lakh people develop TB disease and about 4 lakhs die of it. India accounts for one-fifth of all new TB cases each year globally and tops the list of 22 high TB burden countries.

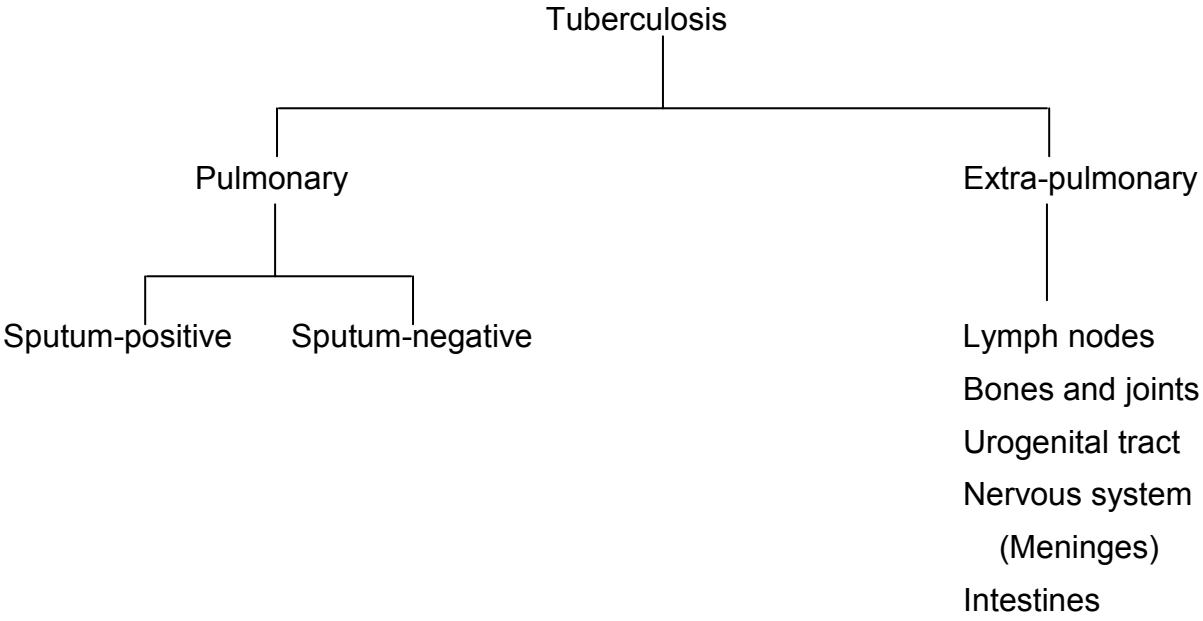
In India, EVERY DAY:

- more than 5,000 develop TB disease
- more than 1,000 people die of TB (i.e. 1 death every 1½ minutes)

It is estimated that in a year, 150–170 cases of tuberculosis per 100,000 population will be diagnosed and treated under the RNTCP

CLASSIFICATION OF TUBERCULOSIS

Please try to understand the following chart:



PULMONARY TUBERCULOSIS

Sputum smear-positive

A patient with at least 2 initial sputum smear examinations (direct smear microscopy) positive for acid-fast bacilli (AFB),

Or: A patient with one sputum examination positive for AFB and radiographic abnormalities consistent with active pulmonary TB as determined by the treating Medical Officer,

Or: A patient with one sputum specimen positive for AFB and culture positive for *M. tuberculosis.*

Sputum smear-negative

A patient having symptoms suggestive of TB with at least 3 sputum examinations negative for AFB, and having radiographic abnormalities consistent with active pulmonary TB as determined by the treating MO, followed by a decision to treat the patient with a full course of anti-tuberculosis therapy,

Or: A patient whose diagnosis is based on culture positive for *M.tuberculosis* but sputum smear examinations negative for AFB.

A sputum smear-positive patient with AFB detected by microscopy is much more infectious than a patient who does not AFB detected by microscopy. Nevertheless, even patients with sputum smear negative for AFB can have pulmonary tuberculosis

EXTRA-PULMONARY TUBERCULOSIS

Extra-pulmonary tuberculosis is TB of organs other than the lungs, such as the pleura (pleurisy), lymph nodes, intestines, genito-urinary tract, skin, joints and bones, meninges of the brain, etc.

Diagnosis should be based on one culture-positive specimen from an extra-pulmonary site, or histological evidence, or strong clinical evidence consistent with active extra-pulmonary TB followed by the MO's decision to treat with a full course of anti-TB therapy.

TB can affect any part of the body

WHEN SHOULD TUBERCULOSIS BE SUSPECTED

Pulmonary tuberculosis

The most common symptom of pulmonary TB is a persistent cough for 3 weeks or more, usually with expectoration. It may be accompanied by one or more of the following symptoms:

- Weight loss
- Chest pain
- Tiredness
- Shortness of breath
- Fever, particularly with rise of temperature in the evening
- Blood in sputum in some case
- Loss of appetite
- Night sweats

The most common symptom of pulmonary tuberculosis is persistent cough for 3 weeks or more (usually with expectoration which is sometimes blood-stained) with or without associated fever and chest pain. Every patient with cough for 3 weeks or more should have sputum sample examined for AFB.

Extra-pulmonary tuberculosis

In persons with extra-pulmonary tuberculosis, the symptoms depend on the organ involved:

- Lymph Node Tuberculosis—Swelling in the neck with or without discharge.
- Tuberculosis Meningitis—Headache, fever, drowsiness, confusion, neck rigidity.
- Spinal Tuberculosis—Back pain, fever and in some cases swelling of the backbone.

In additional general symptoms of like weight loss, fever, with rise of temperature in the evening and night sweats may be present.

HOW SHOULD TUBERCULOSIS BE DIAGNOSED?

Detection of AFB in the sputum is the only reliable method for confirming pulmonary tuberculosis. Therefore, the LT plays a pivotal role in the diagnosis of patients and thus in the success of the programme.

Cases of pulmonary tuberculosis are further divided into sputum smear-positive and sputum smear-negative cases. Whenever TB is suspected, atleast 3 specimens of sputum (spot—morning spot) should be collected over 2 consecutive days and examined by microscopy.

Patients with atleast two positive smear results are diagnosed by the physician as a case of smear positive TB. They are further classified as new or old cases based on their treatment history, and appropriate therapy is prescribed.

For patients with only one sputum positive result on smear examination, chest X-ray is taken. If findings of the X-ray are consistent with pulmonary tuberculosis patient is diagnosed by the physician as a case of sputum positive pulmonary TB.

Patients in whom all 3 samples are negative on sputum smear examination are prescribed symptomatic treatment and broad spectrum antibiotics (such as cotrimoxazole, doxycycline, amoxycillin) for 10-14 days. It must be ensured that antibiotics such as fluroquinolones (ciprofloxacin, ofloxacin, etc.), rifampicin or streptomycin, which are active against tuberculosis, are not used in such cases. Most patients are likely to improve with antibiotics if they are not suffering from TB. If the symptoms persist after a course of broad spectrum antibiotics, repeat sputum smear examination (3 samples) must be done for such patients.

If two or more smears are positive, the patient is diagnosed as having smear positive pulmonary TB. If only one sputum sample is positive, chest X-ray is taken. If findings of the X-ray are consistent with pulmonary tuberculosis, patient is diagnosed by the physician as a case of sputum positive pulmonary TB.

If the results for all the three sputum samples of repeat examination are found negative then a chest X-Ray is taken. If findings of the X-ray are consistent with pulmonary

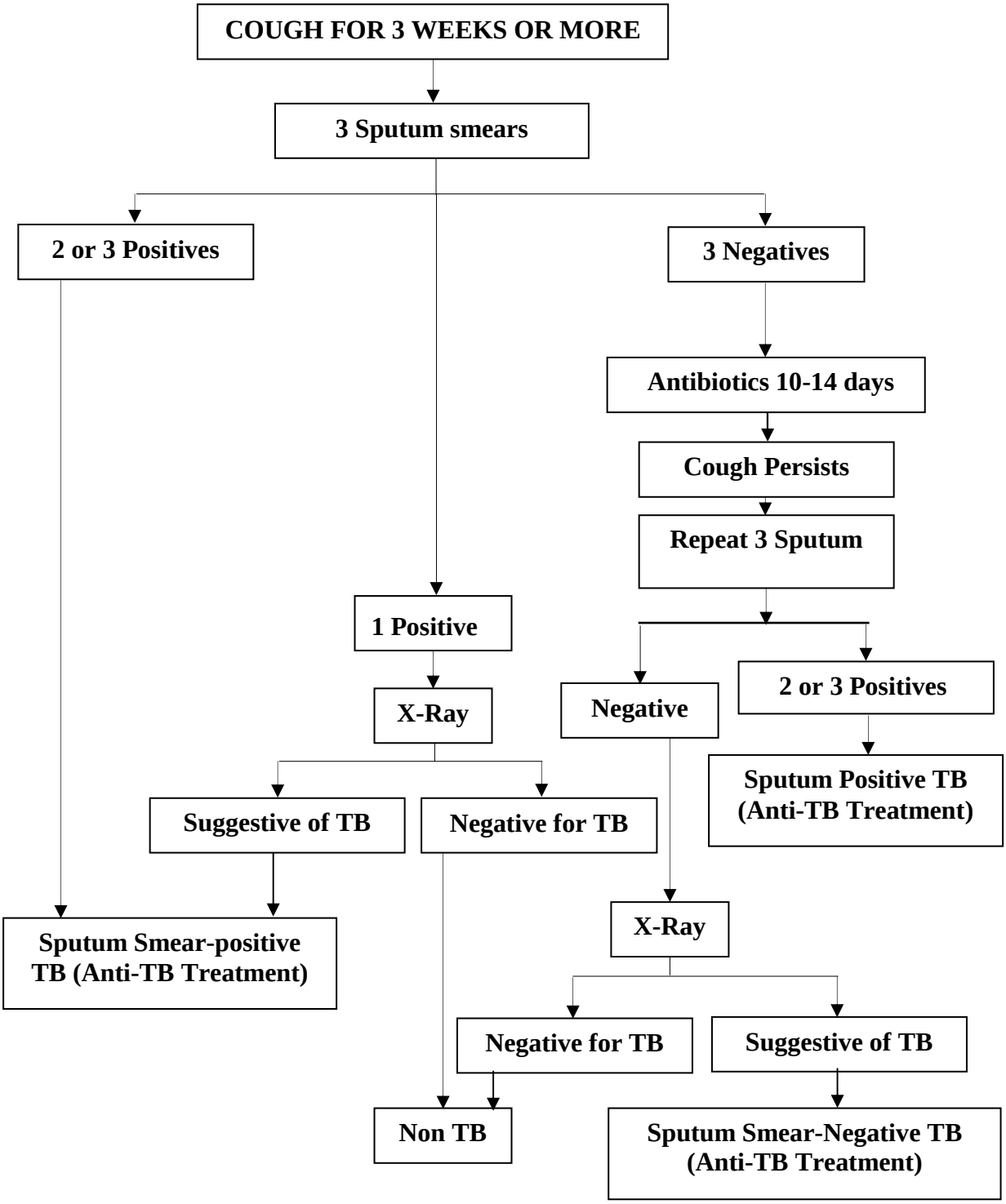
tuberculosis, patient is diagnosed by the physician as a case of sputum negative pulmonary TB.

Diagnosis of extra-pulmonary tuberculosis is made by the Medical Officer. Patients with extra-pulmonary tuberculosis who have cough or any other pulmonary symptoms should have 3 sputum samples examined to determine if they also have pulmonary tuberculosis. A patient may have both pulmonary and extra-pulmonary tuberculosis.

The approach to diagnosing patients with possible tuberculosis is summarized in the diagram below:

Patients who have cough for three weeks or more or other symptoms of TB must be evaluated for tuberculosis by sputum microscopy. It is estimated that 2-3% of new adult outpatients are chest symptomatics. The LT has to ensure that testing of sputum is accurate and results of the sputum microscopy are passed on to the treating physician at the earliest, preferably within a day.

Diagnostic Algorithm for Pulmonary TB



NATIONAL TUBERCULOSIS PROGRAMME

The National Tuberculosis Programme (NTP) in India was implemented in 1962 by establishing District Tuberculosis Centres (DTCs), TB Clinics and TB Hospitals. Since its inception, the programme was integrated with the general health services and service delivery was through the primary health care infrastructure.

