Supervision and External Quality Assurance of Malaria Laboratory Services

OPERATIONAL GUIDELINES FOR DLS

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TABLE OF CONTENTS (To be updated)

PREFACE

The current strategy for malaria control (RBM) relies on early diagnosis through a network of malaria microscopy laboratories. Laboratory diagnosis by microscopical examination of stained blood smears continues to be the method of choice—the gold standard—for confirming a clinical diagnosis of malaria and epidemiological studies (WHO, 2000a, 2004a). While microscopy still remains the mainstay of parasite-based diagnosis in most malaria endemic countries, it is well evident that the strengthened laboratory services are required to meet the needs of malaria control. Microscopy errors are likely to result in failure to detect persons with malaria who will then continue to spread infection in the community, or unnecessary treatment for "non-malaria cases."

The strengthening of malaria microscopy through a network of health facilities in a district implies better-trained staff, enhanced material support, and a system for assuring quality of malaria microscopy services. World Health Organization (WHO) also recognizes the need to address the quality issue by developing country-adapted guidelines and tools for implementing External Quality Assurance for malaria microscopy through a network of health facilities.

National Malaria Control Programme has recommended microscopy-based diagnosis of malaria cases, so effective quality assurance arrangements are required on priority basis. Broader technical resources for performing quality assurance of malaria microscopy are available, including those developed by the WHO1. These technical guidelines are useful reference material for method selection, implementation, interpretation, and issues encountered in an EQA Programme. However, each country is expected to develop context-adapted operational guidelines for implementing these recommended technical procedures.

National Malaria Control Programme has recommended a District-level Laboratory Supervisor (DLS) to plan and carryout EQA at microscopy centers in his respective district. These operational guidelines provide step-by-step instructions and tools for planning; conducting and documenting the EQA related tasks in a district.

These guidelines have been developed through extensive consultation with international experts, national and provincial programme, and districts. The main emphasis has been on suggesting operations considered technically appropriate and logistically feasible in routine programme conditions.

The method proposed here is based on the Lot Quality Assurance System (LAQS). This method of selecting slides for re-examination gives statistically more valid results. The slides are selected irrespective of the initial microscopy result. The total number of slides requiring reexamination has been determined on the basis of estimated annual slide load, slide positivity rate and acceptable level of sensitivity and specificity.

Early implementation and evaluation of the guidelines in four selected districts would enable the programme and its partners to further modify the operations and tools before scaling up in other parts of the country.

SECTION - A OVER VIEW OF EXTERNAL QUALITY ASSURANCE IN MALARIA CONTROL

A.1. Microscopy in Malaria Control

Early diagnosis and prompt and effective treatment, is the key element of the RBM strategy package (WHO, 1993a, 2000, 2004a). Failure to detect persons with malaria can lead to continued spread of infection in the community. Laboratory diagnosis by microscopical examination of stained blood smears continues to be the method of choice—the gold standard—for confirming the diagnosis of malaria (WHO, 2000a, 2004a). Evidence on the effectiveness and feasibility of rapid diagnostic tests, as the prime malaria diagnostic test in different operational situations, is building but still not conclusive (WHO, 2000b, 2003a, 2005).

In light of rapidly increasing resistance to the chloroquine, the use of artemisinin-based combination therapy (ACT) is being encouraged. The rationale use of ACT requires limiting its use to the confirmed falciparum malaria cases. In Pakistan, a network of microscopy centers, with trained staff and equipment, is already in-place. With minimal additional inputs, the microscopy can be used as an effective instrument for confirming malaria cases through a network of about 1200 rural health centers and hospitals.

A.2. Quality of Malaria Microscopy

The quality of microscopy services can only be assured through staff commitment at facility, district, provincial and national levels. At facility level a trained laboratory staff need 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) provides the required support to the facility laboratory staff. The district staff enabling inputs may include 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 staff, whereas the material inputs come mainly from the malaria control programme and the district health offices.

