QUALITY ASSURANCE IN SPUTUM SMEAR MICROSCOPY

District Laboratory Supervisor

OPERATING GUIDELINES

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Section-A	QUALITY ASSURANCE OF LABORATORY SERVICES

A.1 Sputum Smear Microscopy in TB Control

Early diagnosis and effective treatment is the key element of the DOTS strategy package. Failure to detect persons with TB can lead to continued spread of infection in the community. The WHO strategy for tuberculosis control (DOTS) relies on a network of laboratories that provide acid-fast bacilli (AFB) sputum smear microscopy. The AFB result determines the treatment and outcome of an individual TB patient. If the laboratory diagnosis is unreliable, all other activities of the TB control programme are affected. Therefore, quality assurance of laboratory services, including AFB sputum smear microscopy, is essential.

A.2 Quality of Sputum Smear Microscopy

The quality of sputum smear microscopy services can only be assured through staff commitment at facility, district, provincial and national levels. At laboratory level trained staff needs to be supervised and supported, technically as well as logistically. The material support may include reagents, laboratory equipment and supplies, and print materials. An enabled district level senior microscopist (i.e. District Laboratory Supervisor - DLS) provides the required technical support to the laboratory network (both public and private). The district supervisor's enabling requires training and supervision, as well as material and mobility support. A strengthened provincial level laboratory setup provides the training and supervision support to the district laboratory supervisor, whereas the material inputs come mainly from the TB control programme and the district health offices. The national level reference laboratory provides the policy guidelines and overall coordination support for the countrywide network of AFB microscopy facilities.

A.3 Quality Assurance (QA) in Lab. Services

The National Tuberculosis Programme (NTP) recommends that laboratories at all levels be managed by a system of external quality assurance (EQA) and quality improvement that meets international standards.

A.3.1 What is EQA?

External Quality Assurance (EQA) of sputum smear microscopy is an essential requirement for the quality of TB care in a district. The focus of EQA is on the identification of laboratories where there may be serious problems resulting in poor performance, not on the identification of individual slide errors or the validation of individual patient diagnoses**.** It is also a very important tool for communication with and motivation of laboratory staff that may otherwise feel isolated in their work.

A.3.2 Purpose of EQA

The purpose of EQA at public and private laboratories is to improve the quality and the outcome of TB case management. The EQA helps ensuring the trust-worthiness of the smear results through:

- Availability and quality of laboratory inputs including reagents, supplies, print materials, and microscope.
- Continued staff skill enhancement for quality laboratory outputs i.e. smear preparation, staining and examination, slide storage and disposal, and recording and reporting of results.

A.3.3 Methods used in EQA

There are three methods that can be combined to evaluate laboratory performance:

- On-site Evaluation
- Blinded Rechecking
- Panel Testing

An enabled District Laboratory Supervisor (DLS) visits each laboratory (both public and private), on monthly basis. During visit, the DLS performs EQA mainly through the onsite evaluation and blinded rechecking of a sample of TB slides. The assessment is followed by onsite interaction between the staff and the DLS for better understanding and action planning. The panel testing is a method generally used at the level of district by Provincial reference laboratory.

On-Site Evaluation

The observation of laboratory arrangements and working under actual conditions include: quality and functioning of equipment; adequacy and quality of reagents and supplies; slide storage and disposal; record keeping; and laboratory safety. Documentation of the onsite evaluation process, on standardized formats, helps to monitor the changes in laboratory arrangements and practices over a period.

Blinded Rechecking

The DLS, during the monthly visit, would recheck a random sample of slides prepared and examined at the laboratory during the month under review. The method for selecting, rechecking, recording and cross validating the results is described in respective sections.

Panel Testing

In this method, the laboratory person is provided a set of stained and/ or unstained slides for reading, interpreting and reporting the results. This method tests the staff ability to stain and/or read smears, and is not a useful mean to assess routine laboratory performance. In TB control programme, the Provincial reference laboratory would periodically administer the panel testing to assess staff skills preferably by DLS.

A.4 Roles in TB Sputum Smear EQA

The description below outlines the roles of key actors in the EQA process in a district. These set of roles have been extensively discussed and agreed among stakeholders. The main purpose of the proposed arrangements is to build the district capacity for implementing EQA with technical and logistic support from the provinces.

A4.1 DLS Role (EQA Center)

- Planning, conducting, documenting and reporting monthly supervisory visits to each microscopy center in the district.
- Carrying out and documenting the onsite evaluation and providing support to plan/address the material and ability gaps.
- Carrying out and documenting the onsite blinded rechecking of sample slides, as per programme guidelines. This also includes providing feedback to health facility staff i.e. facility in-charge as well as laboratory staff.
- Maintaining communication with the provincial reference laboratory (PRL) including sending them the discordant slides, facilitating their examining a sample of concordant slides (during supervisory visit of the district), and participating in quarterly interaction with them (i.e. PRL staff).

DLS plans and conducts the visits of each laboratory (public and private), on monthly basis, to carry out the agreed role/ responsibility. The following chapters describe the operational details for planning, carrying out, documenting and following-up the EQA activities in the district.

A4.2 Laboratory Staff Role

- Maintaining the laboratory register and storing all slides for re-checking.
- Sharing the technical and logistic issues constraining his AFB testing work.
- Complying with the instructions of the District Laboratory Supervisor and Facility In-charge for improved quality of AFB testing.

A4.3 District TB Coordinator Role

- Supervising (administrative) and supporting the DLS work in the district including approving and monitoring his visit plans, facilitating the availability of laboratory reagents and supplies.
- Contributing in the laboratory related discussions held during the facility review/planning meeting.
- Commenting on the DLS performance presented in the quarterly intra-district meeting.
- Facilitating communication with provincial reference laboratory including:
    - Ensure the discordant slides are sent and feedback is received in-time;
    - DLS attends the quarterly meeting at PRL; and
    - PRL staff quarterly monitoring visit to the district is facilitated.

A4.4 Facility **In-charge Role**

- Selecting a sample of slides, as per programme guidelines, for reexamination by the DLS.
- Entering the facility results (from TB04) into EQA Form, and participate in comparing these with the DLS reexamination results.
- Discussing the EQA findings, and facilitating the process to address the gaps in laboratory arrangements and practices.

A4.5 Provincial Reference Laboratory Role

- Designing and conducting the training of District Laboratory Supervisors (DLS) from all districts in the province.
- Reexamining the discordant slides from the districts, keeping record and providing in-time feedback to the districts.
- Developing and conducting the interaction and build capacity of DLS, when they come for quarterly meeting at PRL.
- Planning and conducting quarterly supervisory visits to all the participating districts in the province.
- Communicating with the National Reference Laboratory for EQA activities in the province.

Section-B	LABORATORY SUPPLY ESTIMATION & REAGENT PREPARATION

The district health office will arrange the laboratory supplies through PTP inputs as well as their own sources and will supply these supplies and materials for quality assured AFB microscopy services through a network of public and private laboratories.

B.1 Estimate the required amount of laboratory supplies in a district

B1.1 The requirements are estimated on the basis of following assumptions:

- Thirty-four (i.e. 30+3+1=34) slides are prepared and examined for every ten suspects assessed. The statistic is based on:
  - Ten suspects are screened to get one smear-positive and one smear-negative case of pulmonary TB. Total 30 slides are examined for screening ten TB suspects.
  - Follow up of one smear-positive and one smear-negative TB patient requires examining four more slides (i.e. 3 for smear-positive and one for smear-negative).

- The reagents required for screening ten suspects and following-up one smear-positive and one smear negative TB patients (i.e. 34 slides) is:

Sr. No.	Reagent	Quantity Required (for 34 slides)
1	25% H_2SO_4	170ml
2	1% Carbol fuchsin	170ml
3	0.3% Methylene blue	170ml
4	Immersion Oil	3.4ml
5	Xylene or Toluene	3.4ml
6	Methylated spirit	34ml

- An average number of TB suspects examined per month, in an average laboratory, are about 50.
- Each district estimates the requirement and arranges supplies for the six months i.e. 3 months supply and 3 months reserve stock.

