

LABORATORY DIAGNOSIS OF MALARIA

OPERATIONAL GUIDELINES FOR LABORATORY TECHNICIANS



समर्थते कर्ते

Government of India

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FOREWORD

The gold standard for diagnosis of malaria all over the world is the detection and demonstration of malaria parasite in the peripheral blood smear. Though, it is a relatively simple technique yet there are many variables in the process, which can affect the result. These include competence of personnel examining the smear and the steps involved in performance of techniques. Optimum, accurate and reliable result is ensured if prescribed quality standards are maintained and followed right from preparation of blood smears, staining and examination.

The document entitled, “**Manual on Quality Assurance for Laboratory Diagnosis of Malaria by Microscopy**” describes step wise the minimum standards which must be followed for accurate and efficient diagnosis of malaria by microscopy. This manual is also to be used for training of personnel involved in diagnosis of malaria.

I congratulate the contributors and experts involved in the development and preparation of this document. We also congratulate the Director and the Officers of NVBDCP for undertaking this activity and completing it successfully.

Dr. R.K.Srivastava

DGHS

Preface

Vector-borne diseases including malaria are a major public health problem in India. Containment of these diseases includes prevention and control measures at the community level and accurate diagnosis and management at the individual case level. As far as malaria is concerned, both the above components require establishment of vector (mosquito) control measures and quality assured diagnostics.

The National Vector-Borne Disease Control Programme has a vast infrastructure of laboratory network consisting of PHC, District, Tertiary care and Medical College laboratories engaged in malaria diagnostics. However, the results produced by different laboratories are not consistent, hence there is a need of national programme to assure and assess the quality of performance by various laboratories.

A successful quality assurance programme for malaria diagnostics is the need of the hour to ensure accurate malaria diagnosis across the country. In this regard, the first step has been the preparation, development and field testing of the manual entitled **“QUALITY ASSURANCE OF LABORATORY DIAGNOSIS OF MALARIA BY MICROSCOPY”** by this Directorate and its field testing. The minimal facilities and standards required for performing microscopy for diagnosis of malaria are given in this manual.

It is envisaged that this manual will help in achieving quality services through capacity building and practice of quality management and quality assurance for laboratory diagnosis of malaria by microscopy.

Dr. G.P.S. Dhillon

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Each and every laboratory working under the NVBDCP must produce accurate, reliable and timely results for better management of vector borne diseases. To achieve this the laboratory personnel must have accurate knowledge of all aspects of diagnostic techniques and its performance. As far as malaria diagnostics is concerned the laboratory diagnosis involves microscopy, rapid diagnostic tests and quality management.

To achieve the objective of providing quality services in malaria microscopy Directorate of NVBDCP undertook the preparation of a laboratory manual entitled, “Quality Assurance of Laboratory Diagnosis of Malaria by microscopy” with the help of the renowned experts from across the country. The document has been prepared, field tested and reviewed and is now ready for use.

The pains taking efforts and technical guidance provided during the preparation of this document by Dr. P.L. Joshi, former Director NVBDCP is greatly acknowledged.

Valuable technical inputs provided by Dr. Krongthong Thimasarn, Regional Adviser, Malaria, WHO (SEARO) are greatly acknowledged. Financial assistance provided by WHO under AUSAID and USAID is also acknowledged.

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Last but not least, Secretarial Assistance provided by Shri Girish Kumar, Ms Nabanita Dutta, Ms Kusum Gairola and Shri Sachin Verma is also acknowledged.

The manual will be used to train laboratory personnel and as a bench manual so as to produce accurate and reliable results.

Valuable inputs provided by the contributors and experts to prepare this manual.

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PROCEDURAL HISTORY

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ACRONYMS USED

Ab	: Antibody
Ag	: Antigen
ATA	: Air Transport Association
DMO	: District Malaria Officer
ELISA	: Enzyme Linked Immunosorbent Assay
EQA	: External Quality Assessment
EQAS	: External Quality Assessment Scheme
EDTA	: Ethylene Diamine Tetra Acetic Acid
HCW	: Health Care Worker
FFP	: Fresh Frozen Plasma
FTD	: Fever Treatment Depot
IQC	: Internal Quality Control
ICMR	: Indian Council of Medical Research
J.S.B. stain	: Jaswant Singh and Bhattacharjee stain
IATA	: International Air Transport Association
LT	: Laboratory Technician
MF	: Malaria Form
MPW	: Multi Purpose Worker
NAMMIS	: National Anti Malaria Management Information System
NIMR	: National Institute of Malaria Research
NRL	: National Reference Laboratory
NIB	: National Institute of Biologicals
NVBDCP	: National Vector Borne Disease Control Programme
PHC	: Primary Health Centre
QA	: Quality Assurance
QC	: Quality Control
QI	: Quality Indicators



