REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME (RNTCP)

Module *for* Senior Tuberculosis Laboratory Supervisors

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

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

MODULE FOR
SENIOR TUBERCULOSIS LABORATORY SUPERVISORS

Successful completion of the "Module for Laboratory Technicians" is a prerequisite for successful completion of this module

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INTRODUCTION

AFB microscopy is the primary diagnostic tool in the Revised National Tuberculosis Control Programme (RNTCP). The Senior Tuberculosis Laboratory Supervisor (STLS) is the person responsible for monitoring day-to-day activities of all the microscopy centres, and is thus essential for the success of the RNTCP.

The STLS is responsible for ensuring that:

- Microscopy services in the sub-district are well organized and the locations of designated microscopy services are known to the Medical Officers in all peripheral health institutions
- There is uninterrupted staffing of designated microscopy centres
- Laboratory technicians have an adequate supply of reagents, sputum containers and other materials, including boxes for storing slides
- Tuberculosis suspects have their sputum examined the correct number of times for tubercle bacilli
- Laboratory technicians understand the importance of limiting administrative errors (for example, keeping the sputum specimens with the proper Laboratory Form for Sputum Examination and slides) and accurately recording results of sputum smear examination
- Each laboratory has a Tuberculosis Laboratory Register which is filled completely and accurately

- All documentation related to sputum smear examinations is accurate and reports of examinations are given to the treating physician promptly
- Laboratory technicians keep the examined slides for your review
- Results of sputum smear examination are accurate.

Visit every microscopy centre in your area for supervision at least once every 4 weeks, and more often if possible.

MONITOR DOCUMENTATION RELATED TO MICROSCOPY

Patients are placed on different treatment regimens based on the results of their sputum smear examinations. If sputum specimens are examined and recorded on the Laboratory Form for Sputum Examination for the wrong person, patients may be prescribed the wrong treatment regimens, treated unnecessarily, or not treated despite having tuberculosis. To limit these errors it is of paramount importance to monitor how the laboratory technicians examine the sputum and record the results, i.e. to make sure they keep the sputum specimens with the proper Laboratory Form for Sputum Examination and slides and accurately record the results of sputum smear examination on the form. Also make sure the laboratory technicians keep all slides until you review all the smear-positive slides and 10% of the smear-negative slides.

Explain the importance of limiting administrative errors

If the patient's sputum specimens are not labelled properly at the health unit or if the Laboratory Form for Sputum Examination gets separated from the specimens, the laboratory technician may not know whose sputum specimens are in the containers when they reach the laboratory.

When you visit the microscopy centre, discuss with the laboratory technicians the method they use to ensure that the Laboratory Serial Number on the Laboratory Form for Sputum Examination matches the Laboratory Serial Number on the sputum container. Also make sure that the Laboratory Serial Number is written on the side of the sputum container (and not on the lid) and that it matches the number reported on the slide which is prepared. The same number should be reported on the Laboratory Form for Sputum Examination.

Other centres which collect specimens and transport them to the microscopy centre should assign Specimen Identification Numbers and write these on the side of the containers.

Make sure laboratory technicians keep slides

Explain to the laboratory technicians that they should keep all the slides after examination until you check all smear-positive slides and 10% of smear-negative slides. The slides should be filed according to the Laboratory Numbers and smear-positive and smear-negative slides kept in separate boxes until your next supervisory visit. On this visit, you should locate and review all smear-positive slides and 10% of smear-negative slides. During your visit to the microscopy centre, check the slide boxes and ensure that all smear-positive and smear-negative slides are being preserved.

Explain the importance of accurate recording of results of sputum smear examination

The laboratory technicians should understand the importance of accurate recording of results of sputum smear examinations on the Laboratory Form for Sputum Examination. Explain to them that patients are diagnosed and placed on the appropriate treatment regimen based on the results of their sputum smear examination. For example, if a laboratory technician records 3 results of sputum smear examination as negative, the patient may be considered a smear-negative case and placed on the Category III treatment regimen. If the results were actually **positive**, the patient would have been placed on the Category I treatment regimen. Also, at the end of the initial intensive phase, patients have their sputum examined to determine whether they have converted to (or

remained) smear-negative. During the continuation phase also, smear-positive patients are monitored by microscopy. If sputum smear examination results are incorrectly recorded, incorrect treatment may be given. As a result, patients may become more ill, may spread tuberculosis to others, and may die from tuberculosis.

When you visit the microscopy centre, speak with the laboratory technicians and make sure they know how to complete the Laboratory Form for Sputum Examination. The following table describes what should be written in the **Results** and **Positive (grading)** columns of the form according to the number of acid-fast bacilli (AFB) seen while examining the slide:

If the slide has:	Results	Positive (grading)
More than 10 AFB per oil immersion field	POS	3+
1 to 10 AFB per oil immersion field	POS	2+
10 to 99 AFB per 100 oil immersion fields	POS	1+
1 to 9 AFB per 100 oil immersion fields	Scanty	Record the exact number seen
No AFB in 100 oil immersion fields	NEG	

The laboratory technician should have little or no difficulty reading slides that contain many acid-fast bacilli. However, when there are less than 10 AFB per 100 oil immersion field, the technician may have difficulty in reading the slide and determining whether the results are scanty or negative. Therefore, if 1–9 AFB are seen in the first 100 oil immersion fields, another 100 oil immersion fields should be examined.

Results should be reported to the treating physician within one day.

EXERCISE 1

Indicate the Result and Grade for each of the following sputum smears of each of the following patients in the space provided.