B1.2 The six months total requirement of reagents and supplies in a district is estimated with the help of the following table.

Estimated Requirement of Laboratory Supplies in a District (6 months)

Item	Laboratory Requirement - 6 month (A)	District Requirement	
Reagents		**# liters of prepared solution (B)** (A x # Labs. in a district)	**Quantity of chemical/ reagent**
1. 25% H_2SO_4 (250 ml acid for 1 liter solution)	5 liters (2 bottle of 2.5 liter each)		(B / 4 liters)
2. 1% Carbol fuchsin (10 gm powder for I liter solution)*	5 liters (2 bottle of 2.5 liter each)		(B x 10 gms)
3. 0.3% Methylene blue (3gm powder for I liter solution)*	5 liters (2 bottle of 2.5 liter each)		(B x 3 gms)
4. Immersion oil (500ml bottle)	1 bottle		
5. Xylene or Toluene (250 ml bottle)	1 bottle		
6. Methylated spirit (1 liter bottle)	1 bottle		
General Supplies			
1. Glass slides	14 packs (72 each)		
2. Sputum containers	1000		
3. Slide storing boxes (100 slides)	2 boxes		
4. TB laboratory register (TB04)	One per laboratory. Replenished only when required.		
5. Wire loop			
6. Diamond pen			
7. Functioning microscope			

* May be repacked in 50 gm packs for distribution to the district. DLS prepares/distribute stain to laboratories.

B.2 Prepare Stains

Preparing and using good quality reagents is required for correctly detecting AFB. DLS prepares reagents, at EQA Center in a district, for distribution to the public and private laboratories. The method below describes the preparation of 1 liter of each reagent. The quantity of ingredients is adjusted according to the required quantity of each reagent being prepared.

The reagent preparation and storage at district EQA center and transport to a network of laboratories require the following equipment and glassware.

No.	ITEMS	QUANTITY
01	Amber bottle (2.5 liter) for storage	3
02	Amber bottle (1 liter) for transport and supply to the laboratories by DLS one each for Carbol fuchsin, Decolorizer solution (25 % H_2SO_4), Methylene blue solution and Xyline)	1 x 4 = 4
03	Conical Flask - 02 liter	4
04	Measuring Cylinder 01 liter	2
05	Measuring Cylinder 500 ml	2
06	Measuring Cylinder 100 ml	2
07	Beaker – 01 liter	1
08	Pipette - 10 ml	5
09	Pipette - 05 ml	5
10	Funnel – Medium size	2
11	Filter Paper - 4 inch.	Sufficient quantity
12	Analytical weigh scale	1

B.2.1 Prepare Ziehl's Carbol Fuchsin

Step-1: Arrange the following equipment and glassware

- Conical flask (02 liter)
- Measuring cylinder (1 liter)
- Filter funnel
- Filter paper
- Weighing scale
- Beaker (01 liter)
- Pipette (10 ml)
- Label
- Spirit Lamp
- Amber bottle (2.5 liter)

Step-2: Arrange the following reagents

- Basic fuchsin powder = 10 gm
- Ethanol (95%) = 100ml
- Phenol crystal = 50 gm
- Distilled Water = 900 ml

Step-3: Prepare Solution A

- Weigh 10 gm basic fuchsin in weighing balance (concentration depends upon the quality of stain).
- Measure 100ml of 95% ethanol in the cylinder.
- Mix the two in conical flask.

Step-4: Prepare Solution B

- Measure 900 ml of warm distilled water in a beaker.
- Weigh 50 grams of phenol crystals and add this to 900 ml of warm distilled water. Stir the solution gently.

Step-5: Prepare 1000 ml Working Solution

- Measure 900 ml of solution B in a conical flask (2 liter) and add 100 ml of solution A.
- Mix well and then filter the solution in amber bottle for storage. Always filter before use (once daily).

B.2.2 Prepare Decolorizer

Sulfuric Acid (25%) or Acid Alcohol (3%) can be used as decolorizer.

B.2.2.1 Prepare Sulfuric Acid (25%)

Step-1: Arrange the following equipment and glassware

- Beaker (1 liter)
- Measuring cylinder (500ml)
- Conical flask = 2 liter
- Transparent bottle (2.5 liter)

Step-2: Arrange the following reagents

- Concentrated Sulfuric Acid = 250 ml
- Distilled water = 750 ml

Step-3: Prepare 1000 ml Sulfuric Acid (25%)

- Measure 750 ml distilled water and put in conical flask.
- Measure 250 ml sulfuric acid in a cylinder and pour it slowly into the flask of water, along its side. This will generate a lot of heat. Usually it is necessary to stop for a few times, swirling the flask to let it cool off a bit.

Caution: Never do the reverse: adding water to acid will make it boil immediately and it may even splash on your face. In case of accident with acid, rinse the body part or cloth immediately with plenty of water.

B.2.2.2 Prepare Acid Alcohol (3%)

Step-1: Arrange the following equipment and glassware

- Conical flask (2 liter)
- Transparent bottle (2.5 liter)
- Cylinder (100 ml)
- Beaker (1 liter)

Step-2: Arrange the following reagents

- Ethanol
- Concentrated HCI

Step-3: Prepare 1000 ml Acid Alcohol (3%)

- Measure 970 ml ethanol in a beaker.
- Measure 30 ml HCI in a measuring cylinder (100ml)
- First add 500 ml of ethanol in conical flask & then add HCI slowly to it.
- Add remaining ethanol to flask & mix.
- Store decolorizer in transparent bottle in refrigerator at 5c.

B.2.3 Prepare Methylene Blue (0.3%)

Step-1: Arrange the following equipment and glassware

- Weighing scale
- Conical flask (2 liter)
- Amber bottle (2.5 liter)

Step-2: Arrange the following reagents

- Methylene blue = 3 gm
- Distilled water = 1000 ml

Step-3: Prepare 1000 ml Methylene Blue (0.3%)

- Measure 1000ml distilled water in the conical flask.
- Weigh 3 gm Methylene blue with weighing scale & add it to flask.
- Mix well & store in amber bottle away from sunlight.
- Let the flask freshly prepared stains stand (covered) till quality control by using positive and negative control slides has been done, then fill the solution in the clean bottle and label them. Label should mention the name of the stain and the date it was prepared.

Caution: Do not use mouth pipetting to measure any liquid reagents during preparation of staining reagents.

B.2.4 Quality Control of Freshly Prepared Stains

- Quality control is necessary to ensure that the stains (especially carbol fuchsin) work well, and that they do not contain contamination of AFB. For the first purpose, two positive smears will be used and for the second purpose two negative or fake smears.
- The stain-preparing laboratory should always do this for each batch prepared. It is advantageous to prepare bigger batches if big flasks are available. The laboratory should keep careful records, to defend itself against possible complaints of bad stains.
- The batches should be identified by name of reagent and preparation date (as on the bottle labels).
- Well-prepared stains can be kept for several months (6 months) even at temperatures over 35^{o}C. However, they have to be stored in clean bottles and out of direct sunlight. It is preferable to keep them in a closed cabinet.
- Keep a record of prepared stains using the following table

Lot No.	**Date of Preparation**	**Result of stain with**		**Remarks**
		(+) Slide	**(-) Slide**	

Section-C	LABORATORY VISIT PREPARATION

Supervision is the process of helping the staff to improve their performance. Supervisory visits give staff and his supervisor an opportunity to share and better understand the situation, and enable the staff to perform at their best. During these visits, the correct performance is reinforced and deviation in practice is identified and corrected. The corrective measures are discussed and agreed with the laboratory staff as well as the laboratory In-charge and District TB Coordinator (where needed). Thorough planning and preparation helps making the DLS visit more productive and efficient.