A.3. Quality Assurance in Laboratory Services

National Malaria Control Programme (NMCP) recommends that laboratories at all levels be supported 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 malaria microscopy is an essential requirement for the quality of malaria 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 diagnosis**.** 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 the microscopy center is to improve the quality and the outcome of malaria case management and control. 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. preparation, examination, storage and disposal, and recording and reporting of smear 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

An enabled District Laboratory Supervisor (DLS) visits all malaria microscopy centers each quarter (i.e. about one third of the total number of microscopy centers to be visited each month). During facility visit, the DLS performs EQA mainly through the onsite evaluation and blinded rechecking of a sample of malaria slides. The assessment is followed by onsite interaction between the facility staff and the DLS for better understanding and action planning.

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; 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 facility visit, would recheck a random sample of slides prepared and examined at the microscopy center during the quarter under review. The method for selecting, rechecking, recording and cross validating the results is described in the section- E of the guidelines.

A.4. Roles in malaria microscopy EQA

Described below are the roles of key actors in the malaria microscopy EQA process in a district:

A.4.1 District Laboratory Supervisor (DLS)

- Planning, conducting, documenting and reporting supervisory visits to each microscopy center in the district. 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 malaria microscopy centers to carry out the agreed role/ responsibility. The subsequent chapters describe the operational details for planning, carrying out, documenting and following-up the EQA activities in a district.

A.4.2. Laboratory Staff

- Maintaining the laboratory register, equipment and storing all slides for re-checking.
- Sharing the technical and logistic issues constraining malaria microscopy work.
- Complying with the instructions of the District Laboratory Supervisor and M.O In-charge for improved quality of malaria microscopy.

A.4.3. District RBM Focal Person

- 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 or after the facility visit. This also includes his review and comments on DLS performance during the month.
- Facilitating communication with provincial reference laboratory including:
- Ensure the discordant slides are sent and feedback is received in-time;
- PRL staff quarterly monitoring visit to the district (where feasible for the programme);

A.4.4 M.O In-charge Role

- Selecting a sample of slides, as per programme guidelines, for reexamination by the DLS.
- Entering the facility results from Lab. Register into EQA Form, and comparing these with the DLS reexamination results.
- Discussing the EQA findings, and facilitating the process to address the gaps in laboratory arrangements and practices.
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A.4.5 Provincial Reference Laboratory Role (where strengthened and supported through programme)

- Designing and conducting the training of District Laboratory Supervisors (DLS) from districts in the province.
- Reexamining the discordant slides received 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 refresher/ meeting at PRL.
- Planning and conducting quarterly supervisory visits to the participating districts in the province (where supported through programme).
- Communicating with the National and Provincial Malaria Control Programmes.

SECTION- B Estimation, Reagent Preparation & Distribution

The District Health Office will arrange laboratory supplies and materials through provincial malaria control programme (PMCP) inputs as well as their own sources. The provision of these materials and supplies for malaria microscopy would be ensured through a network of malaria microscopy centres.

B.1. Estimate the required amount of supplies and reagents

The estimates are based on the assumption that for every suspected malaria case single slide will be examined for thick and thin smears. The requirement of laboratory supplies and reagents (in a district or at a facility) for the next six months (i.e. 3 month supply and 3 months reserve stock) is provisionally estimated as follows:

Arrange and maintain stock of laboratory supplies and reagents required for malaria microscopy centers in the district

Laboratory Item	Microscopy Center Requirement (A)	District Requirement (A x No. of Microscopy centers in a district)
General Supplies		
Functioning Microscope	1	
Glass slides (72 slide packs)	6 packs	
Lens cleaning tissues	1 packet	
Disposable lancets/ prickers	450	
Disinfectant swabs	1 packet	
Disposable gloves	1 box	
Slide storage boxes (Capacity: 100 slides)	2	
Drop bottle (60 ml – glass or polyethylene)	1	
Tally Counter (4 digit)	1	
Timing clock (hand operated)	1	
Staining troughs (for 20 slides)	1	
Xylene glass Jar	1	
Slide drying rack	1	
Pipette 5ml.	1	
Measuring cylinder 100 ml.	1	
Hair dryer	1	
Laboratory register	1	
Laboratory desk-guide and module	1	