Patient	Number of AFB seen	Result	Grade
Parvati Sinha	30 AFB are seen in 100 oil immersion fields	_____	_____
	6 AFB are seen in 200 oil immersion fields	_____	_____
	70 AFB are seen in 100 oil immersion fields	_____	_____
Lakshmi Kumari	150 AFB are seen in 50 oil immersion fields	_____	_____
	80 AFB are seen in 50 oil immersion fields	_____	_____
	25 AFB are seen in 100 oil immersion fields	_____	_____
Lakshmipati Rao	240 AFB are seen in 20 oil immersion fields	_____	_____
	50 AFB are seen in 100 oil immersion fields	_____	_____
	100 AFB are seen in 50 oil immersion fields	_____	_____
Kailash Nath	300 AFB are seen in 25 oil immersion fields	_____	_____
	200 AFB are seen in 50 oil immersion fields	_____	_____
	10 AFB are seen in 100 oil immersion fields	_____	_____
Bhola Ram	400 AFB are seen in 50 oil immersion fields	_____	_____
	60 AFB are seen in 100 oil immersion fields	_____	_____
	0 AFB are seen in 100 oil immersion fields	_____	_____
Man Bahadur Lal	0 per 100 oil immersion fields	_____	_____

Patient	Number of AFB seen	Result	Grade
Lallan	80 AFB are seen in 100 oil immersion fields	_____	_____
Prasad	0 AFB are seen in 100 oil immersion fields	_____	_____
Parmar	0 AFB are seen in 100 oil immersion fields	_____	_____
Kiran	0 per 100 oil immersion fields	_____	_____
Kumar	0 per 100 oil immersion fields	_____	_____

MONITOR MAINTENANCE OF THE TUBERCULOSIS LABORATORY REGISTER

During visits to the microscopy centre, make sure there is a Tuberculosis Laboratory Register that is completely and correctly filled out. Make sure the laboratory technicians know how to complete the Tuberculosis Laboratory Register. You should also review the Tuberculosis Laboratory Register to ensure that patients had the correct number of sputum smear examinations performed.

Laboratory staff should not use the Tuberculosis Laboratory Register to enter results of any other laboratory examinations. All results of AFB examinations should be written in the Tuberculosis Laboratory Register, and these need not be written in any other register.

Check accuracy of the Tuberculosis Laboratory Register

Make sure the laboratory technicians are accurately using the Laboratory Serial Number. A new number should be assigned to a tuberculosis suspect whose sputum is to be examined. The Laboratory Serial Number should begin with 1 each year. When the name of a patient is entered in the Tuberculosis Laboratory Register, "1" should be added to the last Laboratory Serial Number recorded. For example, on 2 January 1992, a laboratory technician records the results of sputum smear examinations of three patients. The laboratory serial numbers assigned to those patients are 1, 2 and 3. On 3 January, the laboratory technician records the results of sputum smear examinations of five patients. The Laboratory Serial Numbers assigned to those patients are 4, 5, 6, 7 and 8.

A Laboratory Serial Number is assigned to a *patient*, *not* to a sputum specimen. Up to *three* sputum specimen results can be recorded for each patient on *one* line of the Tuberculosis Laboratory Register.

The Laboratory Serial Number is written in the Tuberculosis Register. It is used as a cross-reference when you verify whether the results of sputum smear examination in the Tuberculosis Register match those in the Tuberculosis Laboratory Register. By using the name of the patient and his Laboratory Serial Number from the Tuberculosis Register, you can easily find the sputum smear examination results in the Tuberculosis Laboratory Register. Without this Laboratory Serial Number, you would have to look through many pages of the Tuberculosis Laboratory Register for a patient's sputum smear examination results.

During visits to the microscopy centre, look through the Tuberculosis Laboratory Register and make sure all the columns have been completed. For example, you may find that a patient's address or treatment unit is missing in the Tuberculosis Laboratory Register. The laboratory technicians must understand the importance of writing the address of patients examined for diagnosis so that they can be found and placed on treatment. If the sputum smear examination was for diagnosis of a patient with suspected tuberculosis, the name of the treatment unit that *referred* him should be written in the **Name of Treatment Unit** column. If the sputum smear examination was for follow-up of chemotherapy, the name of the treatment unit where the patient is taking the treatment should be written in the **Name of Treatment Unit** column. The TB number of at least all smear-positive patients started on treatment should be recorded in the **Remarks** column, and the TB number of all patients whose sputum is examined for follow-up must be written in the space provided.

At the end of each month, the laboratory technician should summarize the sputum smears done that month. This information should be summarized in the following format, using a blank line in the Laboratory Register itself. The number of **patients** examined and diagnosed, and not the number of slides, should be given.

Number of patients whose sputum was examined for diagnosis	
Number of smear-positive patients diagnosed	
Number of patients on treatment whose sputum was examined for follow-up	
Number of patients whose follow-up sputum examination was found to be smear-positive	

The number of *patients* examined and diagnosed, not the number of *slides*, should be given.

Ensure that New patients had three sputum samples examined and that follow-up cases had two sputum samples examined

When you review the Tuberculosis Laboratory Register, verify that all patients had their sputum specimens examined the correct number of times.

To define a patient as smear-negative, there must be **3** different sputum specimens examined. The results of each

examination must be negative. If you review the Tuberculosis Laboratory Register and notice that only 2 sputum specimens were examined, ask why another sputum smear examination was not done. A smear-positive patient may be missed if the third sputum is not collected and examined. *To minimize the proportion of "false" smear-negative patients, at least 3 sputum specimens must be examined.*

To define a patient as smear-positive, there must be **at least 2 *smear-positive* sputum specimens**. If 2 sputum specimens were examined and only 1 was smear-positive, you are responsible for ensuring that the patient is traced (if the patient has not returned to the laboratory). When the patient returns, another sputum specimen should be collected and examined.

If 3 sputum specimens were examined and 1 was smear-positive, the patient must be found and referred to a Medical Officer for an X-ray examination. Annexure I provides the diagnostic algorithm used in the RNTCP. As can be seen, sputum microscopy is central to the diagnosis of tuberculosis patients.