C.1 Prepare Monthly Plan

- Prepare monthly health facility visit plan, on the format given below, by 20th of every month. The monthly plan of the DLS needs to take into account other facility monitoring activities being planned by the District TB Coordinator during the month.
- Get the monthly visit plan approved. DHQ hospital based DLS will get the approval of plan from the In-charge of the DHQ laboratory, and will get the approved plan endorsed by the District TB Coordinator. The district health office based DLS will get the monthly visit plan approval directly from DTC.
- Facilitate DTC office in dissemination of the plan. DTC office will send a copy of approved DLS visit plan, by 25th of every month, to all the diagnostic centers with a copy to EDO (health) office.
- Get the approval of any change in the visit plan from the same officials and inform the diagnostic centers and EDO office accordingly.
- Maintain a record of all the approved visit plans. Each DLS monthly activities will be assessed in light of his approved visit plan.

DLS Monthly Visit Plan

Date	Health Facility	Remarks

Notes on filling the table:
Date - the date on which the facility is to be visited
Health Facility - the Health facility to be visited on that date
Remarks - Note any particular observation/action for the facility, in light of observations/discussions during the last visit.

C.2 Prepare for on site replenishment of laboratory material

- Check before each laboratory visit the material kit, and replenish the materials as per checklist given below:

Checklist for Laboratory Materials Kit

Item	Quantity for the Kit	Checked and replenished
25% H_2SO_4 (I liter bottle)	1	
1% Carbol fuchsin (I liter bottle)	1	
0.3% Methylene blue (I liter bottle)	1	
Immersion oil (500 ml bottle)	1	
Xylene or Toluene (250 ml bottle)	1	
Glass slides (72 slides per packet)	3 packets	
Sputum container	100	
TB Laboratory register (TB04)	1	
Slide storing box for DC (capacity: 100 slides)	1	
Slide storing box for EQA (capacity: 25 slides)	1	
EQA Forms	5	
Wire Loop	1	
Diamond Pen	1	

C.3 Prepare motorbike for field visit

- Check for:
  - Logbook entries
  - Mobil Oil and petrol
  - Air pressure and tyre condition.
  - Breaks, lights and any visible mechanical problem

C.4 Previous visit report

- Maintain a separate folder for each laboratory to file its monthly EQA visit reports.
- Review the observations and decisions of the last visit report, and make any special preparation/arrangement accordingly.

Section - D	SUPERVISORY VISIT – LOGISTICS & ARRANGEMENTS

D.1 Assess the Laboratory Functioning

- Use the EQA Form-1, Section-1 to record the number of days that the laboratory remained non-functional, reasons for non function, onsite actions taken and actions required at district health office/coordinator levels.

EQA Form-1
Section-1: Laboratory Functioning

No. of days Lab. remained non functional	Reasons	Actions already taken/ Onsite actions	Actions required/agreed

Trouble-shooting checklist/guide for laboratory staff related issues is given as Appendix-1.

D.2 Replenishment of Laboratory Materials

- Use the EQA Form-1, Section-2 to check and replenish the laboratory supplies and keep record.
  - For each laboratory item, fill the quantity of available stock in the "Stock available" column. 1 bottle means one filled bottle. If the Lab has one and half bottles consider it as one. Similarly one pack means one complete pack.
  - Supply the materials and fill in the column "Supplied" and make record on the stock register.
  - Calculate the amount of materials to be arranged and fill in the column "To be arranged"
  - Agree on how to get the "to be arranged" materials and write in the respective column.

EQA Form-1
Section-2: Laboratory Input Replenishment

Item	Minimal Stock Level	Stock available	Stock replenishment (Quantity)		Comments/Actions to be taken
			Supplied	To be arranged	
25% H_2SO_4	2 bottle				
1% Carbol fuchsin	2 bottle				
0.3% Methylene blue	2 bottle				
Immersion Oil	1 bottle				
Xylene or Toluene	1 bottle				
Methlated Spirit	1bottle				
Glass Slides	5 Packets				
Sputum Containers	200				
TB04	1				
Slide Storing Boxes	2 (100 slide capacity each)				
Wire Loop	1				
Diamond Pen	1				
Functioning Microscope	1				
Trained Lab. staff (1)					

D.3 Assess Recording and Reporting

Note that the laboratory staff has written all results of AFB examinations in the Tuberculosis Laboratory Register and not elsewhere.

D3.1 Review TB Laboratory Register (TB04) and record on of EQA Form, Section-3

- Check if all relevant columns of TB04 have been filled for each patient examined. The missing/incorrect entries are identified and discussed with the laboratory staff.

Common Mistakes in filling TB04	
o Laboratory Serial Number is not given continuously	If you find mistakes ask the laboratory staff to consult session 7 of the laboratory training course.
o Lab Serial Number is not started from 01 every year	
o District TB Number is not written in follow-up cases	
o Incomplete name of patient entered (i.e. without referring to father/husband name)	
o Complete patient address is not written	
o Follow-up patients are marked as diagnostic patients	
o Grading is not written according to standard	
o Positive results are not entered with red pen.	

- Check the recording of specimen entries on TB04.

Common Mistakes in recording specimens on TB04	
o Single specimen is not recorded (staff waits for other specimens)	If you find mistakes ask the laboratory staff to consult session 7 of the laboratory training course.
o Second specimen for diagnosis is given new serial number	
o Name is entered without results. This happens when patient name is entered before submission of sputum specimen.	

- Review the TB04 entries to check the number of slides being examined for each TB suspect. Also note the proportion of TB suspects found smear positive on microscopic examination.

Calculate the SPR as follows:

- Count the cells with +ve result entries in sub-columns 1, 2 & 3 column Results of Specimen of TB04.
- Count all the cells with any type of entry in sub-columns 1, 2 & 3 column Results of Specimen of TB04.
- Divide the first count with the second and multiply with 100
- Record the result of calculation in column ‘Smear Positivity Rate in column ‘Statistic’

Calculate the ANSV as follows:

- Add up, Total diagnostic slides + Total follow-up slides
- Record the result in column ‘Statistic’

EQA Form-1
Section-3: Recording & Reporting

Characteristic	Formula	Statistic	Comments
Specimens examined per TB suspect.	Total slides examined / Total suspects		
Proportion of suspects found smear positive on AFB testing. (Suspect positivity rate)	# SS^+ / # suspects examined		
Proportion of total slides found positive (Smear positivity rate)	# positive smears / Total smears examined		
Annual Slide Volume (ANSV)	Total diagnostic slides + Total follow-up slides		
TB04 (Lab. Register) Recording	Complete/Correct		

D.4 Observe Microscope Maintenance, Slide Storage and Waste Disposal

Check and record in EQA Form-1, Section-4.

D4.1 Microscope maintenance

- Check for cleanliness and proper functioning of microscope. The use of microscope is given in Laboratory Training Course in Session-VI. The maintenance of microscope and troubleshooting checklist/guide is given as Appendix-2.

D4.2 Slide Storage

- Check that all slides are stored by the microscopist in the provided slide boxes in the same order as they are listed in the laboratory register. Slides are marked as ‘a’, ‘b’ and ‘c’ along with the lab serial number for first spot, early morning and second spot specimen. In order to maintain consistency with the laboratory register, two blank spaces should be left behind the first slide from a suspect patient so that the second and third slides can be added after they are read. No blank space should be left behind the slide from a follow-up patient.
- Check that slides have been labeled in a manner consistent with the laboratory register to ensure that the correct slide is matched to the result and the result of the smear examination is not appearing on the slide.
- Check that oil has been removed from the examined slides before storage by microscopist. Removal of immersion oil is to be done by placing the slides in xylene jar and then vertically on tissue paper until it drains away. Separate jars should be used for positive and negative slides.