To strengthen and improve tuberculosis control activities, the Government of India launched the Revised National Tuberculosis Control Programme (RNTCP) in a phased manner. Beginning in 1997, the programme has expanded to cover almost the whole country with excellent results. As of July 2005, RNTCP has been extended to 585 districts in 34 States and Union Territories to cover a population of almost 104 crore (93% of the country's population) and by the end of 2005 will cover the entire country.

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Objectives	Strategies to be adopted
1. To achieve and maintain a cure rate of at least 85% among newly detected infectious (new sputum positive) cases	• All cases diagnosed must be registered. All registered cases must be treated till they are cured, with priority for cure given to sputum smear-positive cases. • Directly Observed treatment Short-Course Chemotherapy (DOTS), with observation of treatment done close to the patient's home. • Maintain regular drug supply. • Ensure stipulated smear examinations at specified intervals to monitor progress and cure of the patient. • Implementation of the External Quality Assessment (EQA) system to ensure provision of high quality smear microscopy services.
2. To achieve and maintain detection of at least 70% of such cases in the population	• Diagnosis by sputum microscopy among patients attending health services. • Ensure that all persons attending health facilities with cough or other symptoms of tuberculosis have 3 sputum smears examined. • Implementation of the External Quality Assessment system to ensure provision of high quality smear microscopy services.

WHAT IS DOTS, CURE AND CURE RATE?

DOTS

As the name implies, Directly Observed Treatment, Short-Course Chemotherapy (DOTS) means that the patient swallows short course anti-TB drugs in the presence of a health worker or other trained individual. There are two phases in the treatment of tuberculosis: the **intensive phase**, which is or 2 or 3 months, and the **continuation phase**, which is for 4 and 5 months. The length of treatment depends on the category of treatment the patient is taking. Under the programme, these drugs are swallowed thrice-a week during the intensive phase. Then the sputum is examined and if found negative, the patient is issued anti-TB drugs once a week in a weekly calendared multiblister-combipack to be taken thrice-a-week. The first dose from the weekly pack is to be taken in the presence of a health worker. Drugs for the rest of the week are taken by the patient as directed. The intake of drugs by the patient at his house is monitored by checking the empty blister-packs during the time of collection of drugs for the next week. Sputum examination must be done after two months in the continuation phase, and at completion of the treatment.

Advantages of DOTS

- **Places responsibility for patient cure on the health worker, not on the patient**
- **A service to patients**
- **Prevents drugs-resistant TB**
- **Reduces risk to the community by preventing spread of TB**
- **Cost-effective**
- **The only method which ensures cure**

Cure

A patient who was initially sputum smear-positive, and who has completed treatment and has negative sputum smears on at least two occasions, including one at completion of treatment, is declared as cured. If the sputum is not examined during and at the end of treatment, then the patient is said to have completed the treatment. To be declared **cured,** sputum examinations as per the follow-up schedule are essential.

Cure Rate

The cure rate is the proportion of initially sputum smear-positive patients who are declared as cured based on negative sputum smear results on at least two occasions, including one at the end of treatment. The cure rate of new sputum smear-positive

patients is the most important indicator of the success of the programme. The goal of the RNTCP is to ensure that this proportion is at least 85%.

Treatment for tuberculosis is highly effective, if it taken regularly for the prescribed period. The Medical Officer will classify the patient into one of the three treatment categories -

Category I, II, or III. The category in which the patient is classified determines the drugs to be given and the schedule for follow-up sputum examinations.

Detailed job responsibility of the LT are given in the Laboratory Manual and reproduced in Annexure 8.

EXERCISE 1

Tick the ONE appropriate answer.

1. Tuberculosis is transmitted by
   (a) Blood transfusion _____
   (b) Faecal infection _____
   (c) Droplet infection _____
   (d) Oral infection _____

2. The most infectious form of tuberculosis in adults is
   (a) Extra-pulmonary TB _____
   (b) Smear-positive pulmonary TB _____
   (c) Smear-negative TB _____
   (d) Miliary TB _____

3. The commonest form of tuberculosis is
   (a) Extra-pulmonary TB _____
   (b) Bone and joint TB _____
   (c) Renal TB _____
   (d) Pulmonary TB _____

4. When do you suspect pulmonary tuberculosis? Mention four common symptoms.
   (i) _____
   (ii) _____
   (iii)_____
   (iv)_____

5. How do you classify tuberculosis?
   (i) _____
   (ii) _____
   (iii)_____

6. Which is the surest way to diagnosis pulmonary tuberculosis in an adult?

   (a) Sputum smear examination _____
   (b) X-ray _____
   (c) ELISA test _____
   (d) Tuberculin skin test _____
   (e) ESR _____

7. When do you label a patient as a case of smear-positive pulmonary tuberculosis?

   _____

8. TB can affect any part of the body.

   ❑ True ❑ False

9. Patient with three sputum smears negative for AFB cannot have pulmonary tuberculosis?

   ❑ True ❑ False

IMPORTANT POINTS TO REMEMBER

- **All patients with symptoms of pulmonary tuberculosis should have three sputum examinations done for AFB. By identifying patients with tuberculosis promptly and correctly, severe illness, death and spread of the disease can be prevented.**
- **Patients with negative sputum smears can have pulmonary tuberculosis. However, these patients are less infectious than patients with positive sputum smears.**
- **The most common symptom of pulmonary tuberculosis is persistent cough for 3 weeks or more.**
- **Other symptoms of pulmonary tuberculosis include weight loss, fever, night sweats, chest pain, loss of appetite and coughing up blood in the sputum.**
- **With effective, regular and complete treatment, TB can be cured. Documenting cure requires follow-up sputum examination by smear microscopy. Cure rate is the most important indicator of the success of the programme. The national goal is to achieve and maintain a cure rate of at least 85%, and to achieve and maintain detection of at least 70% of such cases in the population.**

COLLECTING SPUTUM, PREPARING AND STAINING SLIDES, EXAMINING SLIDES, AND RECORDING, REPORTING AND VERIFYING RESULTS

The process of collecting sputum, preparing and staining slides, examining slides, and recording, reporting and verifying results can be divided into six stages.

Stages of sputum smear examination are as follows:

1. Collect the sputum
2. Prepare the slide for examination
3. Examination the slides under the microscope
4. Record the results
5. Report the results
6. Verify the results

STAGE 1: COLLECT THE SPUTUM

Receive patient and laboratory Form. Make sure the form is complete.

The patient should have been referred by a Medical Officer. You should ensure that they have been seen by a Medical Officer.

You must make sure that the Laboratory Form is complete; including the patient's address and reason for examination (Only one form needs to be filled out for all 3 sputum specimens collected from a patient.). Confirm the address of the patient again so that the patient is not lost if follow-up is required. If the sputum is for follow-up examination, the patient's TB Number should have been written on the form.

Reason for examination. If the patient has come to the health facility for the first time, the sputum is examined for diagnosis. In this case, 3 sputum samples are examined (SPOT—MORNING—SPOT). After a patient is diagnosed as a case of tuberculosis, treatment is started. For follow-up examinations, two samples are obtained (MORNING—SPOT). The schedule for sputum examinations is summarized in the table below.

If the health facility is not an RNTCP designated microcopy centre (DMC), then the patient may be referred to the nearest DMC, or else the patient's sputum is collected and transported to the nearest DMC.

The results of follow-up sputum examinations are important. The treatment a patient receives depends on these results. If the first follow-up sputum examination is positive (that is, after the second month of starting treatment for patients receiving Category I

treatment, or after third month of starting treatment for patients receiving Category II treatment), then the treatment is extended for one more month, and the sputum is examined again after that.

Schedule of Sputum Examinations

Category of treatment	**Schedule of follow-up sputum examinations**
Smear-positive Category I	At the end of 2, 4 and 6 months of treatment
Smear-positive Category I (If sputum-positive at the end of Month 2)	At the end of 2, 3, 5 and 7 months of treatment
Smear-positive Category II	At the end of 3, 5 and 8 months of treatment
Smear-positive Category II (If sputum-positive at the end of month 3)	At the end of 3, 4, 6 and 9 months of treatment
Smear-negative Category I or Category III	At the end of 2 months of treatment

Specimen Identification Number

If specimens are being transported to a DMC from another health facility, a Specimen Identification Number is given at the referring facility, because the Laboratory Serial Number can only be assigned at the DMC. Sputum specimens are assigned specific numbers to keep track of each patient's sputum results. After the Laboratory Form for Sputum Examination is filled up, this number is written on the side of the patient's sputum container. (If a sputum specimen is separated from its Laboratory Form for Sputum Examination, a LT can find out whose specimen it is by using the Specimen Identification No. on the sputum container. The laboratory technician can then locate the form by using the date and the identification number.) Each separate specimen will generally have its own unique Specimen Identification No. For example, 3 specimens from a single patient might have Specimen Identification Nos. A1, A2 and A3. The 3 sputum specimens of the next patient may have Specimen Identification Nos. B1, B2 and B3. 1, 2 and 3 correspond to the SPOT-MORNING and SPOT samples, and this sequence of labeling should be ensured by persons collecting the sputum.

Patient's TB number: All patients diagnosed as suffering from tuberculosis are entered in the TB Register maintained by the Tuberculosis Unit. The TB Number is very important. If a patient's sputum is being examined for follow-up, the TB Number should have been written in the space provided on the Laboratory Form. The TB Number should also appear on the patient's Identity Card. If the patient is carrying this card, you can enter the number from this card if it has been omitted from the Laboratory Form.

Demonstrate to the patient how to open and close the sputum container and how to bring up sputum

Give the patient the sputum container with the Laboratory Serial Number written on its side. Show the patient how to open and close the container, and explain the importance of not rubbing off the number you have written on the side of the container.

Explain to the patient that sputum examination is the only sure way to confirm the diagnosis of pulmonary tuberculosis. If it is convenient, you may show AFB-positive slides under the microscope to the patient.

A specimen collected under supervision is likely to yield better results. The person guiding the patient for specimen collection should stand behind and encourage him to cough and produce a good quality specimen. Whenever possible, sputum should be collected in an open place or in a well ventilated room meant for this purpose. Sputum should not be collected in closed rooms, toilets and ill-ventilated rooms. The person collecting the specimen should make sure that no one stands in front of the patient who is trying to cough up sputum. Patients are usually more comfortable if they are separated from other persons at the time of sputum collection

Demonstrate to the patient by action how s/he should bring up sputum. Patient should preferably rinse his mouth as food particles may give false positive results. The patient is instructed to inhale deeply (2–3 times), which will initiate the cough reflex in most patients. The sputum is retained in the mouth and spit into the pre labeled container without spilling. Some patients may not be able to expectorate with deep breathing in which case you should demonstrate to them how they should place their palms on the waist, squat or sit and continue deep breathing again. Tapping or thumping of the back may encourage expectoration. (Sitting and placing hands on the waist fixes the shoulder and pelvic muscles and brings the intercostal muscles of ribcage and diaphragm into action).

Most people do not understand the difference between saliva and sputum. Explain to the patient the characteristics of sputum - that it is thick and mucoid, as compared to saliva which is thin and watery. When a patient has only coughed up saliva or has not coughed up at least 2 ml of sputum, the patient should be encouraged to give good specimen

Please review your Laboratory Manual for detailed instructions on collecting sputum samples.

Write information on the Laboratory Form and on the Sputum Containers

Laboratory Serial Number. A new Laboratory Serial Number is assigned to each of the chest symptomatics whose sputum is examined. The Laboratory Serial Number begins with 1 on 1 January each year and increases by one with each patient until 31 December of the same year. Each set of samples (3 for Diagnosis, 2 for each follow-up examination) is given one Laboratory Serial Number. Diagnosis samples are labelled with a single Laboratory Serial Number with a suffix a-b-c for the spot-morning-spot samples respectively. For the follow-up examinations, the samples are labelled with a serial

number and a suffix a-b with regard to the spot -morning samples respectively. Early morning specimen is always labelled as ‘b’, while the first spot specimen is labelled as ‘a’. Remember that the laboratory serial number is given to a set of slides, and not to individual slides. Enter the Laboratory Serial Number on the side of the sputum container and the Laboratory Form.

It is important to label sputum containers properly. Sputum containers should always be labelled on the side, and never on the lid, as the lid from one container may be placed on another container resulting is specimens being labelled incorrectly. If the labelling is incorrect, a patient who should have been treated may not get treatment, whilst a patient who does not have TB may be put on treatment unnecessarily. Label clearly with a marker that will not be easily erased.