Reagents/Chemicals		
Methyl alcohol (anhydrous, acetone free)	1 bottle (2.5 Litres)	
Xylene	1 bottle (2.5 Litres)	
Giemsa stain (ready made)	1 bottle (2.5 litres)	
Immersion oil	1 bottle (500 ml)	
If 3% Giemsa stain is being prepared at facility level, only then		
At facility level		
Giemsa stock solution for preparing 3% stain (in buffer water)	100 ml	
Buffer solution (prepared by DLS)	02 liters	
pH paper	01 pack	
At district level		
Giemsa stock solution	2500 ml	
Distilled water	50 litres	
Buffer tablets (for preparing buffer water - pH 7.2).	20 tablets	
Correcting Fluid (by PRL) 2% disodium hydrogen phosphate 2% Potassium di-hydrogen phosphate	1 Litre of each solution	
pH paper/ pH Meter	01	

Store the reagents, preferably in amber bottles, at the district health office store.

The required quantity of laboratory reagents and supplies, at district and microscopy center level, is estimated as per table above. The District Malaria Focal Person, with assistance of District Laboratory Supervisor, does the estimation and arranges the required supplies accordingly (mainly through provincial programme support).

B.2. Prepare the Reagents

The program recommends the use of products (i.e. reagents) that involve minimal handling at the district and facility levels. The program encourages the use of ready made Giemsa stain, where possible, to minimize the variation in quality related with preparation methods. However, the following guidelines are for situations where Giemsa stain is to be prepared by malaria program staff.

Arrangements for Giemsa stain at microscopy centers

The provincial reference laboratory staff will prepare the Giemsa stock solution and correcting fluids (for adjusting pH of buffered water) and distribute these to the districts.
The District Laboratory Supervisor will prepare the buffered water (using buffer tablets and distilled water) at respective EQA center. The DLS will then distribute Giemsa stock solution (received from programme) and the buffered water (prepared at district) to each microscopy

centre in 100 ml. amber bottles. The laboratory staff at microscopy centre will prepare 3% Giemsa stain from the stock solution and buffered water.

The method for preparing buffer water, correcting fluids for adjusting pH and 3% Giemsa stain is as follows:

1 Prepare Buffer Water

- Take a liter of distilled water, and put one buffer tablet to make one liter of buffer solution.
- Check pH, using pH paper/pH Meter.
- Adjust pH, if needed, as follows:
  - If pH is below 7.2 (i.e. acidic): Add drop-by-drop 2% di-sodium hydrogen phosphate correcting fluid till pH 7.2.
  - If pH is above 7.2 (i.e. alkaline): Add drop-by-drop 2% potassium-di-hydrogen phosphate correcting fluid till pH 7.2.

2 Prepare Correcting Fluids 2%

- **2% di-sodium hydrogen phosphate**

  Step-1: Weigh 2 g of disodium hydrogen phosphate and transfer to a beaker containing 100 ml. distilled water.

  Step-2: Pour this solution into stoppered glass bottle and mark 2% Disodium hydrogen phosphate.

- **2% potassium-di-hydrogen phosphate**

  Step-1: Weigh 2 g of potassium dihydrogen phosphate and transfer to a beaker containing 100 ml. distilled water.

  Step-2: Pour this solution into stoppered glass bottle and mark 2% potassium dihydrogen phosphate.

Note: Store the bottles away from sunlight in a cool place.

3. Prepare 3% Giemsa

- Fill about half of 100ml cylinder with buffered water (pH 7.2)
- Add 3 ml of filtered Giemsa stock solution into the buffered water by using dry pipette.
- Fill the cylinder to 100 ml mark with buffered water.
- Pour into separate clean and dry bottles.