- Check that slides are stored in boxes that do not allow the slides to touch each other (e.g., do not stack or press slides together) and boxes are placed away from direct sunlight.
- Check that slides are stored according to laboratory serial number in the same box, irrespective of results.

D4.3 Observe Slides/material disposal

This is very important that laboratory personnel follow the NTP protocols for safe disposal of contaminated material i.e. sputum, containers and slides. This reduces the risk of accidental spread of disease from contaminated material.

- Check that the used glass slides and sputum containers are initially disinfected, by boiling in the water for about 20 – 30 minutes or by putting in phenol. Then these disinfected materials are either burned or buried in the ground.

- Check that all the slides are discarded (i.e. positive and negative) on quarterly basis. The slides at diagnostic centers are discarded only when DLS ask the laboratory staff to do so, after DLS quarterly interaction with PRL staff.

All cross-examined slides kept with DLS are discarded only when PRL staff asks the DLS to do so, during their supervisory visit to the district. The used sputum containers are discarded either on daily basis or on alternate days.

EQA Form-1
Section-4: Microscope maintenance, Slide storage and Waste disposal

Characteristic	Acceptable		Action/Comments
	Yes	No	
Microscope cleanliness			
Microscope functioning			
Slide Storage			
Disposal of used slides			
Disposal of used sputum containers			

Section-E	SUPERVISORY VISIT - SLIDES CROSS-EXAMINATION

During the laboratory visit the DLS will cross examine a sample of slides, assess practices and assist the laboratory staff to address technical, operational and logistic issues. The programme recommends the following process for selecting and reexamining a sample of slides.

E.1 Sampling

As a new EQA program, sensitivity of the peripheral laboratory to the controllers is set at 80%. Based on this level of sensitivity, the required monthly sample size for reexamining the slides is 8 at RHC and 10 at district and sub-district hospitals. The calculation of this sample size is based on Lots Quality Assurance System (LQAS) considering provincial average figures of smear positivity rate and negative slides examined annually.

At each monthly visit of the DLS, the Laboratory In-charge (Medical Officer in RHC) selects a sample of slides for the DLS to reexamine and report back. The In-charge keeps the laboratory register with him, while the DLS does the reexamination of sample slides.

Sampling method:

- Check that all sputum slides are kept in sequence in the slide storage box (same sequence as in the laboratory register). This should include both new and follow-up sputum slides.
- Look in the laboratory register for the first and the last serial number of patients examined in the last completed calendar month. (For example, for the month of March 2007 at RHC Barakahu the first patient examined is serial number 201 and the last patient examined is serial number of 288).
- Count the total number of patients examined for the identified range of serial numbers i.e. 201 – 288 (88 patients examined for this range of serial numbers). The required sample size is 8 for rural health centers and 10 for hospitals.
- Divide the total number of patients examined by the required sample size, to get the sample number value “n”. For the above example, divide the number of patients (88) with the sample size for RHC (8). The result is n = 11. This means that a slide of every 11^{th} patient examined at RHC during the month of March is to be reexamined by the DLS.
- Choose randomly the first number to start sampling from. Out of the first “n” group (in this example, 11 patients with serial numbers 201 – 211), select one serial number randomly. In this example “203” is selected out of the first “n” group. Then from the remaining serial numbers, every n^{th} (in this example 11^{th}) serial number is systematically selected (i.e. 203+11=214, 214+11=225, etc.----).

In this way the eight selected serial numbers are identified from the RHC laboratory register (10 slides if a hospital).

- Retrieve the slides with selected serial numbers from the storage boxes. In case of TB suspects with more than one slides examined, the morning slide (i.e. "b" of the same serial number) is taken for reexamination. If a slide is missing, use the next slide. If more than two slides are missing, check whether the slides are being destroyed by the peripheral laboratory technician and take the corrective action
- DLS receives the randomly selected sample of slides from the In-charge. DLS notes the serial numbers of the selected slides in the EQA-1 Form, before taking these slides for the reexamination.

Summary: The sample size of patients to have a slide cross examined is always 8 for RHCs (or 10 for hospitals). However, the sample number ("nth") will be different each time - depending on the number of patients examined in the last completed month. In our example this was 11 because there were serial number 201 – 288 = 88 patients examined in March. That is dividing by 8 (for an RHC) this gives a sample number of 11^{th} patient in this example. (If in the month of April there is 72 patients in the laboratory register, then the sample number will be 9). Also, remember to start with a random number chosen from the first group of "n" patients; by chance patient serial number was chosen 203 in our example, and so the next was 203 + 11 = 214 and so on until all 8 (for this RHC) are sampled.

E.2 Smear Assessment

Check the selected sample of slides for the following seven smear characteristics and note the findings in Section-5 of EQA Form-1. Use Appendix-3 as reference.

1. Specimen Quality:

The presence of dust cells (macrophages) and white blood cells (WBCs) in the smear is an evidence that specimen is sputum not saliva. (Note: Look for WBCs in X 100, and dust cells in X 1,000).

2. Size:

The smear size of approximately 1cm x 2cm is considered acceptable.

3. Thickness:

Acceptable thickness of smear (unstained) can be checked by looking at printed letters through the smear, holding the smeared glass slide 4 – 5cm over a printed-paper. In normal smear thickness letters are blurred but readable. If letters cannot be read, it is too thick. On the other hand, clear print indicates thin smear.

4. Evenness:

Sputum should be spread evenly on the glass slide.

5. Slide Labeling:

The slides need be marked with a permanent marker and writing must be readable. The guidelines for marking/labeling of slides must be followed i.e. laboratory number followed by sequence number e.g. 100-A.

6. Staining:

AFB should be stained in red or dark pink. AFB and background must be clearly distinguished by decolorization. AFB in faint red color is over-decolorization. Remaining of fuchsin color on the background of the smeared part is under-decolorization.

7. Smear Cleanness:

Stained smear must be free from stain deposits, dirt, fuchsin crystals produced by overheating of staining, debris, etc.

Overall quality of smear is determined on the basis of all the sample slides examined for the selected seven main characteristics. The observations on each of seven characteristics of a slide is recorded in section 5 of the EQA-1, by putting tick mark either in “A” for acceptable and “NA” for not acceptable. The overall quality on each of seven characteristics is declared acceptable if only two or less slides (i.e. less than 25%) are judged as not acceptable. The overall quality remarks are recorded in the last row of the table in section 5 of the EQA-1.

EQA Form-1

Section-5: Smear Assessment of Selected Slides

Lab Serial No.	1.Specimen Quality		2.Size		3.Thickness		4.Evenness		5.Labeling		6.Slide Staining		7.Smear cleanness	
	A*	NA**	A	NA	A	NA	A	NA	A	NA	A	NA	A	NA
Total														
Remarks***														

Key:
*A - Acceptable,
**NA - Not Acceptable,
*** Remarks – Gives overall performance of the characteristic under consideration. If for any characteristic, three or more slides are found not acceptable (NA) then overall performance on that characteristic is labeled as Not Acceptable (NA).

If performance on any of the characteristics from 1-5 is found ‘Not Acceptable’ ask the laboratory staff to read Session IV, Smear Preparation from the Laboratory Training Course Module.

If performance on any of the characteristics from 6-7 is found 'Not Acceptable' ask the laboratory staff to read Session V, ZN Staining from Laboratory Training Course Module.

An important performance area for the DLS to consider and guide the laboratory staff is the staining practices. The following table is to help the DLS to identify the possible causes and remedial actions for improved staining practices.