Spot-check sputum results for follow-up

Another important task during your visits to the microscopy centre is to make sure that follow-up sputum smear examinations have been done as per schedule. The schedule of follow-up sputum smear examinations is given in the table on page 13.

Schedule of Follow-up Sputum Examinations

Patient	Follow-up Examinations
Smear-positive, Category I	**After 2, 4 and 6 months of treatment**
Smear-positive, Category I *Sputum smear-positive at month 2 of treatment*	After 2, 3, 5 and 7 months of treatment
Smear-positive, Category II	**After 3, 5 and 8 months of treatment**
Smear-positive, Category II *Sputum smear-positive at month 3 of treatment*	After 3, 4, 6 and 9 months of treatment
Smear-negative, Category I or Category III	**After 2 and 6 months of treatment**

Follow-up sputum smear examinations of smear-positive cases which are carried out at the end of the first (intensive) phase of treatment (2 months for patients on Category I and Category III treatment regimen and 3 months for patients on Category II treatment regimen) are extremely important. If this examination is not done correctly, the patient may receive insufficient treatment and become more ill or may die.

The follow-up sputum smear examination at the end of treatment is essential for evaluation of the outcome of treatment (to determine the cure rate).

To ensure that examinations are actually carried out in accordance with the policy, during visits to the microscopy centre, spot-check the sputum smear examination results of approximately 15 patients in the Tuberculosis Laboratory Register who should have had their sputum examined for follow-up of chemotherapy.

EXERCISE 2

Case 1

During your visit to a microscopy centre in your area you review the Tuberculosis Laboratory Register. You notice that every month the laboratory technicians are beginning with a new Laboratory Serial Number. You also notice that the address column for New patients is never completed.

1. Describe what the laboratory technicians in this microscopy centre are doing incorrectly. Also include what you should tell the laboratory technicians about the importance of maintaining an accurate Tuberculosis Laboratory Register.

Case 2

Review a sample page of the Tuberculosis Laboratory Register given on page 17.

1. List the names of New patients whose sputum was examined three times and **can** be defined as **smear-negative** pulmonary TB provided that a Medical Officer makes the diagnosis of smear-negative tuberculosis, based on clinical and X-ray examinations, and decides on treatment.

2. List the names of New patients who only had 2 sputum specimens examined and **cannot** be defined as **smear-negative**.

3. List the names of New patients who **can** be defined as **smear-positive.**

4. List the names of New patients who **only had 1 positive sputum specimen**. Describe what action you should take.

5. List the patients examined for follow-up whose sputum examination or recording was incorrect and explain.

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Laboratory Register

Year 1997

Lab Serial No.	Date	Name (in full)	Sex M / F	Age	Complete address (for new patients)	Name of Referring Health Centre	Reason for Examination*		Results			Signature	Remarks
							Diagnosis	Follow-up	1	2	3		
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 J.J. 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		
507	2/4	Deepak Dhawan	M	28		Modern TB Clinic	✓		1+	2+		Joshi	No. 346
508	3/4	Preeti Chandra	F	26	H. No. 62, Lane No. 820, Kailash Colony		✓		Neg	Neg	Neg	Joshi	

* If sputum is for diagnosis, put a tick (✓) mark 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”.

ENSURE THAT TREATMENT CARDS ARE CORRECTLY FILLED

Ensure that information on treatment cards is correctly filled in respect of sputum examinations. Sputum results are recorded on every patient's treatment card in the appropriate section. The results of diagnostic samples are written next to "Month 0"; the Date of the sputum sample, followed by the Lab No. (Laboratory Serial Number), followed by the smear result. The smear result should be written in red if the smear was positive, and should denote the grade of positivity (scanty, 1+, 2+, or 3+), and not simply the word "Positive".

If a patient being treated with Category I regimen is smear-positive after 2 months of treatment, forward slashes (/) should be drawn on the Tuberculosis Treatment Card in the **Date**, **Smear result** and **Lab No.** columns next to **Month 2**. The date of the smear examination should be recorded above the slash under the **Date** column. The number associated with the positive results (for example, 2+) should be written above the slash under the **Smear result** column. The laboratory serial number should also be recorded above the slash under the **Lab No.** column. The Medical Officer will continue the intensive phase of treatment for another 4 weeks. At the end of the additional 4 weeks of the intensive phase of treatment (end of 3 months of treatment) a sputum smear should be examined. The date, sputum smear result and laboratory serial number of the sputum smear examination should be recorded below the forward slash under the appropriate columns. Sputum will then be examined at the beginning of 5 months, and at the end of treatment. The date, sputum smear result and laboratory serial number should be recorded in the same way.

Month	Date	Lab No.	Smear result	Weight
2/3	17/3 16/4	164 237	1+ NEG	45 kg

A patient who is treated with Category II regimen will have his sputum examined at the end of 3 months of start of treatment.

If the patient is smear-positive after 3 months of treatment, forward slahses (/) should be drawn on the Tuberculosis Treatment Card in the **Date, Smear result** and **Lab No.** columns. The date of the smear examination should be recorded above the forward slash under the **Date** column. The number associated with the positive results (for example, 1+) should be written above the slash under the **Smear result** column. The laboratory serial number should also be recorded above the slash under the **Lab No.** column. The initial intensive phase of treatment should continue for another 4 weeks. At the end of the additional 4 weeks of intensive phase treatment (end of 4 months of treatment), a sputum smear should be examined. The date, sputum smear result and laboratory serial number of the sputum smear examination should be written below the forward slash under the appropriate columns.

A patient who is treated with Category III regimen will have his sputum examined after 2 months of treatment and at the end of treatment. The date, sputum smear result and laboratory serial number of the sputum smear examination should be recorded next to **Month 2** on the Tuberculosis Treatment

Card. If a smear-negative patient is found to be smear-positive at the end of 2 months, repeat the sputum smear examination and refer the patient to a Medical Officer. If smear positivity is confirmed, the Medical Officer will prescribe the retreatment regimen (Category II).