B.3. Distribute the Reagents and Laboratory Supplies

The distribution of reagents and consumable laboratory supplies to the microscopy centers takes place during the monthly cluster meeting with the microscopy center staff. The consumable laboratory supplies are replenished, along with other malaria materials e.g. ACTs and print materials.

The laboratory staff from each microscopy center reports the available stock levels of consumable laboratory supplies, through the facility staff attending the monthly cluster meeting. The available stock level of few selected laboratory supplies is reported on FM02, whereas the remaining stocks are reported on the following table for other laboratory

supplies (See the cluster meeting guidelines for details of the material replenishment process).

Health facility _____

District _____ **Month/Year** _____

Stock Levels - Other Laboratory Supplies

Item	**End balance**	**Comments**
Consumables		
Sharp Box		
Methanol		
Xylene		
Immersion oil		
Disposable gloves		
Disinfectant swabs		
Lens cleaning tissues		
Non-consumables		
Microscope		
Other items (if any)		

SECTION- C HEALTH FACILITY VISIT PREPARATION

Supervision is the process of helping the staff to improve their performance. Supervisory visits give staff and supervisor an opportunity to share and better understand the situation, and motivate 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 Medical Officer In-charge and District RBM Focal Person (where needed). Thorough planning and preparation help making the DLS visit more productive and efficient. The District Laboratory Supervisor is a regular district staff, enabled by the program, to carry out an agreed set of activities for quality assured malaria microscopy in the district. District Laboratory Supervisor (DLS) is generally a senior malaria microscopist from the district.

C.1. Preparation of Monthly Visit Schedule

- DLS in consultation with the District Malaria Focal Person prepares monthly microscopy center visit schedule, on the format given below, by the 20th of preceding month. Each month, the DLS covers about one third of the total microscopy centers in the district. In this way he visits all the microscopy centers once a quarter.

- The DLS gets the monthly schedule approved from the District RBM Focal Person. Monthly activities of each DLS will then be assessed in light of his approved visit schedule.

- District RBM Focal Person, with DLS assistance, disseminates the visit schedule. The RBM Focal Person will send a copy of approved DLS visit schedule, by 25th of preceding month, to all the malaria microscopy centers to be visited by DLS during the month and a copy to EDO (health) office.

- The DLS gets the approval of any change in the visit schedule from the District RBM Focal Person and inform the malaria microscopy centres and EDO office accordingly.

DLS MONTHLY VISIT SCHEDULE

District: _____ Month _____

Date	Microscopy Center	Remarks

Notes on filling the table:

Date - the date on which the facility is to be visited; Microscopy center – the name of health facility to be visited on that date.

Remarks - Note any particular observation/action for the facility, in light of observations/discussions during the previous visit.

C.2. Preparing for on site replenishment of laboratory material (if needed)

Prior to visit of a microscopy centre, the DLS may call the laboratory staff of the facility to confirm his availability in the facility on the proposed date and ask if there is any material gap that requires urgent replenishment i.e. during the visit. In case one or more laboratory supply items are found out of stock, the DLS may arrange the materials from the district sources, for on-site replenishment during the facility visit.

C.3. Previous visit report

- DLS maintains a separate folder for each microscopy center/health facility to file its monthly EQA visit reports.
- DLS reviews the observations and decisions of the last visit report, to make any special preparation/arrangement accordingly.

SECTION - D HEALTH FACILITY VISIT

D.1. 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 level.

Section-1. Laboratory Functioning (EQA Form-1)

No. of days Lab. remained non functional	Reasons	Actions already taken	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 replenish onsite the requested laboratory supplies and keep record.