TROUBLE-SHOOTING IN STAINING		
Problem	**Possible Cause**	**Remedy**
Smear too Pink	Insufficient decolorization	Decolorize for longer
	Sulphuric Acid concentration < 25%	• Recheck stain preparation with control slides and QC results
	Carbol fuchsin (CF) has been dried on smear	• Add sufficient CF
	Smear too thick	• Prepare new smear
Pale acid – fast bacilli	CF concentration <1%	• Recheck stain preparation with control slides and QC results
	CF insufficiently heated	Heat CF to steaming
	• CF staining time less than 5 minutes	Stain for a minimum for 5 minutes
	Less fixation time	• Pass over flame 3 times, 1-2 seconds each time
	CF reagent has expired or stored in direct sunlight	• Replace reagent • Store stain bottle in the dark
	Over decolorized	Decolorize for proper time
Counter stain too dark	Excessive staining time	Do not exceed 30 seconds
	Inadequate washing step after counterstaining	Extend washing step
	Methylene blue concentration too strong	• Recheck stain preparation and QC results
	Smear too thick	Prepare new smear
Deposits on slide	Stains not filtered	Filter stains prior to use
	Soot deposit on underside of smear	Clean with a moist tissue

E.3 Re-examination Process

- Re-examine the selected sample slides in the same laboratory, without looking at the results recorded in the laboratory register (i.e. blinding). This onsite re-examination provides an opportunity of onsite feedback and skill enhancement of laboratory staff. Record the reexamination results in section-6 of EQA Form-1 in column; Reexamination Result, and takes this back to the Laboratory In-charge.
- The Laboratory In-charge (Medical Officer in RHC) checks the results of the cross-examined slides from the Laboratory register, and records the results in section-6 of EQA Form-1 in column; Diagnostic Centre Result.
- The Laboratory In-charge and DLS jointly fill the 'Errors' column of Section-6.

EQA Form-1
Sectio-6: Reexamination by DLS

S. No	Lab. Serial No.	Reexamine Result	Diagnostic Center Result	Comparison						Remarks
				AG	HFP	LFP	HFN	LFN	QE	
1.										
2.										
3.										
4.										
5.										
6.										
7.										
8.										
9.										
10.										
Summary results										

Notes on Section-6 columns:

- *Lab. Serial Number* as recorded on each selected slide. This is filled by DLS, when he receives the sample slides for reexamination.
- *Reexamine result* refers to the smear results of each selected slide, as reexamined by the first controller (DLS). This column is filled by DLS as he reexamines each of the sample slides.
- *Diagnostic center result* refers to the smear result of each selected slide, as recorded in TB04 of the diagnostic center. The Laboratory In-charge fills this column once he receives the EQA-1 Form with DLS reexamine results.
- *The* results recorded in the two result columns are compared and accordingly recorded in the comparison part of the table. The DLS compares the results and records by putting a "tick " mark in the relevant column.
    - Agreement: refers to that smear positive or smear negative results (given by the diagnostic center), which were found concordant (same) on subsequent cross-examination by the first controller (DLS). In case of smear-positive results, even the grading difference is considered to be the agreed results (i.e. recorded under result agreement).
    - *False positive:* refers to that smear positive results (given by the diagnostic center), which were found smear negative on subsequent cross-examination by the first controller (DLS). More false positive means over-diagnosis of smear-positive cases of TB. False positives can either be high or low false positive.
        a. *High False Positive (HFP): refers to a negative smear misread as 1+ to 3+ (based on IUATLD/ WHO recommended grading of sputum smear microscopy results). This is a major error.*
        b. *Low False Positive (LFP): refers to negative smear that is misread as a scanty (1-9 AFB/100 fields) positive. It is a minor error.*
    - *False negative:* refers to that smear negative results (given by the diagnostic center), which were found smear positive on subsequent cross-examination by the first controller (DLS). More false negative means under-diagnosis of smear-positive cases of TB. False negatives can either be high or low false negatives.
        a. *High False Negative (HFN):* refers to 1+ to 3+ positive smear that is misread as negative. This is a major error.
        b. *Low False Negative (LFN):* refers to scanty (1-9 AFB / 100 fields) positive smear that is misread as negative. It is a minor error.
    - *Quantification Error (QE* : Write down the *number of results with Quantification Error (QE) i.e.* grading difference (more than 2 degrees) but considered as agreed. (I.e. recorded under result agreement). This data could be extracted from EQA Form-1 Sectio-6: by comparing columns 'Reexamine Result' and 'Diagnostic Center Result'.
- *Summary results:* Gives the count of "tick marks" in each column.
- *Remarks:* DLS records the reasons for difference in results and agreed actions

- Discuss the false positive and false negative results with the laboratory staff and take measures to address the gaps in his/her knowledge, skills and practices. Use the following guidelines for discussion and corrective actions.

Sr. No.	Pattern of errors	Possible causes	Suggested investigation steps
1	HFP and HFN	Unusable microscope	Examine a 3+ using that microscope
		Staining problems, poor stains, insufficient staining time or heating	Check stains and staining procedure
		Technician cannot recognize AFB	Test with clear-cut positive & negative slides and good microscope
		Gross neglect, overworked, lack motivation	Exclude other causes
2	HFP	Administrative error	Compare lab-register and verify correct slide number and result? Exclude causes of more frequent HFP, such as low concentration of sulfuric acid, unusable microscope, untrained or inexperienced lab. staff.
		Poor registration routine	Check accuracy of lab-register and other record keeping
		Staining problems/Fading	Check stains and staining procedure, consider re-staining for rechecking. Assess concentration of Basic Fuchsin and Methylene blue.
		Technician unclear on AFB appearance	Look for inconsistent results of suspects (regularly single pos / low positive) in lab register
3	Many LFP, with or without occasional HFP	Problem with controllers Technician unclear on AFB appearance Contaminated stain/ reagents	Evaluate controllers Recheck sample of LFP from laboratory register Test stain with known negative smears, check the distilled water used for stain preparation
4	HFN	Administrative error	Compare lab-register with QC-listing: correct slide number & result?
		Very thick smears and/or poor light	Evaluate quality of smear preparation, check microscope
		Gross neglect	Exclude other causes
		Staining problems	Check stains and staining procedure, consider re-staining for rechecking. Assess concentration of Basic Fuchsin and Methylene blue
		Poor smearing-technique	Test stain with known negative smears
		Problems with microscope	Test stain with known negative smears
		Careless microscopy	Exclude other causes
5	Very high proportion LFN	Reading error	As above
		Concentrated Methylene blue	
6	Many QE	Poor staining	
		Problems with microscope	

E.5 Review with Laboratory Incharge

- Review your report with Laboratory Incharge (Medical Officer in case of RHC) and fill the EQA Form-1, Section-7

EQA Form-1
Section-7: Review with Laboratory Incharge

Agreed actions/comments	
Implementation of previous agreed actions	
Signature:_____ DLS	Signature: _____ M.O/Date

Section-F	COMMUNICATION WITH PRL

E.6 Communication with PRL

E.6.1 Collect all the reexamined slides and bring it back to your station, along with the filled EQA Form-1. Keep all the concordant slides for rechecking by the provincial reference laboratory staff during their quarterly visit to the district. Keep slides from each diagnostic center in a separate storage box, if possible. These slides are kept till the PRL staff asks the DLS to dispose.

E.6.2 Fill up EQA Form-2 and send with the discordant slides to PRL (i.e. false positive and false negative slides), along with a filled EQA Form-2, every month to the Provincial Reference Laboratory (initially through Coordinators). The section 1 of the EQA Form-2 gives the overall summary for EQA work in the district, whereas section 2 gives specific details of the false positive and false negative slides being sent to PRL for reexamination by the second controller at provincial reference laboratory.

E.6.3 Fill up 'Lab Performance Data' form on quarterly basis and submit it to PRL given on page 42.

For filling the Lab Performance Data the period under review would be the previous completed quarter. Fill-up the data using following guidelines.

Name of diagnostic Center:
Write the name and type (DHQ, THQ, and RHC) of the diagnostic center in this column.