PERFORM LABORATORY QUALITY CONTROL

Quality control of the tuberculosis laboratory is an essential part of the RNTCP. Physicians and patients must have confidence in the quality of microscopy services. For these reasons, every positive smear, and 10% of negative smears, must be cross-checked by the STLS. If the number of negative slides is small all should be examined.

These results should be tabulated as follows:

1. Number of smears recorded as positive in the local microscopy centre: _____
2. Of these, number confirmed as positive by supervisor: _____
3. Number of negative smears recorded in the local microscopy centre: _____
4. Of these, number reviewed by supervisor: _____
5. Of the smears which were recorded as negative in the local laboratory and which were reviewed, number confirmed to be negative: _____

Example:

A microscopy centre reads 438 smears in April, of which 40 were positive. The supervisor reviews all 40 positive smears, and confirms that 39 were positive. Of the 398 (438 total – 40 positive = 398 negative) smears read as negative, the supervisor reviews 10%, or 40. Of these 40, two are found on review to be positive, the remaining to be negative. The numbers from above are as follows:

1. Number of smears recorded as positive in the local microscopy centre: *40*

2. Of these, number confirmed as positive by supervisor: *39*
3. Number of negative smears recorded in the local microscopy centre: *398*
4. Of these, number reviewed by supervisor: *40*
5. Of the smears which were recorded as negative in the local laboratory and which were reviewed, number confirmed to be negative: *38*

These figures are included in the *Quarterly Report on Programme Management and Logistics* as shown below:

Laboratory Quality Control Network

Initial reading	Number of slides checked	Supervisor reading		Percentage of Discordance
		Number of positives	Number of negatives	
Positive slides	40	*(a)* 39	*(b)* 1	*(b/[a+b])* 2.5% *[false positives]*
Negative slides	40	*(c)* 2	*(d)* 38	*(c/[c+d])* 5% *[false negatives]*

ENSURE THAT CONTAMINATED MATERIALS ARE DISPOSED OF SAFELY

Sputum specimens examined in the laboratory are potentially infectious and after examination they must be disinfected and destroyed so that risk of infection is avoided. *All disposable containers are to be used only once.*

After the smears are examined, all sputum cups and the removed lids should be kept in a bucket containing 5% hypochlorite or 5% phenol solution. The cups and lids should be fully submerged in the solution. Similarly, used wooden sticks should be put into the same bucket. This bin/bucket should have a lid which is foot operated.

Sputum cups which contain sputum can be disposed of by any of the following methods:

(i) Incineration—wherever incinerators exist.

(ii) Autoclaving in an autoclave or in a pressure cooker. At the end of the laboratory work the sputum cups and lids, with the lids removed along with the wooden sticks can be placed in a pressure cooker of approximately 7 litre capacity containing 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 with other waste.

(iii) If neither of the above is available, use freshly prepared 5% hypochlorite solution or 5% phenol. Caps of the sputum cups must be removed and the cups, caps and wooden sticks submerged in the solution in a secure place overnight.

After this, the solution, cups, caps, and wooden sticks can be discarded with other waste.

(iv) As a last resort, if none of the above is available, sputum cups, caps and wooden sticks can be burnt in a pit at a safe distance from inhabited areas, and the burnt material buried.

CONDUCT VISITS TO MICROSCOPY CENTRES

Regular supervisory visits during which you place emphasis on *helping* staff identify and solve problems will create a good working relationship between you and the staff of your area. Staff will be less worried about your finding things "wrong", and may be more willing to discuss problems and work with you to identify solutions.

Supervisory visits give staff the opportunity to talk with you. It gives you the opportunity to see and better understand problems the staff face. The interest you show during these visits can motivate people to perform their best. When you find problems which cannot be resolved easily by talking with the health unit staff, talk with the Medical Officer of the microscopy centre, the Medical Officer of the Tuberculosis Unit, the District Tuberculosis Officer, or other responsible people to try to resolve the problems.

Good supervision is the process of helping staff improve their performance. During these visits you can observe and reinforce correct performance. You can also identify and correct inadequate performance before it becomes a major problem.

To use your time productively and efficiently during a supervisory visit to a microscopy centre, you will need to prepare carefully for the visit. You will make sure that tuberculosis-related laboratory services are properly performed. Before you visit the microscopy centres, plan your visit thoroughly. During the visits, you will check to see that laboratory activities related to tuberculosis detection and sputum smear monitoring are being correctly performed and recorded by the laboratory technicians.

Prepare for visits to microscopy centres

1. Decide **when to visit** each microscopy centre in the area. Each microscopy centre in your area should be visited for supervision at least once every month. You will be provided with a 2-wheeler to travel for supervision. If the 2-wheeler is out of service, you will have to arrange alternative means of transportation such as public transport. You will be reimbursed for these expenses.

2. Decide **what** to check.
   The specific items you check will depend on the size of the laboratory. Some important items to check are listed on page 29.

3. Decide **when to check** each item.
   Some items, such as the Tuberculosis Laboratory Register, should be checked at each visit. Other items including stocks of sputum containers, slides and reagents may be checked periodically.

4. Decide **how** to check each item.
   Depending on the time available for your visit and the items you have decided to check during the visit, decide the best ways to collect the information:

   a. **Review the Tuberculosis Laboratory Register.** Check the Tuberculosis Laboratory Register to make sure it is filled completely and accurately. Make sure that all smear-positive patients in the Tuberculosis Laboratory Register are also registered in the Tuberculosis Register. Verify that New patients had their sputum examined the correct number of times.

b. **Talk with laboratory technicians.** Make sure they understand the importance of limiting administrative errors and accurately recording sputum examination results on the Laboratory Form for Sputum Examination. Also make sure the laboratory technicians keep the slides of all cases until your next supervisory visit.

c. **Examine supplies**. Check to see if there are adequate numbers of sputum containers, slides, reagents, forms and other laboratory supplies.