Section-2. Onsite Material Replenishment (EQA Form-1)

Item	Quantity replenished	Comments

D.3. Assess Recording and Performance

Review the malaria section of Laboratory Register ((DHS-05)) and relevant record for completing Section-3 of the EQA Form-1. Check if:

- Laboratory staff has entered all results of blood smear examinations in the Laboratory Register (and not elsewhere).
- All the relevant columns of (DHS-05) have been filled for each patient examined. The missing/incomplete/incorrect entries are identified and discussed with laboratory staff.

Common Mistakes in filling (DHS-05)	If you find mistakes ask the laboratory staff to consult session 7 of the laboratory-training course.
o Laboratory Serial Number is not given continuously	
o Lab Serial Number is not started from 01 every year	
o Incomplete name of patient entered	
o OPD/Ward No. Not written	
o Positive results are not entered as per guidelines.	

Based on the above observations, record your comments in the last row of section3 in EQA-1 Form.

- All clinical malaria cases have been examined for confirmed malaria diagnosis (i.e. MP positive). Among those examined, proportion found MP positive and falciparum positive.

Section 3: Recording & Reporting (EQA Form 1)

Characteristic	Formula	Value	Comments
Total number of slides examined for MP			
Proportion of those registered in malaria register, administered microscopy test.	# examined for MP/ # total malaria cases treated		
Proportion of the examined slides found positive for malaria parasite	# MP positive / # total slides examined		
Proportion of P. falciparum among confirmed malaria cases.	# P. falciparum positive / # total MP positive		
Lab. Register (FR8) recording			

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

D.4.1. Microscope maintenance

- Check for cleanliness and proper functioning of microscope. The maintenance of microscope is described in Malaria Microscopy Manual (session 2). A trouble-shooting checklist/guide is given as Appendix-2.

D.4.2. Slide Storage

- Check that the microscopist has stored all slides in the slide boxes, in the same order as they are listed in the laboratory register.
- Check that slides are stored according to laboratory serial number in the same box, irrespective of results.
- Check that slides have been labeled as per programme guidelines, to ensure that the correct slide is matched to the result.
- Check that the result of the slide examination is not appearing on the slide.
- Check that oil has been removed from the examined slides before storage by microscopist. Immersion oil is removed 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.

D.4.3. Observe Slide/material disposal

- Slides and other materials e.g. prickers/lancets need to be disposed of periodically. This reduces the risk of accidental infections from contaminated material.

- Check that the used glass slides and lancets are initially disinfected, by boiling in the water for about 20 – 30 minutes or by putting in phenol. Then these disinfected materials are buried deep in the ground away from the health facility.

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

- The used prickers/lancets are discarded either on daily basis or on alternate days.

All cross-examined slides kept with DLS are discarded only when PRL staff asks the DLS to do so

Section 4: Microscope maintenance, slide storage, material disposal

Characteristic	Acceptable		Action/Comments
	Yes	No	
Microscope cleanliness			
Microscope functioning			
Slide storage			
Disposal of used slides			
Disposal of used lancets/prickers			

SECTION - E SLIDE CROSS-EXAMINATION

During the health facility 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 Programme, sensitivity of the peripheral laboratory to the controllers is set at 80%. Based on the level of sensitivity, the required sample size for reexamining the slides is 20 at microscopy centres. The calculation of sample size is based on Lots Quality Assurance System (LQAS) considering provincial average figures of slide positivity rate and negative slides examined annually.

At each monthly visit of the DLS, the Medical Officer In-charge selects a sample of slides for the DLS to re-examine and report back. Medical Officer In-charge keeps the laboratory register with him, while the DLS does the re-examination of sample slides.