Check the sample to see if it is sputum or saliva only

You must make sure that the sputum sample is of good quality for microscopic examination. Please review your Laboratory Manual for information on how to determine whether samples are good quality.

If the sputum sample is good, the chances of finding AFB are greater. If the sputum sample is only saliva, microscopic examination may be falsely negative for AFB. Poor quality sputum samples will result in patients receiving incorrect treatment or no treatment at all. In this case, patients may become seriously ill or die, and also spread tuberculosis to their family and community. For this reason, it is important that you visually examine every sputum sample and record its appearance on the Laboratory Form.

If the sample is poor, ask the patient to cough again until a good sample is obtained. It may take several minutes for the patient to bring out a good specimen.

A good sputum sample is:	**A poor quality sputum sample is:**
• **Thick (semi-solid), coughed out deeply from the lungs** • **Purulent (yellowish mucus)** • **Sufficient in amount**	• **Contains only saliva (watery) or nasal mucus** • **Is small in quantity (less than 2 ml)**

If you have explained carefully and demonstrated to the patient how to bring out sputum, sample will be of good quality and you will not need to request additional sputum for examination.

Write the visual appearance of the sputum sample on the Laboratory Form

Write the visual appearance of the sputum sample on the Laboratory Form in the space provided. You must make sure that the Laboratory Serial Number on the Laboratory form is the same as the Laboratory Serial Number on the sputum container.

If the patient has provided a sample which is saliva, explain again the importance of a good sputum sample brought out from deep within the lungs. Demonstrate how to bring up sputum, and ask the patient to provide another sample. A patient whose sputum is to be examined for follow-up may only be able to produce saliva, despite their best efforts to produce sputum. These efforts should include having the patient take a series of deep breaths. If this is not successful, try patting the patient gently on the back to help him bring out sputum. If this is not successful, ask the patient to drink something warm and then try to bring out sputum again. If, despite these efforts, the patient is still only able to produce saliva, then the saliva should be examined and the results recorded.

While recording, visual appearance is noted as M- for mucopurulent, B- for blood stained; and S- for saliva.

STAGE 2: PREPARE THE SLIDE FOR EXAMINATION

The next step regarding preparing, staining, examining and reporting a sputum smear are summarized below.

Label the slide with the Laboratory Serial Number

When you are ready prepare the smear, label the slide with the Laboratory Serial Number on the left side. This must be written only with a diamond marker pencil.

Remember that a new Laboratory Serial Number is assigned to each chest symptomatic whose sputum is examined. The Laboratory Serial Number begins with 1 on 1 January each year and increases by one with each patient until 31 December of the same year. Each set of sample (3 for diagnosis, 2 for each follow-up examination) is given the same Laboratory Serial Number. Diagnosis samples are labelled with a single Laboratory Serial Number with a suffix a-b-c for the spot-morning-spot samples respectively. For the follow-up examinations, the samples are labelled with a serial number and a suffix a-b with regard to the spot – morning samples respectively.

Be careful not to leave fingerprints on the slides. Fingerprints can interfere with staining and make accurate examination difficult under the microscope. Only new slides should be used for AFB microscopy, because scratches on old slides can look like AFB, giving a false-positive result.

Spread sputum on the slide using a broomstick and stain the slide

Spreading the sputum correctly on the slide is essential for good staining and accurate microscopic examination.

Explanation of Step in the Preparation and Staining of Slides		
Illustration	**Step of the staining procedure**	**Reasons for/Comments on each step**
	Spread sputum on the slide using a broomstick Smear preparation should be done near a flame. This is required as approximately 6 inches around the flame is considered as a sterile zone which coagulates the aerosols raised during smear preparation.	• If sample are spread too thickly or too thinly, staining and microscope examination will not be accurate. • A different broomstick is used for each smear so that one patient's sputum is not mixed with another patient's sputum mixed with another patient's sputum.
	Allow the slide to air dry for 15-30 minutes	• Heating the slide while it is wet could result in bubbling of TB bacilli into the air.
	Fix the slide by passing it over a flame 3-5 times for 3-4 seconds each time	• Fixation makes the sputum stick to glass slides. • Fixation preserves the shape of the bacilli. • Heating for too long a period could change the shape of bacilli and also cause the slide to break. • Heating for too short a period can result in a false-negative result because the TB bacilli will not be well preserved on the slide.

Illustration	Step of the staining procedure	Reasons for/Comments on each step
	Pour filtered carbol fuchsin to cover the entire slide	• Carbol fuchsin stains the TB bacilli red. • The carbol fuchsin solution must be filtered before use. If it is not filtered before use. If it is not filtered, small particles (sediments) get poured onto the slide and can appear red like the TB bacilli under the microscope.
	Gently heat the slide with carbol fuchsin on it until vapour rises. DO NOT BOIL.	• Carbol fuchsin solution must not be allowed to boil or to dry on the slide, otherwise it will form small particles resulting in a false-positive reading. These particles may look like TB bacilli. • When the slide is heated to 80-90^{0}C, the carbol fuchsin on the slide penetrates the wall of the TB bacilli to stain the bacilli red. • Allowing the carbol fuchsin to boil, will change the shape of the TB bacilli and may result in a false-negative reading.
	Leave carbol fuchsin on the slide for 5 minutes	• The wall of the TB bacillus is thick and waxy. It is essential to give the carbol fuchsin sufficient time to penetrate the wall so that it can stain the bacilli.

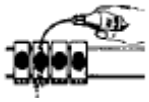
Illustration	Step of the staining procedure	Reasons for/Comments on each step
	Rinse GENTLY with tap water until all free carbol fuchsin stain is washed away	• If water is poured too vigorously, the smear itself will be washed off the slide.
	Tilt the slide to drain off excess water.	• If water is not drained off, it will dilute the next stain/ reagent that is poured, reducing the effectiveness of the next step.
	Pour 25% sulphuric acid onto the slide	▪ Sulphuric acid removes the carbol fuchsin stain from all of the contents of the sputum expect the TB bacilli. For this reason, TB bacilli are known as AFB, or Acid-Fast Bacilli, because the red colour of the AFB from the carbol fuchsin remains after they are decolourized with sulphuric acid.
	Let the slide stand for 2-4 minutes	• Allowing the slide to stand gives sulphuric acid time to wash out the stain from everything except the TB bacilli. • If insufficient time is given, bacteria and sputum contents other than TB bacilli may retain their stain, giving a false-positive result.

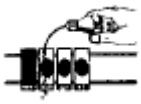
Illustration	Step of the staining procedure	Reasons for/Comments on each step
	Rinse GENTLY with tap water	• Rinsing too strongly can wash the smear itself off the slide. • Sulphuric acid burns the skin. Do not let it splash. • If the slide is still stained red, you may apply sulphuric acid for a second time, letting the slide stand for 1-3 minutes this time. • It is helpful to use sulphuric acid to clean the bottom of the slide, on the opposite side of the smear. This makes it easier to examine the slide under the microscope.
	Pour 0.1% methylene blue onto the slide	• Methylene blue is the counterstain. It colours everything on the smear blue expect the AFB. • The contrast between the AFB which are stained red by the carbol fuchsin and the rest of the smear stained blue by the methylene blue, makes it easier to view the TB bacilli.
	Leave methylene blue on the slide for 30 second	• It takes about 30 second for methylene blue to stain the material on the slide.
	Rinse GENTLY with tap water	• Always rinse gently so that the smear is not washed off the slide.

Illustration	Step of the staining procedure	Reasons for/Comments on each step
	Allow the slide to dry and then examine it under the microscope	• Examining a slide when it is still wet may damage the microscope. • Examining a wet slide will also make it difficult to focus the microscope and read the slide correctly. • Do not dry the slides by blotting.

Keep sputum cups and other materials in a safe place until they are discarded

Do not dispose of the sputum containers until you have examined the slides. In this way, if a repeat smear needs to be prepared from the same specimen, you can do so. However, as soon as all slides are examined, you must dispose of all contaminated materials, including sputum containers.

Place the sputum containers, broomsticks, and other contaminated materials in a container with a foot-operated lid. The materials should be submerged in 5% phenol or phenolic compound containing disinfectant phenyl diluted to 5% and should be kept overnight as per Hospital Waste Management guidelines (Annexure 11 and 12).

Infection Control Measures

You are responsible for ensuring that all biological materials are treated as potentially infectious and are handles as per the Standard (Universal) Precautions (Annexure 12). Hand washing is a simple and effective method of minimizing chances of infection for laboratory personnel. (Read Annexure 12 and familiarize yourself with the correct method of hand washing). Cleanliness of the laboratory and good housekeeping is to be ensured by the laboratory technician.

STAGE 3: EXAMINE THE SLIDE UNDER THE MICROSCOPE

Step-by-step examination of the slide, and reasons for/comments on these steps, is summarized in the table below.

Step	Reasons for/Comments on each step
Put one drop of immersion oil on the left edge of the stained smear	• Immersion oil is necessary for observations under the x100 lens. The oil will bridge the gap between the slide and the lens. • Never let the immersion oil applicator touch the slide. Doing

	so may contaminate the applicator. If the applicator touches the slide, you may spread AFB from one slide to the next resulting in false-positive results.
Bring the slide into focus with the x40, then the x100 lens	• The x40 lens allows you to find a suitable area of the slide to examine. Use the coarse focusing knob for this purpose. • After finding a suitable area, focus the x100 lens with the fine focusing knob. Do not use the coarse focusing knob for final adjustment, as it may break the slide and damage the microscope. • Never let the lens touch the slide. Doing this will damage the lens and may break the slide. In addition, the lens may pick up pieces of sputum and transfer them onto the next slide examined, giving a false-positive result.
Systematically examine at least 100 fields	• Even the most experienced microscopist needs to examine each slide for at least five full minutes. If you examine each slide for too short a period or not carefully enough, you may miss AFB which are present and report the result as negative when it is actually positive. Examine every slide as if it were from one of your family members. • The appearance of AFB is shown in Annexure III of your Laboratory Manual.
Read results as: negative, scanty, or positive (1+, 2+ or 3+)	• See the table below for grading number of fields to be examined. • Grading of sputum smear results is an indicator of the load of infection and also provides epidemiological information.

Grading of slides in AFB Microscopy

Examination	Result	Grading	No. of fields to be examined
More than 10 AFB per oil immersion field	Positive	3 +	20
1–10 AFB per oil immersion field	Positive	2 +	50
10–99 AFB per 100 oil immersion fields	Positive	1 +	100
1–9 AFB per 100 oil immersion fields	Positive	Scanty (Record exact number seen)	100
No AFB per 100 oil immersion fields	Negative	—	100

STAGE 4: RECORD THE RESULTS

The table below summarizes the steps in reporting results, and the reasons for each of these steps.

Step	Reasons for/Comments on each step
Verify the Laboratory Serial Number on the slide and record the result on the Laboratory Form	• Recording results properly is as important as staining and examining a slide correctly. Carelessness can harm patients as well as the programme itself. • Always write the date of the report and sign your name.
Wipe the x100 lens with lens paper	• The x100 lens is a delicate piece of equipment. • Oil will gradually damage the lens unless it is promptly and carefully wiped off after each session of use. • If you take good care of the microscope, it will last for many years. • Never use spirit or xylene to clean the lens, as this may damage it by dissolving the glue.
Write results from the Laboratory Form in the TB Laboratory Register	• For new patients, make sure the address is recorded correctly in the Laboratory Register. • If the patient is a TB suspect being evaluated for diagnosis, you must tick the "Diagnosis" column under the "Reason for Examination" • For patients who undergo repeat sputum examination for diagnosis, you should write "RE" in the Diagnosis column. • You must enter the TB number in the space provided for all patients whose "Reason for Examination" is follow-up. This number should have been recorded on the Laboratory Form, and allows for cross-checking between your Laboratory Register and the Tuberculosis register. • For patients examined for diagnosis, record the TB Number and category of treatment (when known) in the 'Remarks' column. • Every specimen MUST be entered in the Laboratory Register, regardless of where the patient resides or is

	treated. • All positive results should be written in the Laboratory register with a **red pen**. This allows one to find all positive results quickly.

At the end of each month, the laboratory technician should summarize the sputum smears done that month. The format for the monthly abstract is given in Annexure M, which will be written in the last few pages of the Laboratory Register. Both supervisor and laboratory technician should sign and record the date on this list of findings.

STAGE 5: REPORT THE RESULTS

Send the completed Laboratory Form back to the treating physician for information and necessary action. It is important to report these results within one day. The patient's treatment depends on these results, and any delay reduces the value of all the work you have done in examining a slide correctly.