5. Develop a **checklist**.

Once you have decided what you want to look for when you go to the microscopy centre and how to check each item, it will be useful to organize the information into a "checklist". In general, your checklist should be:

a. just long enough to give you the reminders you need to check the important items/activities

b. easy to use.

Include important general information, such as the name of the centre, your name and date of visit. A more comprehensive checklist is given in Annexure II. Turn to Annexure II and review the checklist now. This checklist is longer than the one which you would be likely to use, but is provided for reference.

Conduct the visit

Inform the laboratory technician in advance that you are planning to visit the microscopy centre. He should be there during your visit. When you go to the microscopy centre, use the checklist you prepared. If you find problems, work with

the Medical Officer of the microscopy centre, the Medical Officer-Tuberculosis Control of the Tuberculosis Unit, or the District Tuberculosis Officer to solve them.

Monitor AFB microscopy in all health facilities in the district

In some areas, primary health centres which are not designated microscopy centres may wish to do AFB microscopy. It may be difficult to insist that a primary health centre STOP providing the service (AFB microscopy) and refer patients to a designated microscopy centre which is farther away. However, this is necessary to ensure quality of microscopy as well as uninterrupted availability of reagents, functioning microscope and laboratory staff. If it is absolutely impossible to discontinue AFB microscopy from smaller, non-designated centres, then each and every slide, both positive and negative, must be cross-checked. If the quality of smear, stain, or reading is not optimal, then the laboratory staff must be retrained and their performance improved, failing which the centre must stop performing AFB microscopy.

CHECKLIST FOR LABORATORY SUPERVISION

	Yes	No	Not Assessed
Knowledge: **Does the Laboratory Technician know:** • How to properly complete the Tuberculosis Laboratory Register and the Laboratory Form for Sputum Examination? • How information from the Tuberculosis Laboratory Register is used in the Tuberculosis Register? • How to limit administrative errors (for example, keeping sputum specimens with the proper Laboratory Form for Sputum Examination and slides)?			
Activities: **Does the Laboratory Technician:** • Examine 3 sputum samples for patients diagnosed as smear-negative? • Examine at least 2 sputum sample slides if the first is read as smear-positive? • Maintain an accurate and complete Tuberculosis Laboratory Register? • Use **red ink** to record all positive results in the Laboratory Register? • Maintain separate boxes for all smear-positive and smear-negative slides to be checked during supervisory visits?			
Are the Tuberculosis Laboratory Register and the Tuberculosis Register consistent? • Does the Tuberculosis Register contain all the smear-positive patients recorded in the Tuberculosis Laboratory Register? If the Tuberculosis Laboratory Register contains names of smear-positive patients which are not found in the Tuberculosis Register, make efforts to bring these patients into treatment and registered in the Tuberculosis Register. • Are the smear results for follow-up patients in the Tuberculosis Laboratory Register the same as the results recorded in the Tuberculosis Register?			
Logistics • Is there an adequate supply of sputum containers, slides, reagents, forms, and other laboratory materials? • Is the binocular microscope in good working condition?			

EXERCISE 3

Part A

Draw an outline of your sub-district and map out the location of microscopy centres, labelling them A, B, C, D, etc. Draw out your one-month work schedule of supervisory visits to these locations.

Part B

If your 2-wheeler goes out of order, how will you cover your scheduled visits?

Part C

What is your role as regards the laboratories not designated as "microscopy centres" but which are performing AFB staining in your area?

Part D

In this part of the exercise you will prepare for a visit to a microscopy centre. You will develop a checklist to use during the visit. Include items in your checklist which can actually be checked at the microscopy centre you will visit.

Use the blank page on page 32 to prepare your checklist for the unit. Be sure to include the following information:

- the date
- a space for the name and location of the laboratory
- the items you will check and whether they are correctly or incorrectly performed
- the method to be used to check each item
- a short list of the questions to ask when you are speaking to the laboratory technician(s)
- a space for comments about any problems identified and possible causes
- a space for recommendations, and a space for your signature

Part E

A site visit to a microscopy centre may occur during this training. If so, your facilitator will explain how this visit will take place. Use the checklist you developed. After the site visit, there will be a group discussion about any problems your group found and the solutions you recommend.

WORKSHEET FOR CHECKLIST

MAINTAIN AN ADEQUATE SUPPLY OF ALL MATERIALS NECESSARY FOR MICROSCOPY

The STLS along with the laboratory technician are responsible for determining the amount of reagents and other materials microscopy centres will need each quarter. Place an order four times a year based on the approximate number of sputum specimens the laboratory examined the previous quarter. Also make sure these supplies are distributed in a timely manner, usually on a monthly basis. Because the Revised National Tuberculosis Control Programme depends on the microscopy centres, it is essential that there is an adequate supply of reagents and other materials.

Make sure there is adequate stock of reagents and other materials in the laboratory

It is very important for the laboratory to maintain an adequate stock of reagents and other laboratory materials. Visit each microscopy centre. Ask the laboratory technicians if they have enough supplies. If the laboratory has low stocks of any items, bring or make sure the laboratory is sent supplies from the district or sub-district stock. Remind them to exhaust the old supplies before starting the use of the new supplies.

During the visit, make sure the reagents are in good condition. Re-filter carbol fuchsin if particles have formed. Laboratory workers cannot perform proper sputum examinations with reagents that are not in good condition. Also, ask the laboratory technicians if the binocular microscope is in good working condition and inspect and use the microscope. If it is not working properly, arrange for

appropriate maintenance. If the microscope is still under warranty, get the supplier to repair it.