- Sampling method:
- Check that all blood smear slides are kept in sequence in the slide storage box (same sequence as in the laboratory register).
- Look in the laboratory register and look for the first and the last serial number of patients examined in the last completed calendar month. (For example, for the months of May-July 2007 at a RHC Barakahu the first patient examined is serial number 201 and the last number is 488 for the month).
- Count the total number of slides examined for the identified range of serial numbers i.e. 201-488 (288 patients examined for this range of serial numbers). The required sample size is 20 for RHC and hospitals.
- Divide the total number of slides examined by the required sample size to get the sample number “n”. This means that every “nth” slide is to be reexamined by the DLS. Taking the above example, divide the number of slides examined i.e. 288 with sample size for RHC i.e. 20. The result is n=288/20=14. This means that every 14th slide examined at the RHC during the quarter May-July is to be re-examined by DLS.
- Choose randomly the first number to start sampling from. Out of the first “n” group (in example, 14 slides with serial numbers 201-488), select one serial number randomly. In this example “203” is selected out of the first “n” group. Then from the remaining serial number, every nth (in this example 14th) serial number is systematically selected (i.e. 203 + 14 = 217, 218 +14 = 232, etc. ---). In this way the 20 selected serial numbers are identified from the microscopy centres.
- Retrieve the slides with selected serial numbers from the slide storage boxes. If a slide is missing, use the next slide. If more than two slides are missing the DLS should check if 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 Medical Officer In-charge. DLS notes the serial numbers of selected slides in the EQA-1 Form, before taking these slides for the re-examination.
-

Summary: The sample size of patients to have a slide cross-examined is always 20 for RHCs and 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 14 because there were serial number 201 – 488 = 288 patients examined inform May-July. That is dividing by 20 this gives a sample number of 14th patient in this example. Also, remember to start with a random number chosen from the first group of "n" slides; by chance slide serial number was chosen 203 in our example, and so the next was 203 + 14 = 217 and so on until all 20 are sampled.

E.2. Blood Smear Assessment

Check the selected sample of slides for the following characteristics and note the findings in section 5 of EQA Form-1.

2.1. Smear Quality

Assess the quality of thick and thin blood smears as follows:

Characteristic	Thick smear	Thin smear
Shape and size	Round - 1 cm diameter or Square - 1 square cm.	Tongue shaped – 2-3cm long
Thickness (If placed (wet) over newsprint)	Barely readable.	Easily readable
Smear placement	1 cm from edge of the slide	Uniformly spread over the slide

2.2. Slide Labeling:

Once the slide is dried, label the slide by putting the Lab. serial number on thin smear with lead pencil.

2.3. Staining:

Thick and thin smears are stained with 3% Giemsa stain (pH 7.2) and should have the following characteristics:

Characteristic	Thick smear	Thin smear
Background	Pale gray and free from dust	Clear and free from dust.
WBC nucleus	Deep purple	Deep purple
RBC	Not seen	RBC cytoplasm is gray, pink or pale purple
Parasite nucleus	Deep red	Red
Parasite cytoplasm	Deep blue	Blue

2.4. Smear Cleanness:

Stained blood smear must be free from stain deposits, dirt, and debris, etc.

2.5. Overall Quality:

Overall quality of smear is determined on the basis of all the sample slides examined for the selected six main characteristics (see table below). The observations on each slide is recorded in section 5 (EQA Form-1), by putting codes "A" for acceptable and "NA" for not acceptable. The overall quality on each characteristic 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 (EQA Form-1).

Section - 5: Smear Assessment of Selected Slides (EQA Form-1)

Slide Serial No.	1. Quality of blood smear						2. Labeling of slides		3. Staining of smears		4. Smear Cleanliness	
	Size of smears		Shape of smears		Thickness of smears							
	A*	NA**	A	NA	A	NA	A	NA	A	NA	A	NA
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
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 1-2 is found 'Not Acceptable' ask the laboratory staff to read Session IV, section 3.3. from the Laboratory Training Course Manual.

If performance on any of the characteristics from 3-4 is found 'Not Acceptable' ask the laboratory staff to read Session V, Staining blood films with Giemsa stain from Laboratory Training Course Manual.

E.3. Cross-examination Process

- The DLS Re-examines 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. DLS records the re-examination results in section 6 of EQA Form-1, in column: Re-examination result, and takes this back to the Medical Officer In-charge.