If the patient has been referred from, and will begin treatment at the health unit where the microscopy centre is located, give the results to the treating physician. If the patient was referred from another health unit, ensure that the results of the sputum microscopy are communicated to the treating physician at the referring health facility (a copy of the results/Laboratory form to be sent to the treating physician).

Never give results only to the patient. If the patient fails to bring the results to the Medical Officer or Treatment Centre, s/he may not receive treatment.

STAGE 6: VERIFY THE RESULTS - PRESERVE SLIDES FOR REVIEW BY THE SUPERVISOR

Do not discard any slide until your supervisor has reviewed it. Removal of immersion oil is to be done by placing the slides inverted with the smear surface having the oil immersion on it facing downwards, on tissue paper overnight, or until the immersion oil is completely absorbed. Care may be taken not to rub the slides on the tissue paper as this activity may remove the smear from the slides. Preserve the slides in a cool and dry place in a wooden box to avoid exposure to light and dust. Exposure to light and dust can result in fading of the red colour of the stained TB bacilli. Store the slides in boxes that do not allow the slides to touch each other (e.g. do not stack or press slides together).

Once in a month, your supervisor (STLS) does an on-site evaluation (OSE) of your laboratory (see annexure 2 for check-list). The visit includes a comprehensive assessment of the laboratory safety including Infection Control measures; conditions of the equipment, adequacy of supplies as well as the technical components of AFB smear microscopy employing a simple "Yes" and "No" check-list.

As part of the on-site evaluation, the visiting STLS will review during each visit to the DMC in an unblinded manner, 5 positive and 5 negative slides selected systematically from the RNTCP TB Laboratory Register. The slides selected for un-blinded crosschecking are for the period between the last and current visit of the STLS to the respective DMC. The STLS should indicate the date of the current visit by drawing a line on the left side margin of the laboratory register, below the row with the last laboratory entry. The results of the OSE re-checking are recorded in the "Remarks" column of the laboratory register and also in the STLS OSE forms. The pencil marking on the Laboratory Register for the slides selected for unblended rechecking will be in the form of an "X".

If there are discrepancies between your reading and that of your supervisor, review these together with STLS so that you can learn. Your supervisor will also help you make sure that your staining technique is correct, and is neither too light nor too dark.

The STLS during their monthly OSE visits to the DMCs also need to collect slides from the slide boxes for Random Blinded Re-Checking (RBRC) in a monthly fashion from the routine slides examined by you at your respective DMC. RBRC is a process of re-reading of a sample of routine slides from a DMC to assess whether that laboratory has an acceptable level of performance.

Instructions will be sent by the DTO to all STLS of the district, informing them of the total number of slides to be collected every month from each DMC. The STLS then selects the required number of slides from the RNTCP TB Laboratory Register. It is LTs responsibility to pick out the required slides. If a slide is missing substitute the next slide in the laboratory register regard less of the results and the LT record the results of the selected slides as per Annexure B. The slides collected for RBRC should be from the previous calendar month. Annexure B is then put into an envelope and sealed. The number of slides packed is written on the top of the envelope. Both the slide box and the envelope must be clearly marked with the name of the respective DMC, the name of the TB Unit and district, and the month and the year. The slide box and the sealed envelope are taken by the STLS for handing over to the DTO. The STLS should leave a corresponding number of empty slide boxes for the use of the LT at the DMC. The pencil marking on the Laboratory Register for those slides selected for RBRC should be an "O", so as to differentiate this selection from that of the 5 positive and 5 negative slides selected for un-blinded OSE re-checking.

- **STLS samples and select slides for RBRC**
- **STLS marks RBRC selected slides in the laboratory register with a circle**
- **Laboratory Technician fills out Annexure B for the selected slides**
- **LT seals the filled Annexure B in an envelope**
- **LT marks on the envelope and the slide box the Serial No. of slides, Name of the DMC and TU, Month and year**
- **STLS hands over sealed envelopes and box to the DTO**

The results of the RBRC are intimated to the MO of the DMC through Annexure D by the DTO, and errors found during the RBRC are explained to the LT by the supervisor in order to minimize the chances of such errors recurring in the future and to improve the service provided.

The slides should be stored for a maximum of 3 months during which the STLS rechecks (i) the 5 positive and 5 negative slides on the spot and (ii) collects slides for RBRC. The remaining slides need to be stored for at least 3 months. After that on the advice of the STLS/DTO, the slides are disposed off by burying in the waste-disposal pit as per the RNTCP guidelines. You should never re-use TB slides for TB work including the negative slides. If the slide was read wrongly as negative the first time, AFB may be present and may give an incorrect result the second time. In addition, scratches can cause false-positive reading for AFB. For these reasons, only new slides should be used for TB work.

Remember: False results in AFB sputum smear microscopy during 'diagnosis' and 'follow-up' of treatment affect the quality of laboratory services offered to the patients.

Diagnosis: While false Positive results lead to TB suspect being placed unnecessarily on treatment (a drain on the resources of the programme), on the other hand, false negative results lead to a patient being denied TB treatment and subsequent risk of spreading the TB disease in the society.

Follow-up: False negative results lead to 'incomplete' treatment (termination of intensive phase) and being wrongly declared as cured; false-positive results lead to extension of intensive phase. Both will lead to wrong categorization and incorrect treatment.

Check the quality of Reagents received from STLS

Whenever you receive a new batch of prepared reagents, STLS would give you two unstained control slides, one positive (3+ grade) and one negative. You are to stain these two slides with the new batch of reagents and record results in the stock register for laboratory consumables. These quality control slides are also to be preserved for 3 months.

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Laboratory Register

Lab Serial No.	Date	Name (in full)	Sex M/F	Age	Complete address (for new patients)	Name of Referring Health Facility	Reason for Examination*		Results			Signature	Remarks
							Diagnosis	Follow-up	a	b	c		

If sputum is examined for diagnosis, put a tick (✓) mark in the space under "Diagnosis"
If sputum is examined for repeat diagnosis, put RE in the space under "Diagnosis"
If sputum is for follow-up of patients on treatment, write the patient's TB No. in the space under "Follow-up"

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Tuberculosis Laboratory Monthly Abstract

(Record Numbers)

Month Year 200.	TB Suspects Examined For Diagnosis	TB Suspects Found Positive	TB suspects Undergoing Repeat Sputum Examination	TB suspects Found Positive on Repeat Examination	Follow-up Patients examined	Patients Positive on Follow up	Total Slides Examined	Total Positive slides	Total Negative slides	Signature of LT and STLS
Jan										
Feb										
Mar										
Apr										
May										
Jun										
Jul										
Aug										
Sep										
Oct										
Nov										
Dec										
Total										

Signature of the M.O.

EXERCISE 2

1. How will you explain to a patient to bring up sputum?

   _____

   _____

2. What are the characteristics of a good quality sputum sample?

   (i) _____

   (ii) _____

3. What are the characteristics of a poor quality sputum sample?

   (i) _____

   (ii) _____

4. What may happen if you forget to fix the smear?

   _____

5. What may happen if you fix the smear before waiting for the sputum to dry?

   _____

6. What may happen if you do not filter the carbol fuchsin?

   _____

7. What may happen if you forget to heat the slide after adding the carbol fuchsin solution?

   _____

8. How will you preserve the stained slides for quality control?

   _____

9. Which is the magnification of the oil immersion lens?

   (a) x4

   (b) x10

   (c) x40

   (d) x100

10. You have been told to make sure that the oil immersion lens never touches the slide. You have also been told never to let the oil applicator touch the slide. The reason for both instructions is the same. What is it?

    _____

    _____

11. What do the letters AFB stand for and why is this term used to describe the TB bacillus?

    _____

12. Please indicate the proper grading of each of the following:

    (i) 20 oil immersion fields with 100 AFB seen.

    Result:_____ Grade:_____

    (ii) 20 oil immersion fields with 30 AFB seen.

    Result:_____ Grade:_____

    (iii) 20 oil immersion fields with 200 AFB seen.

    Result:_____ Grade:_____

13. (i) The LT has read first 20 fields and has recorded six AFBs in these fields. What should he do now;

    _____

    (ii) LT has noticed a total of 15 AFBs after reading 50 fields. Can he give any result at this stage?

    _____

    (iii) LT has given a 'positive 1+' result based on the above mentioned finding. What is your comment on this result?

    _____

(iv) LT was asked to read another 50 fields (a total of 100 fields) and he has found 125 AFBs. What result should he record in the Lab register and Lab form? Explain your answer.

_____

_____

_____

14. List 5 causes of false-positive results:

(i) _____

(ii) _____

(iii) _____

(iv) _____

(v) _____

15. What may happen as a result of a false-positive AFB result?

_____

16. List 10 causes of false-negative AFB results:

(i) _____

(ii) _____

(iii) _____

(iv) _____

(v) _____

(vi) _____

(vii) _____

(viii) _____

(ix) _____

(x) _____

16. What may happen as a result of a false-negative AFB result?

_____

17. Is the Laboratory Form on the page 33 correctly filled? If not what is wrong with it?

18. Using the Laboratory Forms on pages 34-36, complete the first three lines of the Laboratory Register on page 37.

19. There is an error in recording or testing in every line of the Laboratory Register on page 38. Find the errors and indicate the possible implications of each.

Error	Possible implications
1.	
2.	
3.	
4.	
5.	
6.	
7.	
8.	
9.	
10	

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Laboratory Form for Sputum Examination

Name of Referring Health Centre: 101 Date: 3-9-05
Name of patient: Lakshmi Kumari Age: 46 Sex: M F X
Complete address: 223 Gandhi Dham, Bapu Nagar

Type of suspect / disease: X Pulmonary
Extra-pulmonary Site: _____

Reason for examination:
X Diagnosis
Repeat Examination for Diagnosis
Follow-up of chemotherapy Patient's TB No _____

(Name and signature of referring person/official)

If sputum samples are being transported:

Specimen identification No.: 1C Date of sputum collection: 4-9-05

Specimen Collector's name and signature Shyam

RESULTS (To be completed in the laboratory of DMC)

Name of DMC: _____

Lab. Serial No.: 102

Date of examination	**Specimen**	**Visual appearance (M, B, S)***	**Results** (NEG or POS)	**Positive (grading)**			
				3+	**2+**	**1+**	**Scanty****
3-9-05	a	B	POS		√		
4-9-05	b	M	POS		√		
4-9-05	c	M	POS			√	

* M = Mucopurulent, B = Blood stained, S = Saliva
** Write actual count of AFB seen in 100 oil immersion fields

Date: 4-9-05 Examined by (signature): JOSHI

The completed form (with results) should be sent to the referring PHI within one day of the examination

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Laboratory Form for Sputum Examination

Name of Referring Health Centre: 237 Date: 3-9-05
Name of patient: Kalavati Age: 46 Sex: M F X
Complete address: 705 Parvati Marg, Gaurgarh, Maharashtra

Type of suspect / disease: X Pulmonary
Extra-pulmonary Site: _____

Reason for examination:
Diagnosis
Repeat Examination for Diagnosis
X Follow-up of chemotherapy Patient's TB No 63

(Name and signature of referring person/official)

If sputum samples are being transported:

Specimen identification No.: Date of sputum collection: 4-9-05

Specimen Collector's name and signature Gopal

RESULTS (To be completed in the laboratory of DMC)

Name of DMC: _____

Lab. Serial No.: 311

Date of examination	Specimen	Visual appearance (M, B, S)*	Results (NEG or POS)	Positive (grading)			
				3+	2+	1+	Scanty**
3-9-05	a	B	POS			√	
4-9-05	b	M	POS		√		
4-9-05	c	M	NEG				

* M = Mucopurulent, B = Blood stained, S = Saliva
** Write actual count of AFB seen in 100 oil immersion fields

Date: 4-9-05 Examined by (signature): JOSHI

The completed form (with results) should be sent to the referring PHI within one day of the examination

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Laboratory Form for Sputum Examination

Name of Referring Health Centre: 237 Date: 3-9-05
Name of patient: Lallan Prasad Parmar Age: 51 Sex: M X F
Complete address: 217 Gali Akara, Near Revoli

Type of suspect / disease: X Pulmonary
Extra-pulmonary Site: _____

Reason for examination:
X Diagnosis
Repeat Examination for Diagnosis
Follow-up of chemotherapy Patient's TB No _____

(Name and signature of referring person/official)

If sputum samples are being transported:

Specimen identification No.: Date of sputum collection: 4-9-05

Specimen Collector's name and signature Kamala

RESULTS (To be completed in the laboratory of DMC)