Number of Patients Examined:

These three statistic are taken from the TB04 column "Reason for examination"

- Column – Total: Fill the next two columns first. Then add the figures in the next two columns to get the total number of patients examined (see below).
- Column – Suspect: Count the number of suspects examined for diagnosis (from TB04), and record the number in this column.
- Column – Follow-up: Count the number of patients examined for follow-up (from TB04), and record the number in this column.

Number of suspects with the following smears examined:

From TB04: Identify all the suspects examined (i.e. for diagnosis) during the period as recorded in "Reasons for examination". For each of the suspect examined count the number of smears examined as recorded in "Results of specimens". According to the number of smears examined, each suspect is recorded in one of the three cells by putting a tally mark. Patients from each page of the TB04 are recorded in a separate row of the table below.

Page (TB04)	Number of smears examined		
	One smear	Two smears	Three smears

Total number of tally marks in column “one smear” of the above table are counted and recorded in the column labeled “one” in the laboratory performance data sheet.

Total number of tally marks in column “two smears” of the above table are counted and recorded in the column labeled “two” in the laboratory performance data sheet.

Total number of tally marks in column “three smears” of the above table are counted and recorded in the column labeled “three” in the laboratory performance data sheet.

Total number of Diagnostic smears examined:

- Multiply the number entered in column ‘One’ with 1
- Multiply the number entered in column ‘Two’ with 2
- Multiply the number entered in column ‘Three’ with 3
- Add up three statistics and enter the total number in this column

Number of suspect with following +ve Smear:

From TB04: Identify all the suspects examined (i.e. for diagnosis) during the period as recorded in “Reasons for examination”. For each of the suspect examined count the number of smears found positive and recorded in “Results of specimens”. According to the number of smears found positive, each suspect is recorded in one of the three cells by putting a tally mark. Patients from each page of the TB04 are recorded in a separate row of the table below.

Page (TB04)	Number of suspects found with		
	One +smear	Two + smears	Three + smears

Total number of tally marks in column “one +smear” of the above table are counted and recorded in the column labeled “one” in the laboratory performance data sheet.

Total number of tally marks in column “two + smears” of the above table are counted and recorded in the column labeled “two” in the laboratory performance data sheet.

Total number of tally marks in column “three + smears” of the above table are counted and recorded in the column labeled “three” in the laboratory performance data sheet.

Total # of +ve diagnostic smear:

- Multiply the number entered in column ‘One’ with 1
- Multiply the number entered in column ‘Two’ with 2
- Multiply the number entered in column ‘Three’ with 3
- Add up three calculations and enter in this column

Follow-up Examination

Take the number entered in column ‘Number of patients examined’ sub column ‘Follow-up and enter in ‘Total Smear’.
Count the follow-up cases with +ve smears from TB04 column ‘Results of specimens’ and enter the count in column ‘+ve smear’

Total # of +ve Suspects:

Add up the entries in sub columns ‘One, Two, Three’ of columns ‘# of suspects with following positive smears’ and enter the added number in this column.

Smear Positivity Rate (%):

Divide the figure entered in column ‘Total # of positive diagnostic smears’ with figures in column ‘Total # of Diagnostic smears examined’ and multiply with 100. Enter this calculated result in this column.

Smear examined / Suspect:

Divide the figure entered in column ‘Total # of Diagnostic smears examined’ with figures in column ‘Number of Patients Examined’ sub column ‘Suspects’ and enter this calculated result in this column.

E.6.4 Fill up QA Entry Form on quarterly basis and submit it to PRL given on page 48.

Date:

Write down the date of preparing the report.

Functioning Diagnostic Centers

Enter the number of functioning diagnostic centers in the concerned district. The centers without a trained person or non availability of Lab. supplies would be considered non functional.

# of Diagnostic Centers checked:

Enter the total number of diagnostic center checked by DLS during his routine monthly visit. This information could be found from the filled EQA form-1.

# of Diagnostic Centers with major errors:

Enter the number of diagnostic centers with major errors found i.e. (HFP, HFN) in the district.

Laboratory:

In this column write down the name of the diagnostic centers checked in the district functional or non functional.

ANSV (Annual Smear Volume):

This statistic shows cumulative number of smears examined during the current year. Take the reading from sub column 'Statistic" of EQA Form-1Section-3: Recording & Reporting and record in this column.

Smear Positivity Rate (SPR):

Record Smear Positivity Rate (SPR) in QA Entry Form, column 'SPR'. This information can be extracted from EQA Form-1 Section-3, column 'Statistic'. Alternatively these figures can also be noted from Lab. Performance Data Sheet.

# of Slides Collected:

Column 'Pos' (Positive):
In this column write down the total number of positive slides collected in previous quarter. This information could be extracted from **the three relevant monthly** EQA Form-1 Section-6: Reexamination by DLS (Column: Diagnostic center results).

Column 'Scn, (Scanty):
In this column write down the total number of scanty positive slides collected in previous quarter. This information could be extracted from **the three relevant monthly** EQA Form-1 Section-6: Reexamination by DLS (Column: Diagnostic center results).

Column 'Neg' (Negative):
In this column write down the total number of Negative slides collected in previous quarter. This information could be extracted from **the three relevant monthly** EQA Form-1 Section-6: Reexamination by DLS (Column: Diagnostic center results).

Column-Total':
Add up the entries in sub columns 'Pos, Scn, and Neg 'of columns '#of slides collected' of QA entry form and enter the added number in this column.

Slides correct:

Write down the number of *correct* slides which refer to those smear positive or smear negative results (given by the diagnostic center), which were found concordant (up to 2 degrees) on subsequent cross-examination by the first controller (DLS). Get this information from; the three relevant monthly EQA Form-1, Section-6, column comparison, sub-column "AG"

Errors:

- Column –HFN**:** Write down the number of *High False Negative (HFN)* smear results. It refers to 1+ to 3+ positive smears that were misread as negative. This data could be extracted from EQA Form-1 Section-6: column comparison, sub-column "HFN"
- Column – HFP: Write down the *number of High False Positive (HFP)* smear results. It refers to a negative smears misread as 1+ to 3+. This data could be extracted from the three relevant monthly EQA Form-1 Sectio-6: column comparison, sub-column "HFP"

- Column – LFN: Write down the *number of Low False Negative (LFN)* smear result. It refers to scanty (1-9 AFB / 100 fields) positive smear that is misread as negative. This data could be extracted from the three relevant monthly EQA Form-1 Section-6: column comparison, sub-column "LFN"
- Column – LFP**:** Write down the *number of Low False Positive (LFP) smear result. It refers to negative smear that is misread as a scanty (1-9 AFB/100 fields) positive.* This data could be extracted from the three relevant monthly EQA Form-1 Section-6: column comparison, sub-column "LFP"
- Column – QE: Write down the *number of results with Quantification Error (QE) i.e.* grading difference (more than 2 degrees) but considered as agreed. (I.e. recorded under result agreement). This data could be extracted from the three relevant monthly EQA Form-1 Section-6 sub column 'QE".
- Column –Total:
  Add entries of the columns HFN, HFP, LFN, LFP, QE and write down their sum in this column.

The following indicators will automatically be calculated on data entry at PRL level.

Column - Agg %(Agreement %)

Mj E (Major Error):

Mn E (Minor Error)

FPR% (False Positive Rate %)

FNR% (False Negative Rate %)

Sen % (Sensitivity %)

Spc % (Specificity %)

F. GENERAL GUIDANCE

The corrective actions depend on the reasons for deviation in practices.

- *Problem with staff knowledge, skills or attitude* – onsite guidance, refresher training or retraining (depending on nature and level of gap in knowledge/skills of staff)
- *Problem with quality of laboratory reagents* – replace with quality reagents.
- *Problem with laboratory equipment and supplies* – arrange the laboratory supply found missing or inadequate, and get the non-functioning microscope repaired or replaced (example is microscope with less than 100X lens or uni-ocular).
- *Feedback* – The PRL will return a copy of the EQA Form-2 with comments to the DLS and the DTC. The DLS will act in light of those comments. Any further discussions can be done in cluster meetings.