RDT	: Rapid Diagnostic Test
RF	: Reporting Forms
ROH & FW	: Regional Office of Health & Family Welfare
RRL	: Regional Reference Laboratory
RMRC	: Regional Medical Research Centre
RT	: Redical Treatment
RBC	: Red Blood Cell
SOP	: Standard Operating Procedure
SRL	: State Reference Laboratory
ZMO	: Zonal Malaria Officer
RRL	: Regional Reference Laboratory



GLOSSARY

Quality is defined as a set of processes/ procedures which ensure that whatever function/assay is undertaken produces an outcome/result/product which is valid, accurate, reliable, reproducible and has met all the quality standards laid down for the said function/assay.

Competency in microscopy, competence is the skill of a LT for performing an accurate examination and reporting of a malaria blood film.

External Quality Assessment (EQAS) involves specimens, of known but undisclosed content being introduced into the laboratory by designated “Apex/Reference” laboratory and examined by the staff of participating laboratory/ies using the same procedures as used for routine/normal specimens of the same type. This method checks the accuracy of the test results produced by the participating laboratories.

Internal audit is the process of critical review of all the functions of the laboratory to establish whether all activities that ensure quality are being carried out. Internal audits are also called first party audits i.e. those audits which are performed by the staff of laboratories themselves to inspect their own system.

Internal Quality Control (IQC) describes all the activities taken by a laboratory to monitor each stage of a test procedure to ensure that tests are performed correctly, that is accurately and precisely.

Negative Predictive Value (NPV) is the probability that the disease is absent when the test is negative. The lower the prevalence, the greater the likelihood of high NPV.

Prior Probability or Prevalence is the probability of the disease before the test is carried out that a subject has the disease.

Performance of Laboratory Technician is the accuracy of a LT examining malaria slides in routine practice. For assessment of the performance of a LT setting standards of performance is a prerequisite.

Positive Predictive Value (PPV) is the probability that the disease is present when the test is positive, the higher prevalence the *higher PPV* since there exists lower probabilities of false positive results in a populations where there are few true negatives.

Quality Assurance is a wide ranging concept covering all components that individually or collectively influence the quality of a product. It is the totality of the arrangements made with the objective of ensuring that the product is of the required quality for its intended use. It denotes a system for continuously improving reliability efficiency and utilization of products and services.

Quality Control (QC) describes all the activities taken by a laboratory to monitor each stage of a test procedure to ensure that the tests are performed correctly and results produced are accurate and precise. QC must be practical, achievable and affordable.

Standard Operating Procedures (SOP) are the most important documents in a laboratory. These describe in detail the complete procedures for performing tests and ensures that consistent and reproducible results are generated.

Sensitivity is the probability that it will produce a true positive result when used in an infected population (as compared to a reference or “gold standard”)

A highly sensitive test detects all the individuals who are infected but may also detect as positive few individuals who are not infected.

Specificity is the probability that it will produce a true negative result when used on a non infected population (as determined by a reference or “gold standard”)

A highly specific test correctly identifies all the individuals who are not infected as negative, but may detect few infected cases (early infection, low parasitemia cases) also as negative.



Chapter-1

INTRODUCTION

Malaria is one of the most widespread parasitic diseases all over the world. The disease present in 102 countries is responsible for over 100 million reported cases annually and 1-2 million deaths, especially in children. Normally, diagnosis of malaria is based on clinical symptoms such as presence of chills and rigors, intermittent fever, etc. which are non-specific, leading to false diagnosis and over use of anti-malarial drugs, thus increasing the potential of drug resistance, as well as the number of malaria cases.

Early diagnosis, followed by prompt and effective treatment is the key to reducing malaria mortality and morbidity. Consequently, it is essential to recognize the importance of this aspect in the control programme. Laboratory diagnosis of malaria greatly facilitates the management of the disease by confirming the clinical diagnosis and also aids in monitoring drug resistance. Laboratory diagnosis is desirable in all suspected cases of treatment failure and severe forms of the disease, as well as for diagnosis of uncomplicated malaria during low transmission seasons.