Name of DMC: _____

Lab. Serial No.: 102

Date of examination	**Specimen**	**Visual appearance (M, B, S)***	**Results** (NEG or POS)	**Positive (grading)**			
				3+	**2+**	**1+**	**Scanty****
3-9-05	a	M	POS			√	
4-9-05	b	M	NEG				
4-9-05	c	M	NEG				

* M = Mucopurulent, B = Blood stained, S = Saliva
** Write actual count of AFB seen in 100 oil immersion fields

Date: 4-9-05 Examined by (signature): JOSHI

The completed form (with results) should be sent to the referring PHI within one day of the examination

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Laboratory Form for Sputum Examination

Name of Referring Health Centre: 237 Date: 3-9-05
Name of patient: Srinivasa Rao Age: 36 Sex: M X F
Complete address: WB 2451, Gali Pathanwali, Loni Village

Type of suspect / disease: X Pulmonary
Extra-pulmonary Site: _____

Reason for examination:
X Diagnosis
Repeat Examination for Diagnosis
Follow-up of chemotherapy Patient's TB No _____

(Name and signature of referring person/official)

If sputum samples are being transported:

Specimen identification No.: Date of sputum collection: 4-9-05

Specimen Collector's name and signature Kamala

RESULTS (To be completed in the laboratory of DMC)

Name of DMC: _____

Lab. Serial No.: 109

Date of examination	**Specimen**	**Visual appearance (M, B, S)***	**Results** (NEG or POS)	**Positive (grading)**			
				3+	**2+**	**1+**	**Scanty****
3-9-05	a	M	NEG				
4-9-05	b	M	NEG				
4-9-05	c	M	NEG				

* M = Mucopurulent, B = Blood stained, S = Saliva
** Write actual count of AFB seen in 100 oil immersion fields

Date: 4-9-05 Examined by (signature): JOSHI

The completed form (with results) should be sent to the referring PHI within one day of the examination

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Laboratory Register

Lab Serial No.	Date	Name (in full)	Sex M/F	Age	Complete address (for new patients)	Name of Referring Health Facility	Reason for Examination*		Results			Signature	Remarks
							Diagnosis	Follow-up	a	b	c		

If sputum is examined for diagnosis, put a tick (✓) mark in the space under "Diagnosis"
If sputum is examined for repeat diagnosis, put RE in the space under "Diagnosis"
If sputum is for follow-up of patients on treatment, write the patient's TB No. in the space under "Follow-up"

Revised National Tuberculosis Control Programme

Laboratory Register

Lab Serial No.	Date	Name (in full)	Sex M/F	Age	Complete address (for new patients)	Name of Referring Health Facility	Reason for Examination*		Results			Signature	Remarks
							Diagnosis	Follow-up	a	b	c		
499	30/3	Sita Dixit	F		H. No.211, Pocket III, Mayur Vihar	Modern TB Clinic	√		Neg	Neg	Neg	Joshi	No. 326
500	30/3	Krishna Kanth	F	54	H. No. 40, Sector II, Jamnagar	Jamnagar Health Centre	√		Neg	Neg		Joshi	
501	30/3	Aswani Rai	F	39	225 Block 4, Bapu Nagar	Chest Disease Health Centre	√		3+			Joshi	No. 341
502	30/3	Abdul Hazan	M	44	Gali No.7, JJ Ram Rani Colony	Aligarh Dispensary		20	Neg			Joshi	
503	1/4	Bhim Singh		38	H. No. 422, Sector III, Rohini	Modern TB Clinic		102	Neg	Neg		Joshi	
504	1/4	Alex Chopra	M	45		Good Health Centre	√		1+	1+	2+	Joshi	
505	1/4	Renu Sharma	F	37		Jamnagar Health Centre		√	1+	2+		Joshi	
506	2/4	Kumar Bhatia	M	58	BB22/Block 4, Nehru Place	Chest Disease Health Centre	√		Neg	Neg	Neg	Joshi	
507	2/4	Deepak Dhawan	M	28		Modern TB Clinic	√		1+	2+			No. 346
508	3/4	Preeti Chandra	F	26	H. No. 62, Lane No. 820, Kailash Colony		√		Neg	Neg	Neg	Joshi	

If sputum is examined for diagnosis, put a tick (✓) mark in the space under "Diagnosis"
If sputum is examined for repeat diagnosis, put RE in the space under "Diagnosis"
If sputum is for follow-up of patients on treatment, write the patient's TB No. in the space under "Follow-up"

20. Next to each step, indicate the reason for the step and whether not performing it correctly could lead to a false-positive result, a false-negative result, both, or neither, and indicate the reason for this.

Steps of staining procedure	Reason for the step	Consequences if not performed correctly
Spread sputum on the slide using a broomstick		
Allow the slide to air dry for 15-30 minutes		
Fix the slide by passing it over a flame 3-5 times for 3-4 seconds each time		
Pour filtered carbol fuchsin to cover the entire slide		
Gently heat the slide with carbol fuchsin on it until vapours rise. DO NOT BOIL		
Leave the slide for 5 minutes		
Rinse GENTLY with tap water until all free carbol fuchsin stain is washed away		
Tilt the slide to drain off excess water		
Pour 25% sulphuric acid onto the slide		
Let the slide stand for 2-4 minutes		
Rinse GENTLY with tap water until all free stain is washed away		
Pour 0.1% methylene blue onto the slide		
Let the slide stand 30 seconds		
Rinse GENTLY with tap water		
Allow the slide to dry and then examine it under the microscope		

HEALTH EDUCATION AND COMMUNICATION WITH PATIENTS

Dos	DON'Ts
• Communicate respectfully and patiently with patients • Explain and demonstrate to patients by actions the method of bringing out sputum • Examine the quality of sputum samples before patients leave the laboratory • Tell patients that TB is curable if regular and complete treatment is taken • Tell patients that treatment for TB is free of cost • Tell patients to tell others with symptoms of TB to contact the health facility	• Tell patients that their test for TB is negative • Tell patients that they are cured • Be impatient or rude with patients • Refuse to accept sputum from patients at any time of the day • Give the results of sputum examination only to the patient • Make tuberculosis patients fell rejected

Patients with tuberculosis may be highly infectious, but if they take effective treatment using DOTS, they will be cured and they will also not infect others. The LT must be aware that patients with negative sputum smears may have tuberculosis. Therefore, they should never tell patients that their test for tuberculosis is negative. Patients with negative sputum smears must be further examined by a Medical Officer. The Medical Officer will determine if they have tuberculosis or not. Similarly, when follow-up sputum is examined, the LT must not tell patients that their tuberculosis is cured. Although smears become negative within 2-3 months in most patients on DOTS, they will not be cured unless they complete a full course of treatment. Patients who stop treatment before they have completed a full course of treatment are likely to develop even more severe disease.

Collection of sputum in the correct manner is essential both for diagnosis and monitoring treatment of tuberculosis. By communicating effectively with patients, the LT improves patients' diagnosis and treatment. By showing respect and patience when talking with patients, the LT can encourage patients to take treatment until they are cured.

Annexure 1

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Laboratory Form for Sputum Examination

Name of Referring Health Centre: _____ Date: _____
Name of patient: _____ Age: _____ Sex: M F
Complete address: _____
_____

Type of suspect / disease: Pulmonary
Extra-pulmonary Site: _____

Reason for examination:
Diagnosis
Repeat Examination for Diagnosis
Follow-up of chemotherapy Patient's TB No _____

(Name and signature of referring person/official)

If sputum samples are being transported: _____

Specimen identification No.: _____ Date of sputum collection: _____

Specimen Collector's name and signature

RESULTS (To be completed in the laboratory of DMC)

Name of DMC: _____

Lab. Serial No.: _____

Date of examination	**Specimen**	**Visual appearance (M, B, S)***	**Results** (NEG or POS)	**Positive (grading)**			
				3+	**2+**	**1+**	**Scanty****
	a						
	b						
	c						

* M = Mucopurulent, B = Blood stained, S = Saliva
** Write actual count of AFB seen in 100 oil immersion fields

Date: Examined by (signature):

The completed form (with results) should be sent to the referring PHI within one day of the examination

Annexure 2

On-Site Evaluation Checklist for STLS

I General Information

DMC:	
District:	
Number of Technicians:	
Qualifications of current staff: (Separate sheet to be attached to indicating information for each of Lab staff, if they are different from the previous visit)	
Supervisor/MO of DMC:	
Date of Visit:	
Name of visiting STLS:	

II Data on Slide volume for the last month:
This information is necessary to (i) select slides for Blinded Rechecking for the current month and as cumulative number for (ii) next annual SPR, (iii) next annual negative slides and (iv) annual total slides.

Sl. No.	**Type of slide** (Includes diagnosis and follow up slides)	**Number**
1	Positive slides	
2	Negative slides	
3	Total	

III Action required as per the previous visit:

IV Current visit particulars

Sl. No	Item	Adequate/ Acceptable	Problems Identified
1	Standard Operating Procedure (charts, manuals and modules)	Y / N	
2	Separate area for TB Lab work	Y / N	
3	Separate platform / tables for specimen receipt / smear preparation / microscopy	Y / N	
4	Power supply	Y / N	
5	Running water supply	Y / N	
6	Waste containers with lid	Y / N	
7	Waste disposal by Autoclave/burning/buried	Y / N	
8	**Adequate Stock and Supply of**: Specimen cups	Y / N	
9	Slides	Y / N	
10	Lens Tissue	Y / N	
11	Spirit lamp or Bunsen burner	Y / N	
12	Filter paper	Y / N	
13	Smearing / Staining Equipment (staining racks, sticks etc)	Y / N	
14	Slide boxes	Y / N	
15	Staining reagents:	Y / N	
15 (a)	1% Carbol fuchsin	Y / N	**Within expiry date Y / N**
15 (b)	25% Sulphuric acid	Y / N	**Within expiry date Y / N**
15 (c)	0.1% Methylene Blue	Y / N	**Within expiry date Y / N**
16	Immersion oil		
17	Label on sputum container	Y / N	
18	New slides used for AFB microscopy	Y / N	
19	Slides labeled with Lab Sl. No.	Y / N	
20	Number of specimens collected for diagnosis and for re-examination for diagnosis	Y / N	
21	Number of specimens collected for follow up examination	Y / N	
22	Smears air-dried prior to fixing	Y / N	
23	Staining procedure	Y / N	
24	Follow grading chart	Y / N	
25	Are positive results entered in Red ink	Y / N	

Sl. No	Item	Adequate/ Acceptable	Problems Identified
26	Control smears are used for each new batch of stains received at DMC	Y / N	
27	Binocular Microscopes	Y / N	
28	Maintenance of microscope	Y / N	
29	Laboratory Register	Y / N	
30	Write TB number of 'Follow up' patients in all cases	Y / N	
31	Write TB number and category of smear positive patients in the remarks column when this becomes available	Y / N	
32	Laboratory forms	Y / N	
33	Any change in lab staff since last supervisory visit.	Y / N	
34	Personnel	Y / N	
35	Training status	Y / N	
36	Has each staff member participated in refresher training within past two years	Y / N	
37	Safety Practices	Y / N	
38	General order / cleanliness	Y / N	
39	Timely reporting of results to clinicians	Y / N	
40	Does the TB Register contain all smear positive patients recorded in the TB Lab Register	Y / N	
41	Are the smear results for follow up patients in the TB Lab Register the same as the results recorded in the TB Register	Y / N	

42	**Are all slides kept as required by the RNTCP EQA Programme?**	Yes	No
43	Are slides collected for EQA, do the number in the slide box correlate with the number in the Lab Register	Yes	No

V Review of five positive and five negative slides from RNTCP TB Lab Register:

(Systematic sampling, separately for positive and negative slides)

a) Of the 5 Pos slides, number re-read as positive by STLS _____

b) Of the 5 Neg slides, number re-read as negative by STLS _____

Tick appropriate column or write letter as indicated below table

Sl. No.	Slide No.	AFB result / Grade by		Specimen Quality		Staining		Size		Thickness		Evenness	
		STLS	LT of DMC	≥10 WBC/ field	< 10 WBC/ field	Good	Poor (U/O)	Good	Poor (B/S)	Good	Poor (K/N)	Good	Poor
		1		2		3		4		5		6	
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													