G. SUMMARY ACTIVITIES OF THE PROVINCIAL REFERENCE LABORATORY

The role and operations for the provincial reference laboratories to manage and support the EQA work in their respective provinces have been documented in a separate document/ guidelines. The summary below is just to provide the district staff an outline of the PRL role in managing EQA activities in districts.

- DLS Training and Supervision
    1. Provincial Reference Laboratory trains each selected DLS, for 5 days, on core set of EQA activities and operations at district level. The Provincial Reference Laboratory uses a standard set of tailor-made training materials.
    2. A senior staff from the Provincial Laboratory visits each district, on quarterly basis, to supervise and support the DLS working. During his visit to EQA Center, the PRL staff reviews mainly the: a) stain preparation/ storage and quality control practices, b) EQA related records, c) storage of slides reexamined by DLS, d) cross-check a sample of concordant slides and discuss the disagreements, including quantification errors, with DLS.
- Second Controller for DLS
    1. The provincial reference laboratory receives the discordant slides, on monthly basis, along with EQA Form-2 from each district.
    2. A second controller at provincial reference laboratory will perform rechecking of all discordant slides received from a district. PRL will re-stain slides, where needed. The results of the provincial reference laboratory are recorded in the last two columns of EQA-2 form received from each district and will be considered as final. The provincial reference laboratory informs each DLS about results of reexamination (by second controller).
    3. If agreement rate for a district is more than 95%, PRL staff becomes more watchful about the DLS observation (i.e. both discordant and concordant results).
- Monitoring and Continued Capacity Building
    1. Quarterly review of the overall performance of EQA work in each district. This may be done in a provincial level event (Laboratory Day), where each DLS presents/ discuss his district situation/ issues and PTP/PRL staff and peer DLS contribute by suggesting measures for improved EQA performance.

APPENDICES

Appendix-1

Trouble Shooting-Laboratory Staff

Reasons	Analyze	Further requirements
Technician transferred to another place or left the job	• Action already taken by the RHC MO Incharge and replacement is expected shortly.	• Follow-up the matter with the Incharge RHC
	• No action taken by the RHC Incharge and no replacement provided so far.	• This is a serious situation and requires urgent attention. Report the matter to the district RBM focal person for immediate replacement or to make temporary arrangement by providing alternate from the district office till availability of permanent Lab. staff.
	• Alternate already provided	• Check if the alternate is trained and has desired level of skills. If not arrange for the training.
Lab. Technician on leave	• Trained alternate staff provided	• No further action
	• No action taken by the RHC Incharge so far and no alternate provided so far	• Bring this issue into the notice of RBM focal person for arranging an alternate on priority. • If Focal person is unable to arrange an alternate the matter should be brought into the notice of PMCP through district coordinator
Laboratory Technician too busy with other laboratory tests e.g. Malaria slides and could not give time to TB slide examination	• Bring the matter into the notice of RHC Incharge to solve the problem and follow. • If the problem still exists	• Report the matter to the district RBM Focal Person to solve the problem.

MICROSCOPE

The microscope area should be:

- Free from dust
- On a steady level platform
- Away from centrifuges and refrigerators
- Away from water, sinks or chemical to avoid splashes or spills
- Ergonomically correct work position

Parts of microscope

1. Binocular eye pieces.
2. Diopter ring adjustment.
3. Tube/ body
4. Nose piece.
5. Lenses/ objectives.
6. Stage.
7. Condenser diaphragm.
8. Light source
9. Stage movement screw.
10. Coarse focus.
11. Fine focus.
12. Voltage regulator.
13. Power on/ off

Setting up the microscope

1. Set the variable voltage regulator to minimum
2. Turn the power on
3. Slowly adjust until the desired light intensity is reached
4. Place a stained slide onto the stage
5. Rotate the nose-piece to the 10x objective
6. Bring the smear into focus with the coarse adjustment knobs

Always use the focusing adjustment knob to lower the stage away from the lens

7. Adjust the inter-pupillary distance until the right and left images merge
8. Focus the image with the right eye by looking into the right eye-piece and turning the dioptre ring

9. Focus the image with the left eye by looking into the left eye piece and turning the dioptre ring
10. Open the condenser iris diaphragm so that the field is evenly lit
11. Place one drop of immersion oil onto the smear and rotate the 100x objective into place
12. Focus using the fine adjustment knob
13. Use the variable voltage regulator to achieve a comfortable illumination
14. Once that smear has been read, rotate the 100x objective away, locate the 10x objective over the slide, and then remove the slide
15. When finished, reset the voltage regulator to a minimum, and turn the power off
16. At the end of each day, use lens paper, muslin cloth, or fine tissue paper to carefully remove immersion oil from the 100x lens, cover the microscope, or put it in the microscope box and return to the humidity controlled cupboard

Do's and Don'ts

- The 100x objective is the only lens requiring immersion oil
- Keep immersion oil away from other lenses
- Immersion oil must have medium viscosity and a refractive index (RI) greater than 1.5. Any synthetic, non-drying oil with an RI>1.5 is suitable (refer to manufacturer's instructions).
- Do not use cedar wood oil as it leaves a sticky residue on the lens
- Do not use liquid paraffin because it has a low refractive index resulting in an inferior image

MAINTENANCE

➢ Cleaning lenses

- Use a minimum amount of cleaning fluid (xylene for oil objective and alcohol for all other parts), never a dip a lens into cleaning fluid
- Lens paper is best for cleaning optical surfaces as it does not scratch the lens
- Alternatives are muslin cloth, silk, or fine quality toilet paper
- Do not use ordinary paper or cotton wool to clean lenses
- Keep the microscope covered when not in use
- Keep the eye-piece in place
- Fungus or dust may enter through holes where objectives in the nose-piece are missing

- If the image appears hazy with black dots, check for dust or dirt on the lenses (eye-pieces, objectives, condenser and illuminator lens). If:
    - The black dot moves when the eye-piece is rotated, then the dust is on the eye-piece
    - The black dot moves when the slide is move, then it is on the slide
    - These two are ruled out, then assume the dust is on the objective (if inside the objective, it appears as dots: if on the outside, then as a hazy image)
- Dust can be removed using a camel-hair/artist brush or by blowing over the lens with an air brush
- Some cleaning agents will damage lenses over time

➢ **Light source**

- Never touch the glass bulb surface as skin oils will burn, reducing light intensity
- Use lens paper to hold the bulb when inserting into the microscope

➢ **Mechanical parts**

- Never disassemble the microscope
- Stiffness of movement may be due to an accumulation of dust in the sliding channel, or in the rack and pinion
- Remove the dust with an air brush to artist rush, clean with a solvent such as petrol, then polish and apply high-quality silicone grease to lubricate the moving parts
- Stiffness may be due to bending of some part. The up and down movement of the mechanical stage will loosen over time. Both problems need to be assessed by a service engineer

➢ **Fungal growth**

- Fungus grows on the lenses, the eye-piece tube and prisms causing the microscope image to become hazy and unclear
- To check for fungus turn the microscope on:
    - Rotate the 10x objective into the light path
    - Take out both eyepiece, look down the eyepiece tubes for fungus

- To prevent fungal growth, the microscope should be kept in a warm cupboard
    - A cupboard with a tightly fitting door, heated by a light globe (maximum 40W), located at the top of the cupboard near to the microscope head
    - Always leave the cupboard light on, even when the microscope is not in the cupboard
    - Check the temperature inside the cupboard is at least 5^{o} C warmer than room temperature
    - Microscope must be kept in the cupboard even if the laboratory is air-conditioned
- Where power is unreliable, store the microscope in its (or tight fitting cover) with silica gel, salt, or rice (~100 grams) placed in an open on the microscope stage
    - Replace the salt when it begins to look wet
    - Replace the rice when it is no longer dry and crisp
    - Silica gel changes color from blue to pink when it is unable to absorb any more moisture
    - Dry gel by placing in a hot air oven or heating in a saucepan until the blue color reappears
- If proper storage not available, keep the microscope in the shade and with good air circulation