The following is a list of reagents which should always be available in the laboratory:

- Carbol fuchsin
- Methylene blue
- Sulphuric acid
- Immersion oil
- Xylene
- Phenol
- Methylated spirit

The following is a list of other materials that should always be available in the laboratory:

- Microscope slides, and separate boxes for storing positive and negative slides
- Marking pencils, diamond pencils and grease pencils
- Wooden sticks (broomsticks) for making smears
- Plastic bottles for reagents
- Universal glass containers
- Glass (or metal) rods for holding slides during the staining process
- Sputum containers
- Spirit lamp or bunsen burner
- Weighing balance (if reagents are prepared in the centre)

EXERCISE 4

The following is a list of reagents and supplies for the microscopy centre:

Carbol fuchsin	Sputum containers
Methylene blue	Boxes of microscope slides
Sulphuric acid	Boxes of grease pencils
Xylene	Wooden sticks (broomsticks)
Phenol liquid	Universal glass containers
Methylated spirit	

What, if anything, is missing from the laboratory's stock?

Let your facilitator know when you have completed the Exercise. He will review the answers with you.

Make sure there is adequate stock of sputum containers and slides

It is very important for every health unit in your sub-district that collects sputum specimens to have an adequate stock of sputum containers. Every microscopy centre must also have an adequate stock of sputum containers to collect sputum specimens and slides to examine the specimens.

Results of sputum examination are used to diagnose patients with pulmonary tuberculosis. If sputum examinations cannot be done, people who have pulmonary tuberculosis may not be identified and may continue to spread the infection to others. They will become sicker and may die. Patients who are under treatment for pulmonary tuberculosis are also given follow-up sputum examinations.

To keep health units and microscopy laboratories supplied with sputum containers and slides, calculate the number of sputum containers needed for diagnosis and for follow-up examinations each quarter. Then determine the number of slides needed. Place an order for the sputum containers and slides with the appropriate source. Visit each health unit that collects sputum specimens and microscopy laboratories to make sure there is an adequate stock of sputum containers and slides.

Calculate the number of sputum containers needed

During the first week of each quarter, calculate the quantity of sputum containers your sub-district/Tuberculosis Unit will need for the current quarter. There are six steps (see page 39) required for this calculation:

STEP 1. Determine the number of New smear-positive cases registered and treated last quarter. Use Block 3 of the *Quarterly Report on New and Retreatment Cases.*

STEP 2. Determine the quantity of sputum containers needed for diagnosis.

Follow the steps below:

a. Multiply the number of New pulmonary smear-positive cases by 10. The number of smear-negative, extra-pulmonary, retreatment smear-positive cases should not be considered, because 10 symptomatic cases include all types of patients and because patients with failure and default are examined as follow-up. Ten is the average number of symptomatics required to be examined for detecting one case of New pulmonary smear-positive tuberculosis. Fewer patients may need to be examined per case diagnosed at the District Tuberculosis Centre. This is the approximate number of symptomatic patients whose sputum will need to be examined for tubercle bacilli.

b. Multiply the number obtained in *STEP 2a* by 3. (Three sputum specimens are usually taken for each symptomatic patient.)

Example:

Last quarter, 40 smear-positive cases were registered and treated in a sub-district. To determine the number of sputum containers needed for diagnosis, multiply 40 by 10. 40 x 10 = 400. Multiply 400 by 3. 400 x 3 = 1200. The total number of sputum containers needed for diagnosis is 1200, plus an allowance for wastage.

STEP 3. Determine the number of sputum containers needed for follow-up examinations.

Follow-up specimens are taken for the majority of smear-positive patients *3 times* during their treatment (at the end of 2 months, 4 months and at the end of treatment). Two sputum containers are needed for follow-up examination because 2 sputum specimens are taken for each follow-up sputum examination.

For each pulmonary smear-negative case, follow-up sputum is taken *2 times*, so multiply the number of pulmonary smear-negative patients by 4 (2 sputum samples each at 2 months of starting treatment and at the end of treatment).

The number of sputum containers for the examination of patients who remain smear-positive at 2 months and of retreatment patients who remain smear-positive at 3 months is not calculated. This number is usually very small, and will not influence the stock.

STEP 4. Add the number of sputum containers needed for diagnosis to the number of sputum containers needed for follow-up examinations.

After you determine the number of sputum containers needed for diagnosis and follow-up examinations, add these numbers together for the approximate number of sputum containers needed for the quarter.

STEP 5. Allow for reserve stock. Add 10% to account for wastage of sputum containers.

STEP 6. Account for the sputum containers in stock.

On the last working day of the quarter, count the number of sputum containers currently in stock. Then, during the first week of the new quarter, subtract the number of sputum containers in stock from the quantity of sputum containers calculated to be needed for diagnosis and follow-up examinations (*STEP 4*).

Calculate the number of slides needed

There should be approximately the same number of slides in stock as there were sputum containers, because one slide is used to examine one specimen in a sputum container. Therefore, once you have determined the number of sputum containers needed for the next quarter, order the same number of slides. There may be a need for slightly more slides than containers because of unavoidable breakage of slides.

Order sputum containers and slides

After you have calculated the number of sputum containers and slides needed for your sub-district, order the supplies. Order the sputum containers during the first week of the quarter so that the health units and microscopy laboratories have enough sputum containers to collect sputum specimens and the microscopy laboratories have enough slides to conduct sputum examinations. In the RNTCP, these supplies will be procured by the District TB Society. It is important that good quality slides, containers and reagents are purchased.

Distribute the sputum containers and slides

After you receive the supply of sputum containers and slides for the quarter, distribute the sputum containers to each health unit that has facilities for sputum collection. Also distribute the sputum containers and slides to the microscopy laboratories in the sub-district. Keep the supply which is not distributed to the health units and microscopy centres in reserve stock for any facility which requires additional sputum containers and/or slides during the quarter.

Make sure there is an adequate supply of sputum containers and slides

When you go on visits to health units and microscopy laboratories, check the supply areas to see if there is an adequate stock of sputum containers and slides. Ask the health workers or laboratory technicians if they think the stock is sufficient. Estimate if there is enough stock to last until the end of the quarter, and there is sufficient reserve stock.