- The Medical Officer In-charge checks the results of the re-examined slides from the Laboratory register, and records the results in section 6 of EQA Form 1 in column: Malaria Microscopy Center Results.
- Medical Officer In-charge and DLS jointly write comments in "Comparison" column of Section-6 of EQA Form-1.

Section 6: Cross-examination by DLS (EQA Form 1)

S. No.	Lab. Serial Number	Cross-exam Result	Microscopy Center Result	Comparison			Remarks
				AG	FP	FN	
-							
-							
20							
Cross-examination Summary							
AG = In agreement, FP=False positive, FN=False negative							

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

Notes on columns in Section-6:

- *Lab. Serial Number* as recorded on each selected slide. This is filled by DLS, when he receives the sample slides for re-examination.
- *Re-examine* result refers to the smear results of each selected slide, as re-examined by the first controller (DLS). This column is filled by the DLS as he re-examines each of the sample slides.
- *Malaria Microscopy Center* result refers to the blood smear result of each selected slide, as recorded in laboratory register of the microscopy center. The Medical Officer In-charge fills this column once he receives the EQA Form-1 with DLS re-examination 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 one of the relevant columns i.e. AG, FP, FP.

- Agreement: refers to that slide positive or slide negative results (given by the malaria microscopy centre), which were found concordant (same) on subsequent re-examination by the first controller (DLS).
- False positive: refers to that slide positive results (given by the malaria microscopy centre), which were found negative on subsequent re-examination by the first controller (DLS). More false positive means over-diagnosis of confirmed malaria cases.
- False negative: refers to that slide negative results (given by the malaria microscopy centre), which were found positive on subsequent re-examination by the first controller (DLS). More false negative means over-diagnosis of confirmed malaria cases.

o *Summary results:* Gives the count of "tick marks" in each column.
o *Remarks:* DLS records the reasons for difference in results and agreed actions.

Error	Possible Causes	Suggested Investigations/steps
False Positive (FP)	Artifacts may be read as malarial parasites on peripheral smear. Dirty slides Unfiltered stain	Check stain concentration and pH and staining procedure
	Microscopist – lack of skills	Explain/demonstrate the positive and negative slides and microscope use.
	Microscopist: Gross neglect, over worked, lack of motivation	Counsel the laboratory staff, and discuss with M.O In-charge.
	Low quality or poorly prepared stain.	Replace the stain. Note the problem for discussion with District RBM Focal Person.
	Problem with microscope	Check and rectify problem with the microscope (use Appendix-2)
	Administrative error	Compare Lab. register and verify correct slide number and result.
False Negative (FN)	Microscopist – lack of skills: In adequate smear and/or staining	Refer to page 41-47 and 52-53 of Malaria Microscopy Manual. Also demonstrate, if required, the smear preparation and staining procedure.
	Microscopist: gross neglect, over worked, lack of motivation	Counsel the laboratory staff, and discuss with M.O In-charge.
	Storage of blood in anticoagulant before preparing the smear.	Discuss and explain to the microscopist
	Low quality or poorly prepared stain.	Replace the stain. Note the problem for discussion with District RBM Focal Person.
	Problem with microscope	Check and rectify problem with the microscope (use Appendix-2)
	Administrative error	Compare Lab. register and verify correct slide number and result.

E.4 Review with PRL

Review your report with Focal person RBM and fill the EQA Form-1, Section-7

Section-7: Review with RBM Focal Person (EQA Form-1)

Agreed actions/comments	
Implementation of previous agreed actions	
Signature DLS: _____	Signature (RBM FP): _____ Date: _____

E.5. Communication with PRL

- DLS collects all the re-examined slides and brings them back to his station, along with the filled EQA Form-1 (Sections 1-6). Keep all the concordant slides for re-checking by the provincial reference laboratory staff during their supervisory visit to the district. Keep slides from each microscopy center in a separate storage box. If possible these slides are kept till PRL staff asks the DLS to dispose the slides.