Since 1880, when malaria parasites were first detected in the blood of a patient, light microscopy has been the definitive tool for routine malaria diagnosis, especially because clinical diagnosis has low specificity, gives rise to over diagnosis and misuse of antimalarial drugs, resulting in increased cost to the health services. Microscopic examination of blood smears stained with JSB stain (and /or Giemsa, Leishman), continues to be the method of choice-the “Gold Standard”, for confirming the clinical diagnosis of malaria. Microscopy is a reasonably affordable, sensitive and specific technique. It not only allows the differentiation of *Plasmodium* species but also provides an estimate of the parasite load i.e. number of parasites per micro liter of blood. With the advent and spread of antimalarial drug resistance, particularly of multidrug resistant *P. falciparum*, the need and the importance of accurate microscopic diagnosis has been felt more acutely.

In India, under the National Vector Borne Disease Control Programme (NVBDCP), both microscopy and newer RDTs are being used across the country for diagnosis of malaria. Irrespective of the technique employed, establishment and maintenance of a reliable diagnostic service depends on operational feasibility of the test, availability of adequate trained personnel, equipment and laboratory management systems at all levels. Quality Assurance (QA) and adequate monitoring of laboratory services at the peripheral level have been perceived as one of the important but weak components under NVBDCP which needs to be strengthened. Therefore, it is essential to build and incorporate a Quality Assurance Programme under NVBDCP. As a first step to achieve this goal, the development of Standard Operating Procedures (SOPs) was felt imperative and the SOPs have since been developed. This document describes the Quality Assurance Programme for malaria microscopy for diagnosis of malaria. There are two more documents which describe the Quality Assurance Programme for malaria Rapid Diagnostic Tests and network of laboratories.

Chapter 2

QUALITY ASSURANCE OF MALARIA DIAGNOSTIC TESTS

Quality Assurance (QA) is a wide ranging concept covering all components that individually or collectively influence the quality of a product. It is the totality of the arrangements made with the objective to ensure that the product is of the required quality for its intended use. It denotes a system for continuously improving reliability, efficiency and utilization of products and services. The activities encompass all those factors in any health care organization that are concerned with inputs, processes and outcomes of the health care system. They must not only involve every department and every health care worker but should also be integrated into the routine work of any health care organization or service, both in public and private sectors. In short, QA refers to the sum total of all activities that are performed to ensure quality of a product or service by opting and implementing Good Laboratory Practices (GLP) and Good Manufacturing Processes (GMP).

2.1 Quality assurance programme

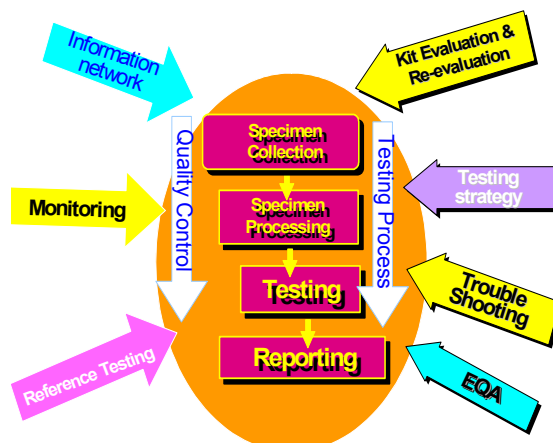
A QA programme deals with the dynamic ongoing process of monitoring the diagnostic laboratory's testing system for reproducibility in order to permit corrective action when established criteria are not met. This includes sampling specifications, testing methods, reporting and documentation for procedures ensuring that the necessary and relevant steps have been taken for quality services. The essential components are as in Figure 1.

2.1.1 Objectives

The objectives of QA programme are to

- assess the quality of the specimen/sample collection and processing.
- document the validity of the test methods

Fig 1. Essential Components of QA



- monitor reagents, stains, equipment, the performance of test procedures and personnel
- review test results of microscopy
- provide feedback for corrective action

This can however be attained only by active participation of everyone working in the system.

Following are the components of a QA programme:

- adhering to Standard Operating Procedures
- ensuring correct methods of specimen/sample collection
- ensuring quality of reagents used and calibration of equipment
- performing the tests with proper precision and accuracy
- interpreting of the results correctly
- monitoring and evaluation
- coordinating and supervising
- adequate training and re-training (experienced personnel)
- giving timely feedback
- detecting errors in the techniques and taking corrective steps
- documenting procedures, results, etc.

2.1.2 Structural set up for Q A

2.1.2.1 Leadership

The whole ethos of QA is teamwork. If one link fails in the QA process, the whole laboratory fails. Interpersonal relationship is an important aspect of teamwork. Good interpersonal relations establish trust and credibility through demonstration of confidentiality, courtesy, responsiveness and empathy.