If a health unit is treating more cases than usual during this quarter, or a microscopy centre is examining more sputum specimens, consider sending them more supplies. When this happens, be sure to check the records for possible reasons for the increased use of supplies. If you determine that a health unit or microscopy centre will definitely need more supplies before the end of the quarter, check your reserve stock to see if it will meet the increased need. If the reserve stock is not sufficient, order the supplies needed and distribute them as quickly as possible.

If a health unit or microscopy centre has too many sputum containers (or slides) you can take the excess supply back to your store. Or, you may keep them at the facility, but deduct the excess number from the number you would distribute to them the following quarter.

EXERCISE 5

In this exercise you will calculate the number of sputum containers and slides needed by a sub-district for the current quarter. According to the *Quarterly Report on New and Retreatment Cases*, the sub-district began treatment of 80 pulmonary smear-positive cases last quarter. There were 125 sputum containers and slides currently in stock in the beginning of the quarter. Calculate the number of sputum containers and slides needed for the quarter. Use the Table below to complete the calculations. Then answer the questions at the end of the worksheet.

STEP	Description of Step	Resulting Number
1		
2a		
2b		
3		
4		
5		
6		

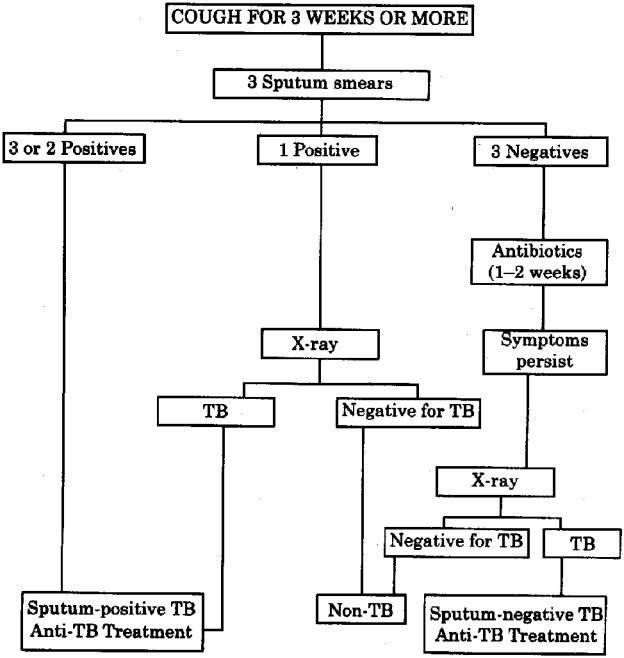
Answer the questions below:

1. How many sputum containers should you order for the sub-district?

2. How many slides should you order for the sub-district?

Annexure I

Diagnosis of TB in Chest Symptomatics



Annexure II

ITEMS TO MONITOR ON SUPERVISORY VISITS

1. Is every smear-positive patient recorded in the Tuberculosis Register?
2. Is patient information on the Laboratory Forms, including patient address and reason for examination, complete and legible?
3. Are patients having follow-up examinations at recommended intervals?
4. Are there sufficient reagents for the expected number of slides to be prepared and examined in the next quarter?
5. Are the Medical Officers and other staff of the centre aware of the importance of sputum smear microscopy for all chest symptomatics?
6. Are three sputum samples being examined for diagnosis of chest symptomatics?
7. Are two sputum samples being examined for follow-up of diagnosed patients?
8. Are laboratory safety precautions followed correctly?
9. Are sputum containers and other potentially infectious materials disposed of properly?
10. Are results reported promptly to the referring facility?
11. Is the Tuberculosis Laboratory Register being properly and completely filled?

Observe the laboratory technician during the sputum-collection process

1. Did the laboratory technician check to ensure that the *Laboratory Form for Sputum Examination* was complete?
2. Is the address listed clearly on the Laboratory Forms?
3. Is the sputum container clearly labeled on the side, not on the lid?
4. Is the Laboratory Serial Number entered correctly, starting with 1 on 1 January of the year, and continuing until 31 December?
5. Is each set of samples from a single patient given a single Laboratory Serial Number?
6. Is the TB Number written in the space provided for all patients whose Reason for Examination is Follow-up of Chemotherapy?
7. Does the laboratory technician demonstrate to patients how to bring up sputum?
8. Does the laboratory technician supervise patients when they provide spot sputum specimens?
9. Does the laboratory technician visually examine the sputum provided to determine if it is sputum or saliva only?

Observe the laboratory technician preparing smears for the examination

1. Does the laboratory technician use only new slides?
2. Does the laboratory technician either engrave each slide or label it with a grease marker?
3. Does the laboratory technician use a different wooden stick for each smear?
4. Are the smears made on the slide of the correct size (2 x 3 cm) and thickness?
5. Does the laboratory technician wait for the slide to dry before heating the slide to fix it?
6. When the laboratory technician fixes the slide by heating, does he do it for the proper duration of time?
7. Is the carbol fuchsin free of particles, and properly filtered?
8. When the laboratory technician heats the carbol fuchsin, does he do it properly, avoiding boiling?
9. Does the laboratory technician tip water off the slides after rinsing with water?
10. Is the sulphuric acid allowed to stand on the slide for the appropriate period of time (2–4 minutes)?
11. Is the methylene blue allowed to stand on the slide for the appropriate period of time (1 minute)?

Observe the laboratory technician examining slides under the microscope

1. In placing immersion oil on the slide, does the laboratory technician take care to avoid touching the slide with the applicator?
2. In examining the slide with the 100x lens, does the laboratory technician take care to ensure that the lens does not touch the slide?
3. Does the laboratory technician examine negative slides for at least 5 minutes?
4. Does the laboratory technician correctly complete the Laboratory Form and Laboratory Register?
5. Does the laboratory technician clean the 100x lens with cotton after completing the examination?
6. Are slides correctly cleaned and maintained for review by the supervisor?
7. Are all positive results recorded in red ink in the Laboratory Register?
8. After examining the slides, does the laboratory technician put the sputum containers and lids, with lids removed, along with the wooden sticks, into a foot-operated bucket containing either 5% phenol or 5% hypochlorite?
9. Does the laboratory technician break all positive slides after they have been reviewed by his supervisor?
10. Does the laboratory technician ensure that negative slides are not being re-used for AFB microscopy?