- All the discordant slides (i.e. false positive and false negative slides), along with a filled EQA Form-2, are sent to the Provincial Reference Laboratory. 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 re-examination by the second controller at provincial reference laboratory.

PRL will evaluate discordant slides and their results will be considered as final.

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 RBM Focal Person. The DLS will act in light of those comments. Any further discussion can be done in cluster meetings.

G. SUMMARY ACTIVITIES AT PROVINCIAL REFERENCE LABORATORY

- The provincial reference laboratory receives the discordant slides, on monthly basis, along with EQA Form-2 from each district.
- A second controller at provincial reference laboratory will perform rechecking of all discordant slides received from a district. PRL may re-stain the slides, if necessary. The results of the provincial reference laboratory are recorded in the last two columns of EQA-2 form received from each district.
- If agreement rate for a district is more than 95% (Which is exceptionally good), the PRL staff should be alert and to be watchful about the DLS observation (i.e. both concordant and discordant results). All discordant slides to be re-examined at PRL. A random sample

of about 10 or more concordant slides is re-examined during PRL staff visit of the district??.

- The provincial reference laboratory staff discusses the results of re-examination (by the second controller) with the DLS, during their quarterly interaction. These re-examined slides are returned to the respective DLS, after this interaction.

Annexure - I

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. Onsite Material Replenishment

Item	Quantity replenished	Comments

Section 3: Recording & Reporting

Characteristic	Formula	Value	Comments
Total number of slides examined for MP			
Proportion of those registered in malaria register, administered microscopy test.	# examined for MP/ # total malaria cases treated		
Proportion of the examined slides found positive for malaria parasite	# MP positive / # total slides examined		
Proportion of P. falciparum among confirmed malaria cases.	# P. falciparum positive / # total MP positive		
Lab. Register (FR8) recording			

Section 4: Microscope maintenance, slide storage, material disposal

Characteristic	Acceptable		Action/Comments
	Yes	No	
Microscope cleanliness			
Microscope functioning			
Slide storage			
Disposal of used slides			
Disposal of used lancets/prickers			

(For subsequent use with District RBM Focal Person)

Section-7: Review with RBM Focal Person

Comments on performance:	
Suggested measures:	
Signature: _____ DLS	Signature RBM FP _____ Date: _____

Section - 5: Smear Assessment of Selected Slides

Slide Serial No.	1. Quality of blood smear						2. Labeling of slides		3. Staining of smears		4. Smear Cleanliness	
	Size of smears		Shape of smears		Thickness of smears							
	A*	NA**	A	NA	A	NA	A	NA	A	NA	A	NA
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
Total												
Remarks***												

Section 6: Cross-examination by DLS

S. No.	Lab. Serial Number	Cross-exam Result	Microscopy Center Result	Comparison			Remarks
				AG	FP	FN	
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
Cross-examination Summary							

AG = In agreement, FP=False positive, FN=False negative

Annexure – II

MCP: External Quality Assurance (EQA) – Form-2

Name of District: _____ Month of Review: _____

Number of malaria microscopy 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:					
		Size	Shape	Thickness	Evenness	Staining	Cleanness

2. Comments by the PRL.

3. Discordant Slides

S. No	Name of Facility	Lab. Serial No.	Cross-exam Results	Health Facility Results	Reference Lab. Result	Error of: Lab. 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

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.
Laboratory Technician too busy with other laboratory tests e.g. TB slides and could not give time to malaria 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.

Appendix-2

Trouble-Shooting - Microscope Use

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, and 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 • Clean the lens
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
Headaches/incomplete binocular vision	• Eye-pieces are not matched • Improper adjustment of interpupillary distance • Dioptre adjustment was not done	• Use matched eye-pieces • Adjust the interpupillary distance • Adjust dioptre settings
Fuse blows frequently	• Fuse incorrectly rated. • Unstable line voltage	• Replace with correctly rated fuse • Use voltage protection device