1: Write smear and grade
2: Tick appropriate column
3: Tick if good; write 'U' if under-decolourized, 'O' if over-decolourized
4: Tick if good; write 'B' if too big, 'S' if too small
5: Tick if good; write 'K' if too thick, 'N' if too thin
6: Tick appropriate column
*** Please carefully review all discordant slides with the LT**

Overall summary (please tick appropriate alternative):

Specimen quality: Needs improvement ❑ Yes ❑ No
Smear size: Needs improvement ❑ Yes ❑ No
Smear thickness: Needs improvement ❑ Yes ❑ No
Smear evenness: Needs improvement ❑ Yes ❑ No
Staining: Needs improvement ❑ Yes ❑ No

Name of STLS: _____ Signature of STLS: _____

Name of LT: _____ Signature of LT: _____

Name of MO-in-charge: _____ Signature of MO-in-charge: _____

Date_____

Annexure 3

Annexure B of EQA: RNTCP Smear Results Sheet for Random Blinded Rechecking

Microscopy Centre: _____ District: _____

Name of TU_____ Month/Year: _____

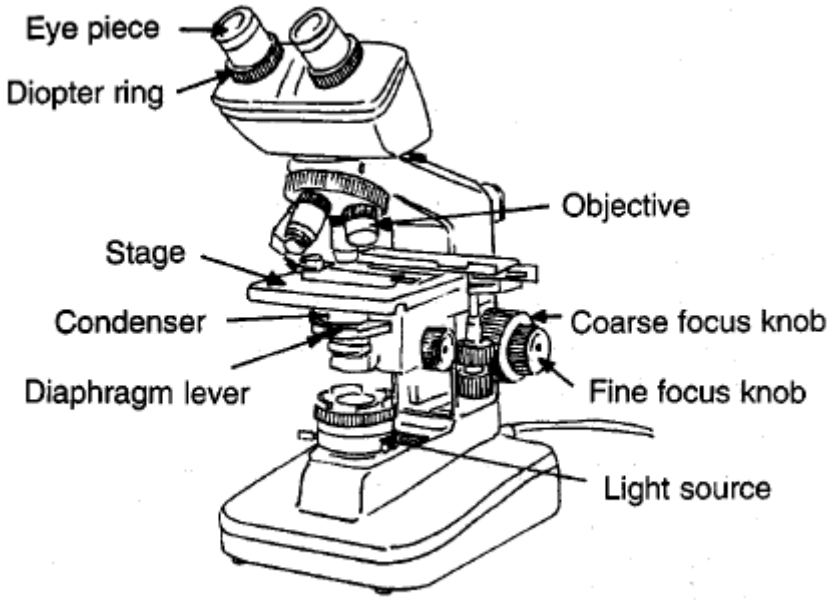
Sl. No.	Lab No.	Result of LT of DMC, including grade for positive smears
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		
15.		
16.		
17.		
18.		
19.		
20.		
21.		
22.		
23.		
24.		
25.		

Name of Lab Technician: _____

Signature: _____ Date _____

Annexure 4

Microscope and its parts



Annexure 5

Care of the Microscope

The microscope is the lifeline of the Revised National Tuberculosis Control Programme. Proper handling and maintenance of the microscope, particularly of its lenses, is very important. The following points should be observed:

1. **Place and store the microscope in a dry, dust-free and vibration-free environment, which is specially built in the laboratory, kept warm with a light source to prevent growth of fungus**

- Vibration damages the microscope.
- When the microscope is not being used, cover or keep it in the box so as to keep it free from dust.
- Avoid exposing the microscope to direct sunlight.
- Avoid exposing the microscope to moisture. Humidity may allow fungus to grow on the lens and cause rusting of the metal parts.
- To protect the microscope against the harmful effects of humidity, it is preferable that it be kept overnight in a specially designed storage cupboard. A bulb holder for a 15 watt bulb (candle bulb) will be fixed on the rear wall of the storage cupboard on the right hand side top corner, such that the microscope does not come in contact with the bulb while storing or removing the microscope. The operating switch for the bulb will be located in the storage space on the right side wall towards the front of the storage space (just behind the shutter) for ease of operation. The bulb should remain on when the microscope is stored inside.
- An alternative may be to place plenty of dry blue silica gel into a shallow plate and place it in the box when the microscope is kept in it. Silica gel is blue in colour when it is dry but when it becomes wet it turns pinkish. As soon as the silica gel becomes pink, change or heat it until it turns blue again and then reuse it.

2. **Keep the microscope and lenses clean**

- All the lenses should be cleaned with dry lens paper or fine silk cloth or lint cloth, immediately after use and at the end of a day's work. Do not wipe the lens with an ordinary cloth.
- Do not leave immersion oil on the surface of the immersion lens.
- Never use spirit or alcohol or xylene to clean the lenses, as these can damage them.
- Never let the oil immersion lens touch the smear.
- Use the fine focusing knob only while using the oil immersion lens.

Annexure 6

Items Needed for Staining and Examining Slides for AFB

For preparing the sputum smear:

- Wooden broomsticks for spreading sputum
- New microscopy slides
- Carbol fuchsin solution in a plastic squeeze bottle (500 ml capacity)
- 25% sulphuric acid (H_2SO_4) in a plastic squeeze bottle (500 ml capacity)
- 0.1% methylene blue solution in a plastic squeeze bottle (500 ml capacity)
- Staining rack
- Heat source (spirit lamp or gas burner)
- Tap water

For microscope examination:

- Diamond marker pencil to label slides
- Immersion oil for x100 examination & applicator
- Lens paper or fine silk cloth or lint cloth to clean the microscope lens
- A notebook to record the number of AFB in each field of the slide

For preservation of slides:

- Soft tissue (toilet tissue) rolls to drain oil
- Slide boxes

Annexure 7

Formulation of Reagents

Preparation of 1% carbol fuchsin

- Potency correction factor: Note down the dye content – this should be available on the container. The dye content should be approximately 85% - 88%. To calculate the required amount of basic fuchsin, divide the actual amount required by the dye content. For example: dye content = 85%, actual amount required = 5gms, required amount of dye = 5/0.85 = 5.88 gms.
- Weigh potency corrected amount of basic fuchsin dye (5.88gms in the above example) in a balance and transfer it to a 250ml Erlenmeyer glass flask.
- Add 50 ml of methylated spirit and shake to dissolve the dye.
- Heat 25 grams of phenol to melt it and add it to the above solution.
- Heat the flask containing basic dye dissolved in spirit and phenol gently in a water bath at about 60^0C. **Do not heat directly on a flame.**
- Transfer the contents into a 500 ml measuring cylinder.
- Add distilled water to make up a final volume of 500 ml.
- Pour the solution through filter paper (Whatmann No. 1) and store filtered solution in a glass bottle. Label the bottle as 1% carbol fuchsin with the date of preparation.

Though the expiry date is four months from the date of preparation, it is preferred to use the reagents within one month of preparation.

Any time particles start to form in the carbol fuchsin solution, the solution must be filtered again

Preparation of 25% sulphuric acid

- Pour 375 ml of distilled water into a 1 litre glass flask.
- Measure 125 ml of concentrated sulphuric acid and transfer it slowly into the flask containing water.
- Always add acid to water. Never add water at acid.
- Store the sulphuric acid solution acid solution in a glass bottle.

Preparation of 0.1% methylene blue solution

- Potency correction factor: Note down the dye content – this should be available on the container. The dye content should be approximately 82%. To calculate the required amount of methylene blue, divide the actual amount by the dye content. For example:

dye content = 82%, actual amount required = 0.5gms, required amount of dye = 0.5/0.82 = 0.61 gms.

- Weigh potency corrected amount of methylene blue (0.61 gms in the above example) in a balance and transfer to a 1 litre glass flask.
- Add 500 ml of distilled water.
- Shake well to dissolve.
- Store in a glass bottle with the label showing name of the reagent and date of preparation.

After each batch of reagents is made, slides known to be positive and negative should be stained as internal quality control of the reagents. Control smears are a panel of two slides: One known positive (3+, labeled as QCP) and one known negative (Neg, labeled QCN). It is preferred to select a negative specimen having ≥10 pus cells/ field, from a patient with negative results in three specimens. A batch is the volume of reagent prepared at one time. Each batch of new reagent should be tested with control smears and should be found correct. Do not mix remaining quantity of old batch of reagent with newly prepared batch. Though the expiry date is four months from the date of preparation, it is preferred to use the reagents within one month of preparation.

Annexure 8

Prevention and Consequences of False-positive and False-negative Sputum Results

HOW TO PREVENT FALSE-POSITIVE SPUTUM RESULTS

- Always use new, unscratched slides
- Use a separate broomsticks for each sample
- Always use filtered carbol fuchsin
- Do not allow the carbol fuchsin to dry during staining
- Decolorize adequately with sulphuric acid
- Make sure there no food particles or fibres in the sputum sample.
- Never allow the oil immersion applicator to touch a slide
- Label sputum containers, slides and Laboratory Forms accurately
- Cross-check the number on the Laboratory Form and sputum container before recording
- Record and report results accurately

Consequences of false-positive sputum results

- Patients are begun on treatment unnecessarily
- Treatment is continued longer than necessary, in the case of follow-up examinations
- Medications will be wasted
- Patients may lose confidence in the programme

HOW TO PREVENT FALSE-NEGATIVE SPUTUM RESULTS

- Make sure the sample contains sputum, not just saliva
- Make sure there is enough sputum (at least 2 ml)
- Select thick, purulent particles to make the smear
- Prepare smears correctly-not too thick, too thin or too little material
- Fix the slide for the correct length of the time, not too short or too long
- Stain with carbol fuchsin for the full 5 minutes
- Do not decolourize with sulphuric acid too intensively
- Examine every smear for at least five minutes before recording it as negative
- Label the sputum containers, slides and Laboratory Forms carefully
- Cross check the number on the Laboratory Forms and sputum container before recording
- Record and report result accurately

Consequences of false-negative sputum results

- Patients with TB may not be treated, resulting in suffering, spread of TB and death
- Intensive phase treatment may not be extended for the required duration, resulting in inadequate treatment
- Patient may lose confidence in the programme

Annexure 9

Job Responsibilities of the Laboratory Technician (LT) in the Revised National Tuberculosis Control Programme.

1. Sputum collection

- Instruct and demonstrate to patients the proper methodology on how bring out good quality sputum.
- Label the sputum container properly.
- Before the patient leaves, check the sample to see if it is sputum or only saliva.
- Coordinate with other staff to ensure that patients with productive cough for three weeks or more undergo three initial sputum examinations for diagnosis and those under treatment undergo two sputum examinations for follow up.

2. Sputum processing and examination

- Prepare Smears from the thickest part of sputum, stain read and record results.
- Write the Laboratory No. and visual appearance of the sputum on the Laboratory Form.
- Always use new slides.
- Spread the smear and heat it in order to fix it on the slide.
- Stain the smear by the Ziehl-Neelsen method.
- Examine the stained smear under the microscope.

3. Recording and reporting

- Enter the result of each microscopic examination on the Laboratory form and in the Laboratory Register.
- Maintain the Laboratory Register properly, including the reason for sputum examination.
- Send the Laboratory Form with results recorded to the treating physician promptly.
- Enters the data in monthly lab abstract (Page 28) & signs
- Assist the MO-PHI in completing the monthly PHI report

4. Quality control

- Preserve month-wise in a slide box all the slides according to the entries in the RNTCP TB Laboratory Register.
- Obtain the feedback on the 5 positive and 5 negative slides re-checked by the STLS during their monthly on-site evaluation visit to the respective DMC.
- Collect the slides for random blinded re-checking (RBRC) as requested by the STLS and arrange the slides serially in a slide box, complete Annexure B with

the results of the corresponding slides that are selected for RBRC. Place Annexure B (see following page) in a sealed envelope. Label the slide box "with "LQAS slides", "number of slides", "month/year", "TU and DMC names" Handover the slide box and sealed envelope to the STLS.

- Implement the corrective actions suggested by STLS and DTO, if any error is found in any of the EQA activities i.e., un-blinded, random blinded re-checking of slides and during on-site evaluation by STLS.
- Inform your MO the corrective action taken as per the suggestion of STLS and DTO.
- MO will submit every month the action-taken report on EQA to DTO.

LT is an important staff in RNTCP and the purpose of EQA is to identify the errors occurring in smear microscopy and taking corrective action immediately so that errors are not repeated.

5. Safety

- Keep the laboratory clean.
- Do not eat, drink, or smoke in the laboratory.
- Safely dispose of all contaminated materials including sputum cups.
- Discard all the slides after they have been verified by the STLS and the EQA results (RBRC) with likely cause of error, if any, is identified and corrective action is implemented and intimated by him/her to do so.