TROUBLE-SHOOTING MICROSCOPY

Problem	Cause	Remedy
Light flickers or does not turn on	• Loose plug or connection. • Loose light bulb. • Dirty bulb. • Erratic voltage supply. • Faulty on-off switch. • Fuse blown or transformer blown. • Discolored bulb/burn out.	• Check wall sockets, transformer, power supply. • Reinstall the bulb – do not touch bulb with fingers • Replace bulb • Use a voltage stabilizer • Replace the switch • Replace the fuse • Replace the bulb – Do not touch bulb with fingers.
Uneven Illumination	• Field of view partially blocked. • Iris diaphragm is almost closed or condenser is not aligned • Dirty lenses • Heavy fungal growth on lenses	• Rotate the nose-piece until it click into position • Recalibrate microscope • Gently wipe the lenses with lens paper/soft cloth. If the trouble persists clean with lens paper soaked in the recommended lens cleaning fluid
Excessive image contrast	• Iris diaphragm is almost closed	• Open diaphragm
Unclear image with glare	• Iris diaphragm too far open	• Close the iris diaphragm to make the opening smaller
Specimen focused at 10x but not at higher magnification	• Slide upside down	• Turn it over
Specimen goes out of focus more than usual at high magnification	• Slide is not flat on the stage	• Clean the stage and underside of slide
Mechanical stage cannot be raised	• Lock set too low	• Adjust to proper height and lock
Mechanical stage is loose or stiff	• Poor tension adjustment on the mechanical stage • Solidified lubricants	• Adjust tension with tension adjustment device • Microscope requires service
Oil immersion objective does not give a clear image	• Is oil being used? • Light source collector lens dirty • Poor quality immersion oil (low refractive index) • Surface of the lens is dirty • Water on slide • Bubbles in immersion oil • Oil inside lens	• Apply immersion oil • Clean using lens paper and cleaning fluid • Use quality immersion oil(as described in microscope details) • Clean lens with lens paper • Air dry slides • Remove oil from slide and carefully reapply oil • Clean or replace lens
Dust/dirt visible in the field of view	• Dust on the collector lens of the light source • Dust on the top-most lens of the condenser • Dust on the eye-piece	• Clean all surfaces • Clean all surfaces • Clean all surfaces
Cracked objective lens	• Lens has been dropped • Lens forced into slide or stage	• Replace lens • Replace lens
Regular or semi regular crescent shapes that may be confused for AFBs	• The glass slide is scratched	• Learn to recognize glass artifacts
Headaches/incomplete binocular vision	• Eye-pieces are not matched • Improper adjustment of inter-pupillary distance • Dioptre adjustment was not done	• Use matched eye-pieces • Adjust the inter-pupillary distance • Adjust dioptre settings
Fuse blows frequently	• Fuse incorrectly rate. • Unstable line voltage	• Replace with correctly rated fuse • Use voltage protection device

NTP
External Quality Assurance (EQA) Form-1

Name of Health Facility _____ Date of Visit _____

Section-1: Laboratory Functioning

No. of days Lab. Remained non functional	Reasons	Actions already taken	Actions required/agreed

Section-2: Laboratory Input Replenishment

Item	Minimal Stock Level	Stock available	Stock replenishment (Quantity)		Comments/Actions to be taken
			Supplied	To be arranged	
25% H_2SO_4	2 bottle				
1% Carbol fuchsin	2 bottle				
0.3% Methylene blue	2 bottle				
Immersion Oil	1 bottle				
Xylene or Toluene	1 bottle				
Methylated Spirit	1bottle				
Glass Slides	5 Packets				
Sputum Containers	200				
TB04	1				
Slide Storing Boxes	2 (100 slide capacity each)				
Wire Loop	1				
Diamond Pen	1				
Functioning Microscope	1				
Trained Lab. staff (1)					

Section-3: Recording & Reporting

Characteristic	Formula	Statistic	Comments
Specimens examined per TB suspect.	Total slides examined / Total suspects		
Proportion of suspects found smear positive on AFB testing. (Suspect positivity rate)	# SS^+ / # suspects examined		
Proportion of total slides found positive (Smear positivity rate)	# positive smears / Total smears examined		
Annual Slide Volume (ANSV)	Total diagnostic slides + Total follow-up slides		
TB04 (Lab. Register) Recording	Complete/Correct		

Section-4: Microscope maintenance, Slide storage and Waste disposal

Characteristic	Acceptable		Action/Comments
	Yes	No	
Microscope cleanliness			
Microscope functioning			
Slide Storage			
Disposal of used slides			
Disposal of used sputum containers			

Section-5: Smear Assessment of selected slides

Lab. Serial No.	Specimen Quality		Size		Thickness		Evenness		Labeling		Slide Staining		Smear cleanness	
	1		2		3		4		5		6		7	
	A*	NA**	A	NA	A	NA	A	NA	A	NA	A	NA	A	NA
Total														
Remarks***														

Key:
*A - Acceptable, **NA - Not Acceptable, *** Remarks – Gives overall performance of the characteristic under consideration. If for any characteristic, three or more slides are found not acceptable (NA) then overall performance on that characteristic is labeled as Not Acceptable (NA).

Section-6: Reexamination by DLS*

S. No	Lab. Serial No.	Reexamine Result	Diagnostic Center Result	AG (# Slides Correct)	Errors					Remarks
					HFP	LFP	HFN	LFN	QE	
11.										
12.										
13.										
14.										
15.										
16.										
17.										
18.										
19.										
20.										
Summary results										

* Write grading of positive slides

Section-7: Review with Medical Officer

Agreed actions/comments	
Implementation of previous agreed actions	
Signature:_____ **DLS**	**Signature: _____** **MO/Date**

External Quality Assurance (EQA) Form-2 (Page 1)

Name of District: _____ Month of Review: _____

Number of diagnostic centers in a district: _____

Name of DLS: _____

1. Summary EQA Activity – Smear Assessment

Name of Diagnostic Centers	# Slides reexamined	Number of Slides with Acceptable Smear:					
		Quality	Size	Thickness	Evenness	Staining	Cleanness
Total							
%age							

2. Comments by the PRL

External Quality Assurance (EQA) Form-2 (Page 2)

3. Discordant Slides

S. No	Name of Facility	Lab. Serial No.	Cross-exam Results	Health Facility Results	Reference Lab. Result	Error of: DC staff/DLS
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
9.						
10.						
11.						
12.						
13.						
14.						
15.						

Signature (DLS):_____	**Signature (DTC):** _____

3. Feedback to the District

Signature: _____

Manager PRL

Lab Performance Data

				Diagnostic Examination (TB Suspects)								Follow up Examination				
Name of diagnostic centers	Number of patients Examined			# of suspects with following smear examination			Total # of Diagnostic Smear examined	# of suspect with following +ve smear			Total # of +ve Diagnostic Smear			Total # of +ve suspect	Smear positivity Rate %	Smear exam / suspect
	Total	Suspect	Follow up	One	Two	Three		One	Two	Three		Total Smear	+ve smear			

QA Entry Form

Date							# Functioning Diagnostic Centers													
# Diagnostic centers checked							# Diagnostic Centers with Major Error													
Laboratory	ANSV	SPR	# Slides Collected				# slides correct	Errors						Agg. %	Mj E	Mn E	FPR %	FNR %	Sen %	Spc %
			Pos	Scanty	Neg	Total		HFN	HFP	LFN	LFP	QE	Total							