Annexure III

LABORATORY REQUIREMENTS FOR SPUTUM SMEAR EXAMINATION

A binocular microscope for use with either daylight or electric power illumination, with oil immersion objective (100x), eyepieces (8x or 10x) and spare bulbs and fuses will be provided for each designated microscopy centre.

Equipment needed in each microscopy centre	Number
Slide holder made of metal	2
Spirit lamp, cotton wool plug or metal wire	1
Diamond markers	1
Timer, 30 or 60 minutes, with alarm	1
Forceps, stainless steel for slides, 15 cm	2
Slide rack made of glass, plastic, or metal for 12-25 slides	2
Slide boxes for 100 slides	2
Funnel glass, 45 mm or 60 mm diameter	4
Funnel glass, 90 mm or 125 mm diameter	4
Drop bottles, glass, 100 ml	4
Bottles, brown glass, 100 ml	4
Flasks, glass or pyrex, 500 ml	2
Flasks, brown glass, 1000 ml	2
Bowl made of plastic, 50 x 30 cm	2
Wash bottles, made of plastic, 500 ml	6
Drop plastic bottles, 10 ml for immersion oil	2
Bucket, metal or plastic, 12 litre, foot-operated	2

Supplies needed in each microscopy centre for every 2000 slides examined	**Quantity**
Disposable plastic sputum containers	2200
Slides for microscope, 25 X 75 mm, 1.1–1.3 mm thick	**2200**
Wooden sticks	2200
Laboratory Forms for Sputum Examination	**1000**
Tuberculosis Laboratory Register	1
Pens, ball point, black or blue ink	**4**
Pens, ball point, red ink	2
Adhesive labels for sputum containers	**2200**
Lens paper	2 rolls
Ball of white absorbent cotton	**500**
Filter paper, no. 1 packs of 100	4 boxes
Toilet tissues	**2 rolls**
Towel and clean rags	as needed
Sodium hypochlorite	**10 litres, freshly prepared**
Grease pencils	6

Reagents needed for every 2000 examinations, to be prepared/procured at the district or sub-district (Tuberculosis Unit) level and distributed monthly	**Quantity**
Sulphuric acid (25%)	**10 litres**
Carbol fuchsin (1%) for Ziehl–Neelsen staining	10 litres
Aqueous methylene blue (0.1%)	**6 litres**
Immersion oil	200 ml
Xylene	**4 litres**

Other equipment needed at the district/sub-district (Tuberculosis Unit) level	**Quantity**
Pressure cooker	**1**
Apparatus for distilling water	1
Methylated spirit, for every 2000 examinations	**2 litres**

Annexure IV

JOB RESPONSIBILITIES OF THE SENIOR TUBERCULOSIS LABORATORY SUPERVISOR (STLS)

- Maintain the quality of sputum microscopy and smooth functioning of laboratory services
- Organize smear examination at the microscopy centres of the sub-district
- Organize regular training and continuing education of the laboratory technicians
- Maintain a list of all microscopy centres in the sub-district which carry out TB activities, including distribution (map of the area) and staff responsible (name, position and address)
- Supervise the microscopy centres at least once every month, and perform quality control of slides as per the Laboratory Manual, registering the number of slides checked and the proportion of discordance for positive and negative results
- Check the record-keeping (Tuberculosis Laboratory Register) and compare the workload for case-finding with the general OPD attendance of symptomatic patients in the health facilities
- Arrange for coverage of the microscopist in case of leave, so that there is regular and uninterrupted availability of smear examination at that microscopy centre
- Prepare and distribute reagents, and ensure regular and sufficient supply of reagents and sputum containers in each health facility
- Ensure proper storage and transport of sputum specimens, safety of laboratory staff and maintenance of microscopes
- Prepare and forward reports to the DTO, in collaboration with the STS, regarding implementation, quality control, supervision and management of laboratory supplies
- Collaborate with the laboratory of the DTC in smear examination and record-keeping to maintain quality control
- Whenever the STLS is not accompanied by the STS, check that sputum-positive cases have been put on treatment and if not, inform the Medical Officer in-charge and the STS so that they may take necessary action
- Make a monthly tour programme in advance so that all the field units are covered at least once a month and get it approved from the Medical Officer-Tuberculosis Control
- Maintain a diary recording the details of field visits

Annexure V

WORKSHEET FOR STLS DURING MONITORING MISSION TO A MICROSCOPY CENTRE

1. General condition of laboratory: ❑ Good ❑ Poor (specify: _____)

2. Condition of the microscope: ❑ Good ❑ Poor
   - Condition of eyepiece ❑ Good ❑ Poor
   - Condition of 10x objective ❑ Good ❑ Poor
   - Condition of 100x objective ❑ Good ❑ Poor

3. Specimen quality:
   - LT observation made on gross examination ❑ Yes ❑ No
   - Quality of smear on microscopic examination ❑ Good ❑ Poor

4. Staining quality ❑ Good ❑ Poor (specify: _____)

5. Slides maintained clean and in order for crosschecking ❑ Yes ❑ No

6. Smear correct size ❑ Yes ❑ No (specify: ❑ Too big ❑ Too small)

7. Smear even ❑ Yes ❑ No

8. Thickness of smear ❑ Good ❑ Poor (specify: ❑ Too thick ❑ Too thin)

9. Comment/suggestions for improvement:

   a) _____

   b) _____

   c) _____

   d) _____