6. Materials management

- Keep the microscope in good working condition.
- Prepare and store solutions and reagents properly.
- Maintain records of the Lab consumables and reagents and Order supplies well in advance to avoid shortages.
- Use freshly prepared reagents within expiry date

Wash your hands every time you handle contaminated material

Annexure 10

Ziehl–Neelsen Staining Procedure

1. Select a new unscratched slide and label the slide with the Laboratory Serial Number with a diamond marking pencil.
2. Spread sputum on the slide using a broomstick.
3. Allow the slide to air dry for 15–30 minutes.
4. Fix the slide by passing it over a flame 3–5 times for 3–4 seconds each time.
5. Pour filtered carbol fuchsin to cover the entire slide.
6. Gently heat the slide with carbol fuchsin on it, until vapours rise. Do not boil.
7. Leave carbol fuchsin on the slide for 5 minutes.
8. Gently rinse the slide with tap water until all free carbol fuchsin stain is washed away. At this point, the smear on the slide looks red in colour.
9. Pour 25% sulphuric acid onto the slide.
10. Let the slide stand for 2–4 minutes.
11. Rinse gently with tap water. Tilt the slide to drain off the water.
12. A properly decolourised slide will appear light pink in color .If the slide is still red, reapply sulphuric acid for 1–3 minutes and rinse gently with tap water.
13. Pour 0.1% methylene blue onto the slide.
14. Leave methylene blue on the slide for 30 seconds.
15. Rinse gently with tap water.
16. Allow the slide to dry.
17. Examine the slide under the microscope using x40 lens to select the suitable area and then examine under x100 lens using a drop of immersion oil.
18. Record the results in the Laboratory Form and the Laboratory Register.

If the slide has:	Result	Grading	No. of fields to be examined
More than 10 AFB per oil immersion field	Pos	3+	20
1-10 AFB per oil immersion field	Pos	2+	50
10-99 AFB per 100 oil immersion fields	Pos	1+	100
1-9 AFB per 100 oil immersion fields	Pos	Scanty - record exact number seen	100
No AFB in 100 oil immersion fields	Neg		100

19. Invert the slides on tissue paper till the immersion oil is completely absorbed. Do not use xylene for cleaning the slides, as it may give false results of repeat examination after storage.
20. Store all positive and negative slides serially in the **same slide-box** until instructed otherwise by the supervisor.
21. Disinfect all contaminated material before discarding.

Annexure 11

HOW TO DISPOSE OF CONTAMINATED MATERIALS SAFELY

Sputum specimens examined in the laboratory are potentially infectious and after examination, they must be disinfected and disposed of so that risk of infection is avoided. **All disposable containers are used only once. Slides should never be used again and should be disposed of correctly.**

After the smears are examined, remove the lids from all sputum cups and put the cups and removed lids in a bucket containing 5% phenolic compound containing disinfectant solution. The cups and lids should be fully submerged in the solution. Similarly, used broomsticks should also be put in the same bucket containing 5% phenolic compound containing disinfectant solution. The bin/bucket should have a foot-operated lid. Thereafter, the used sputum cups, lids and wooden sticks can disposed off by any of the following methods.

1. Autoclaving in an autoclave or in a pressure cooker. At the end of the laboratory work the sputum cups and the removed lids, along with broomsticks, can be placed in a pressure cooker of approximately 7 litres capacity containing an adequate amount of water to submerge the contents, and boiled for at least 20 minutes using any heating source, electrical or non-electrical. After proper cooling, the material can be discarded along with the other waste.
2. If autoclaving cannot be done, use chemicals such as 5% phenolic compound containing disinfectant solution. Caps of the sputum cups must be removed and the cups, caps and broomsticks submerged in the solution in a secure place overnight. After this the solution, cups, caps and broomsticks can discarded along with the other waste.
3. As a last resort, if none of the above is available, sputum cups, caps and broomsticks can be buried in a pit at a safe distance away from inhabited areas.

Disposal of sputum containers with specimen and wooden sticks

Step 1: After the smears are examined, remove the lids from all the sputum cups. Use gloves whenever you handle infected material.

Step 2: Put the sputum cups, left over specimen, lids and wooden sticks in foot operated plastic bucket/bin with 5% phenol solution. The cups and lids should be fully immersed in the solution.

Step 3: At the end of the day, drain off the 5% phenolic compound containing disinfectant solution into the drain.

Step 4: Take out the sputum cup/lid/ wooden sticks and put into a reusable metal or autoclave-able plastic container/red bag. The red bag should have a

biohazard symbol, and be of adequate strength so that it can withstand the load of waste, and be made off non PVC plastic material.

Step 5: Put this container/bag in to the autoclave with other autoclavable BMW and the contents be autoclaved at 121^0C at 15 psi pressure for 20 minutes. The autoclave shall comply with the standards stipulated in the rules. Under certain circumstances, if autoclaving is not possible, boil such waste in water for at least 20 minutes. However, the District hospital/ CHC/PHC etc. shall ultimately make the necessary arrangements to impart autoclaving treatment on regular basis.

Step 6: After adequate cooling, the material can be safely transported to the common waste treatment facility for mutilation/shredding/disposal.

If a common waste treatment facility is not available in the area, the sputum cups/ lids/wooden sticks after autoclaving, can be deep buried in a deep burial pit.

Disposal of used syringes/needles/broken vials

Step 1: Immediately after administering an injection, cauterize the needle on site using a suitable **needle destroyer/cutter**, followed by cutting of the plastic hub of the syringe without detaching the needle from the syringe.

Step 2: Put the cauterized needles and broken vials, ampoules in a **sturdy puncture proof** white translucent plastic/card board container.

Step 3: Segregate and store cut plastic syringes in reusable metal or autoclave-able plastic container/red bag. If a red bag is used, its strength should be such that it can withstand the load of waste inside, and be made of non PVC plastic material.

Step 4: Label both containers with a biohazard symbol as stipulated in the Schedule III of the Biomedical Waste (Management & Handling) Rules 1998.

Step 5: Put both the containers into the prescribed bag and transport through a dedicated vehicle to the Common Waste Treatment Facility (CWTF) for autoclaving, mutilation/shredding/disposal.

Step 6 : If a CWTF does not exist, put both the sharps container (needles) and the metal/plastic container/red bag (syringes) into an autoclave along with the other BMW, and autoclave at 121^0C at 15 psi pressure for 15-20 minutes. Under certain circumstances if autoclaving is not possible, boil such waste in water for at least 20 minutes. However, the District hospital/CHC/PHC etc. should ultimately make necessary arrangements to autoclave the waste on regular basis.

Step 7: Dispose of the autoclaved waste as follows:
- I Dispose the needles and broken vials into the sharps pit.
- II Send the syringes for shredding/mutilation or landfill in deep burial pit.

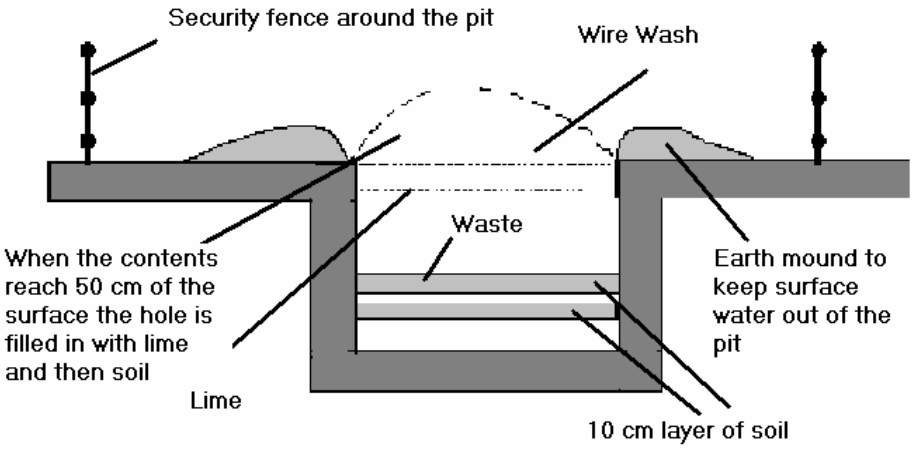
Disposal of used slides.

Step 1: Place the slides into a puncture proof container or red bag. The red bag should have a biohazard symbol and it should be made of non-PVC plastic material.

Step 2 : Dispose of the slides in the sharps pit .

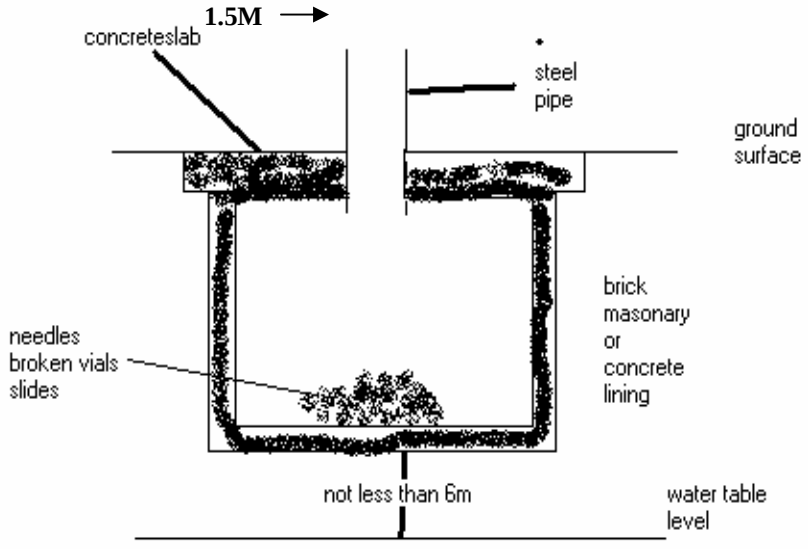
Under no circumstances the slides should be broken.

Design for Deep Burial Pits



Pit for onsite disposal of Sharps

The treated needles/broken vials should be disposed in a circular or rectangular pit as shown in figure below. Such rectangular or circular pit can be dug and lined with brick, masonry or concrete rings. The pit should be covered with a heavy concrete slab, which is penetrated by a galvanized steel pipe projecting about 1.5 meters above the slab, with an internal diameter of up to 50mm or 1.5 times the length of vials, which ever is more. The top opening of the steel pipe shall have a provision of locking after the treated waste sharps has been disposed in. when the pit is full it can be sealed completely, after another has been prepared.



Annexure 12

Standard (Universal) Precautions

Standard precautions are used in the care of all patients and apply to blood, all body fluids, secretions and excretions except sweat, regardless of whether they contain visible blood.

Standard precautions include:

- Hand washing
- Barrier protection
- Safe handling of sharp items
- Safe handling of specimens (blood etc)
- Safe handling of spillage of blood/body fluid
- Use of disposable/sterile items

Hand washing

This is an ideal safety precaution and gloves should not be regarded as a substitute for hand washing.

For General patient care (hand decontamination)

- Wash hands thoroughly in running water with soap without missing any area. For effective hand washing first wash palms and fingers followed by back hands, knuckles, thumbs, fingertips and wrists. Rinse and dry hand thoroughly.
- Wash hands immediately after accidental contamination with fluid, before eating and drinking and after removing gowns/coat
- Leave soap bars in dry container to prevent contamination

For Surgical care (Surgical Scrub)

- Wash hands up to the elbows.
- Scrub hands for minimum of 2 minutes
- Prevent dripping down of water from unwashed area of arms to washed hands.
- Put on gowns and gloves after drying only.

Barrier Protection

Gloves

- Wear while collecting/handling blood specimens and blood soiled items.
- Wear while disposing waste
- Remove before handling door knobs, telephone, pen, performing office work
- Discard if cracked, discoloured or punctured.
- Discard if blood spills on them.

- Don't reuse disposable gloves.
- Wash hands when gloves are removed or changed.

Masks

- Wear masks and protective glasses if splashing or spraying of blood/body fluids is expected.
- Masks of cotton wool, gauze, or paper mask are ineffective. Paper masks with synthetic material for filtration are an effective barrier against microorganisms.

Caps

Cover hair completely in aseptic units, operating rooms or performing selected invasive procedure.

Gown and aprons

- Wear clean clothes made up of a material easy to clean.
- Change after exposure to blood and body fluids.
- Wear gown or apron of plastic water resistant paper when splashes of blood or other body fluids are likely to occur e.g. during surgery, obstetric procedures, invasive procedures, post mortem and embalming.

Occlusive bandage

- Cover all skin defects e.g. cuts, scratches or other breaks with waterproof dressing before patient care.

Safe Handling of sharps

- Take extra care to avoid autoinoculation.
- Discard all chipped or cracked glassware in appropriate containers.
- Never use hands to pick up broken glass. Use a brush and pan.
- Don't manipulate disposable needles. Never bend, break, recap or remove needle from syringe.
- Dispose your own sharps. Don't pass used sharps directly from one person to another.
- Discard of needles into puncture proof rigid containers (Plastic or cardboard boxes) after disinfection in 5% phenol solution. Use needle shredder if available for needles or needles along with syringe nozzle.
- Send sharp disposal containers for disposal when three fourths full.

Safe handling of specimen

- Collect specimens, especially blood and body fluids, in pre sterilized containers properly sealed to prevent leakage or spillage.
- Use autoclaved/pre-sterilized disposable syringes and needles for venupuncture and lancets/cutting needles for finger pricks.
- Cover cuts in hands properly with water proof adhesive bandages.
- Wear disposable gloves while collecting blood/body fluids and maintain proper asepsis.

- Wash hands thoroughly with soap and water, particularly after handling specimens.

Safe handling of blood/body fluids spills

- Cover spills of infected or potentially infected material on the flow with paper towel/ blotting paper/ newspaper.
- Pour 5% phenol solution on and around the spill area and cover with paper for at least 30 minutes.
- After 30 minutes, remove paper with gloved hands and discard in general waste.

Use of Disposable Sterile Items

- Ensure proper handling of disposable/ sterile item before/ during use. There should be no re-circulation of disposable items.

Annexure 13

ROLE PLAYS FOR
LABORATORY TECHNICIANS (LTs)

Introduction

Example Role Play

You are a busy LT and your patient comes with a poor sputum sample

Sample Key Messages

Role Play Scenarios

1. LT is seeing a patient who has come in for initial diagnosis but who is having trouble producing an adequate sputum sample
2. LT is seeing a patient who wants to give 3 sputum samples on the same day
3. LT is educating a patient who has been found to be sputum positive at diagnosis
4. LT is educating a symptomatic patient who has been found to be sputum negative at diagnosis
5. LT is educating a patient who has been found to be sputum positive at follow-up
6. LT is educating a patient who has been found to be sputum negative at follow-up

INTRODUCTION

For developing good interpersonal communication (IPC) skills, you, the trainer, will need to be aware of the duties that the LTs have to perform. These include explaining to patients about TB and the importance of having their sputum examined, and helping them produce a good sputum sample. They also include developing a strong bond with patients to help motivate them to continue participation in the treatment, especially submitting good quality sputum samples at the defined times during treatment.

In this chapter, you will help the LT participants become better at these duties through role plays. Through the role plays, poor IPC skills and good IPC skills will be demonstrated. Demonstrating poor IPC skills develops insight into common behaviours that occur in real situations. Identification of these will help in working towards developing good IPC skills. Therefore, for the role plays to be effective, two sessions will have to be done for each scene; one highlighting poor IPC skills and the other showing good IPC skills.

In order to help the participants understand the importance and potential pitfalls of non-verbal communication, perform the following exercise: Tell the participants to just observe you without making any comments. Then, sit down in a chair with your arms and legs crossed, your body turned slightly away from the participants, and an annoyed expression on your face. Swing your legs and gaze around the room.

After about 30 seconds, ask the participants to describe what they were feeling when you were sitting in front of them. List their responses on the board or flip chart.

Then discuss:

- Do we communicate without words?
- Describe ways that we communicate without words.

Discuss with them that we need to be aware of what we are communicating non-verbally, for example, boredom, dislike, superiority, impatience. We also need to be aware of what our patients and others communicate non-verbally, such as fear, embarrassment, discomfort and shame.

After this discussion, you will tell the participants that you are going to enact a role play scene for them. Tell them to watch for behaviours that depict poor IPC skills.

Next, choose another trainer (if available) or one of the participants (if no other trainer is available) to play the part of the patient in the following role play. A trainer should play the part of the LT. You will then enact the following role play scene using as many poor IPC skills as possible (for example, you will yell at the patient, you will have them stand while you sit, you will tell them facts using big words that they don ’t understand, etc.).

Role Play Scene

L T: You are a busy LT and your patient comes with a poor sputum sample.
Patient: You are a patient who is having trouble understanding how to produce a good sputum sample. You think you just need to spit into the sputum cup. You also don’t want to move away from other patients to give the sputum sample.

After you have completed enacting the scene, ask the participants to list the poor IPC skills. Write these on the chalk board or flip chart. Then, go through each item listed and discuss the ways in which the poor behaviours could be improved. Spend as much time as needed to thoroughly discuss the poor behaviours. Be sure to discuss non-verbal communication elements such as eye contact, posture, nodding, encouraging or discouraging sounds, etc.

Also discuss the messages about the RNTCP that were conveyed during the scenario. Discuss the accuracy of the messages and, for inaccurate messages, discuss how they could be more accurately conveyed.

Once the discussion is finished, perform the scene again using your best IPC behaviours. Afterwards, ask the participants to discuss the differences in the two role play scenes. Encourage them to discuss how the two different scenarios made them feel and how they think the patient and LT felt.

After this discussion, inform the participants that everyone in the group is now going to practice IPC skills by doing role plays themselves, with the other participants. Tell them that you will be handing out their roles and that they will perform the scene twice; once using poor IPC skills, followed by a group discussion n how the behaviours can be improved, and then again using good IPC skills.

Split the group of participants into smaller groups of no more than six people per group. Make sure each small group contains an even number of participants. Then, choose scenarios from the list of “Role Play Scenarios for LT’s ”which can be found at the end of this chapter and write the roles on separate pieces of paper to give to the participants in each small group. You can also use your own experiences to come up with ther role play scenarios and roles. Make sure that everyone receives a role.

After you have handed out the roles, give the participants a few minutes to think about how they will act out their role. Then, have the participants play each scenario in front of their small group using good IPC skills.

During the play by the trainees, circulate to each group to ensure that participants are exhibiting the appropriate IPC skills, such as smiling, sitting with the patient or other person, looking at the other person when speaking, pausing after asking questions, asking open-ended questions, etc. Also, use the following list of “Key Messages” to guide you as you watch the role play. After each role play by the participants, stop and have the group discuss the good ideas and IPC skills that were exhibited in the role play scene, and also discuss things that could improve IPC skills and improve the accuracy of RNTCP messages.

SAMPLE KEY MESSAGES

Listening and understanding

- “Please sit down.”
- “How are you feeling?”
- “How many children do you have?”
- “What are their ages?”
- “How is your wife/husband?”
- “Are they doing well?”
- “What do you do for a living?”
- “Does anyone in your family also have cough?”
- “What do you think this illness might be?”
- “Do you think you have a serious illness?”
- “What do you think may have caused your illness?”
- “Have you heard of tuberculosis?”

- "What do you understand tuberculosis to be?"
- "What do you think causes tuberculosis?"
- "Have you heard of the microscope sputum test to diagnose TB?"
- "Do you know that we need to test your sputum three times to confirm whether you have TB?"
- "Do you know that TB can be cured?"
- "Do you know that TB can be completely cured even if it has reappeared?"
- "Do you know that TB can spread from one person to another if it is not properly cured?"
- "Do you know that other people in your house can contract TB from you?"
- "Do you know that till complete investigations are done we cannot assess the degree of damage that has been caused?"
- "The tests to detect TB are simple and will have to be done at regular intervals to monitor improvement in your condition."
- "You will have to take your medicines as prescribed so that your illness does not get worse."
- "If you do not take medicines as prescribed, you can develop an even more dangerous form of TB which you can then spread to your family."
- "You can prevent the spread of TB to others by covering your mouth when you cough."

Demonstrating caring

- "I want to make sure that you get the best medicines. That's why a sputum test is so important —so that we can be sure that you are getting the right medicines."
- "To prescribe the right treatment for you the doctor needs a sputum examination."
- "If you have any doubts regarding sputum examination or how to bring out sputum, you can ask me. I will be happy to clarify your doubts and help you."
- "If the sputum test confirms your disease you will get regular attention and treatment."
- "Treatment cannot be started until the results of sputum examination are available."
- "We want to make sure that you are completely cured."

Motivating and Problem solving

- "Sputum examinations do not cause any harm or discomfort."
- "You just have to have three sputum examinations done as all treatment will be based on their results."
- "Yes, your symptoms suggest that you MAY HAVE TB, but we cannot be sure till we test your sputum."

- “An ordinary cough does not last that long. You have been coughing for a month and we must find out why. Only when we know the cause can we cure it completely.”
- “The reason for conducting 3 sputum examinations is because one or two tests may not be accurate enough to detect the TB germs.”
- “TB is a fairly common disease and should not be a cause for worry as it is fully curable now but it should be diagnosed early so that it doesn’t spread to other parts of the body or to others. Therefore, it is necessary to have your sputum tested.”
- “With the sputum test we can actually see whether there are TB germs in you.”
- “If I or my wife/husband had your symptoms, I would certainly have 3 sputum examinations done.”
- “Sputum tests are free here, and of excellent quality. Our microscope is better than many even in private laboratories.”
- “The test here is better than what you can get even in a private laboratory.”
- “The sputum test is much more accurate than an X-ray. We can actually see whether you have TB germs when we look at your sputum with a microscope. This is why sputum examination is known as the gold standard.”
- “If your test is positive. I’ll be happy to show you what the germs actually look like under the microscope if you like.”
- “It’s not just you but everyone with cough for 3 weeks or more has to have the sputum tests, so that we can know exactly what your problem is and treat you accordingly.”
- “Yes, you may have to miss work for 1 –2 days because of sputum examinations. But if you are not fully cured, the loss of work and earnings will be far more.”
- “If it is convenient for you to come for your follow-up sputum tests on your off days, we could make adjustments for you accordingly. However, you must come for your tests on the appointed day without fail.”

ROLE PLAY SCENARIOS

(These are only some examples. Use your own experiences to come up with other scenarios and roles.)

Scenario 1: LT is seeing a patient who has come in for initial diagnosis but who is having trouble producing an adequate sputum sample

Write the following instructions on two separate pieces of paper and hand them out to two participants. Give male roles to female participants and female roles to male participants, if possible.

LT: You are an LT who is seeing a patient for initial diagnosis. The patient is having trouble producing an adequate sputum sample.

Patient: You are a patient who has had a cough for several weeks and your doctor has asked you to come for a sputum test. You are having trouble producing a good sputum sample.

✶ ✶ ✶

Scenario 2: LT is seeing a patient who wants to give 3 sputum samples on the same day

LT: You are an LT who is seeing a patient for suspected TB. The patient does not want to return tomorrow but wants to submit 3 sputum samples today.

Patient: You are a patient who has been asked by your doctor to come for a sputum test. You are busy with work tomorrow so you want to submit 3 sputum samples today.

✶ ✶ ✶

Scenario 3: LT is educating a patient who has been found to be sputum positive at diagnosis

LT: You are an LT who is seeing a new patient whose sputum is positive for TB.

Patient: You are a patient who has come to get the results of your sputum test. Your father had TB and died from it when you were a child. You think that TB is inherited.

✶ ✶ ✶

Scenario 4: LT is educating a symptomatic patient who has been found to be sputum negative at diagnosis

LT: You are an LT who is seeing a patient whose first 3 sputum samples are negative for TB.

Patient: You are a patient who has had a cough, fever and expectoration for weeks. You want to be admitted to the hospital because you feel so sick.

✶ ✶ ✶

Scenario 5: LT is educating a patient who has been found to be sputum positive at follow-up

LT: You are an LT who is seeing a patient whose sputum was positive at follow-up.

Patient: You are a patient who has been very regular in your treatment, but you are tired of it now and want to stop your treatment.

✶ ✶ ✶

Scenario 6: LT is educating a patient who has been found to be sputum negative at follow-up

LT: You are an LT who is seeing a patient whose sputum is negative at follow-up.

Patient: You are a patient who is feeling good and who does not understand why you should continue treatment if your sputum is negative.

✶ ✶ ✶

Scenario 7: LT must talk with an MO of his microscopy centre who is not referring patients for sputum examination

LT: You are an LT who is meeting with an MO at a hospital where no patients are being referred for sputum examination.

MO: You are an MO who does not believe in sputum examinations to diagnose TB. You believe that X-rays are the best method to diagnose TB.

✶ ✶ ✶