Thus, the person in charge who exhibits good interpersonal skills is likely to achieve the goal, as junior staff will respond positively to him/her. Everyone, therefore, in the laboratory should understand the goals and aims of QA. The principle of the QA cycle should be explained to everyone.

The laboratory in charge should realize that the performance of QA system would depend on him/her; it is his/her responsibility to ensure that SOPs are followed on daily basis.

The senior laboratory personnel should strive to achieve a consistently high standard of work through their knowledge and understanding of the principles of QA.

2.1.2.2 Confidentiality

All results should be processed according to the SOPs. Laboratory staff, should not under any circumstances, disclose the results of laboratory tests to unauthorized individuals. Corrective action should be taken against defaulters.



2.1.2.3 Procedure

- **Identification of the members of the QA team**

It includes the laboratory staff, staff in the health facility whose work requires interaction with the laboratory e.g. Medical Officers, paramedical staff and community volunteers, who transfer specimens and results. There should be representation from management, who have the responsibility for the efficient and effective working of the laboratory and also for ensuring that the laboratory services meets the wider needs of the end users.

- **Setting standards and targets**

Simple quality indicators (QI) should be defined for monitoring by competent authority whether the standards laid are being met or not. In addition to Internal Quality Control (IQC) standards, laboratories should participate in External Quality Assessment Schemes (EQAS), referring batches of specimens for cross checking and comparing results obtained with designated Reference Laboratories of the medical colleges.

- **Selecting the priority issues for quality monitoring and improvement**

- i. Seeking views of the competent authority and/or quality assurance team of the referral laboratories,
- ii. Collecting data on the quality indicators for laboratory functioning and their remedial actions are necessary to improve the service.

- **Analysing the problems for quality**

Once the issues pertaining to quality in the laboratory service have been identified, the QA team should engage in analysis of the problems such as:

- i. What are the factors contributing to the problems?
- ii. At which stage in the process are interventions available for solving the problem (s) that lead to poor quality?
- iii. Who are the personnel involved?
- iv. How feasible it is to make changes to overcome the problems?

- **Developing solutions to the problems**

For resolution of problems that arise from time to time, meetings/brainstorming sessions involving all team members should be held to ensure improvement in quality. Once a particular solution has been arrived at, a clear plan should be drawn up that identifies the action required to implement the chosen solution and delegating responsibility to designated personnel for carrying out those corrective actions. Further, the “Action Plan” should indicate a timetable to implement and clearly set out a monitoring process which would ensure that the remedial actions are being implemented.

As a rule, no change or deviation in the implementation of SOPs are permitted and it is necessary to ensure that all activities are carried out in accordance with the procedures laid out in the SOPs.



- **Evaluating the quality improvements**

Periodically, QIs should be measured to evaluate the success of the Action Plan by an expert team drawn from National/ Regional/ State resource to be identified by the Dte. of NVBDCP.

2.1.3 Main components

A QA programme should have two important parts: IQC and EQAs. Differences between these two are as follows:

Salient points	Internal quality control (IQC)	External quality assessment (EQAS)
Nature	Concurrent and continuous	Retrospective /prospective and periodic
Performed by	Laboratory staff	Independent agency
Objective	Provide reliable result on day to day basis	Ensure inter-laboratory comparability and assesses proficiency of participating lab.

2.1.4 Expected outcome

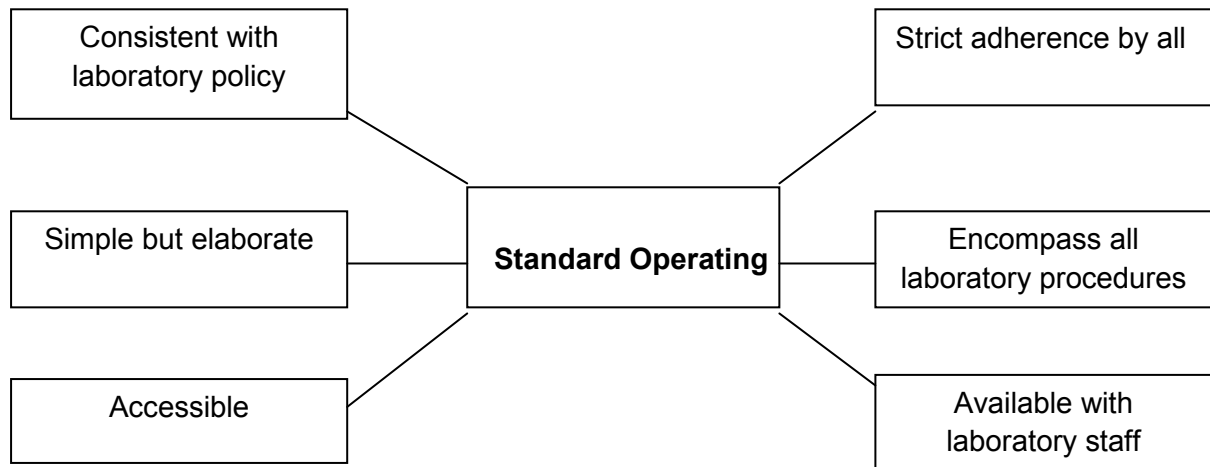
The expected outcome of a QA programme are as follows:

- generation and provision of a standardized laboratory service for malaria diagnostics
- reliability of laboratory results, thereby helping the physician in establishing proper and rapid diagnosis, leading to better management of patients
- creation of a good reputation for the laboratory
- enhancing motivation of staff
- accreditation of laboratories

2.2 Standard operating procedures in malaria diagnosis

The Standard Operating Procedure (SOP) is the most important document in a laboratory. It describes in detail the complete procedure for performing tests and ensures that consistent and reproducible results are generated. The instructions given in a SOP must be strictly adhered to by all those who are related with the functioning of the laboratory. The important factors in respect to the SOP are depicted in Figure 2.

Fig. 2 Important factors to design SOP



It should be reviewed periodically and any change required should be documented, validated and duly signed by the competent authority.

2.2.1 Broad structural components of SOP

- Administrative set up of the laboratory.
- Laboratory safety instructions including emergency measures.
- Techniques for collection, transportation and storage of samples. It should also include criteria for the rejection of a specimen and the action to be taken in case the sample is rejected.
- Details of all the procedures indicating different tests and recording of results.
- Quality control programme, including the laboratory's QA procedure, stating time and frequency of performing QC activities. Instructions must indicate acceptable IQC results and the actions to be taken when deviations occur.
- Clear cut instructions about reporting results
- Documentation of all results.
- Participation in National EQAS programme if any is available.



Chapter 3

CURRENT STATUS OF QUALITY ASSURANCE FOR MALARIA MICROSCOPY

3.1 Microscopy

It is the most widely used diagnostic test in India, since the inception of a structured malaria control programme in our country. It is till today the “Gold Standard” for laboratory diagnosis, yet it does have some disadvantages, the most important being the subjectivity in interpretation of the result by the examiner.

3.2 Strategy of cross-checking of malaria microscopy under NVBDCP

- There has been a well established programme for cross verification of the laboratory results of microscopy under Dte. of NVBDCP, wherein all the blood smears found **positive** at the Primary Health Centres (PHC) or other peripheral laboratories are supposed to be cross-checked for parasite species and stage by the designated centers. The **negative** slides are also cross checked as well. It was envisaged that all positives and 10% of all negative blood smears examined at PHC/ Malaria Clinic would be cross-checked.
- **Coding:** One of the responsibilities of the Zonal Malaria Officers (ZMO) is the coding of the examined slides. The code number (last digit) for cross checking is issued by ZMO (in the first week) of every month, for the negative slides examined in the previous month. If the code for the month is 5, the slide numbers ending with 5 from each section are cross checked.
- **Cross checking:** The PHC/ malaria clinic laboratory technician is supposed to collect all negative slides examined during the previous month with number ending with the code digit and dispatch to the concerned cross-checking laboratory by 10th of every month. All positive blood smears are cross checked in the Regional Office of Health & Family Welfare (ROH&FW), Govt. of India and State Headquarter laboratories. Depending on the workload, it is shared 50:50 between these laboratories. The negative slides are distributed between state/zonal and ROH&FW laboratories, at the ratio of 8.5: 1.5 between former and latter. Instructions are issued to the PHC/malaria clinic laboratory to preserve the rest of the slides, until the cross-checking results are received back.
- **Supervision of laboratories for cross checking:** In 1975, the expert committee on malaria recommended very strongly for supervision of efficiency of laboratories. For this, there was a provision for posting a supervisory laboratory technician at district/zonal/state laboratories. His function was to visit every PHC laboratory to inspect and conduct on the spot corrections in regard to laboratory records, returns, materials and equipment. He was also supposed to cross-check the laboratory procedures and to assist the technicians to improve their efficiency.
- **Results and feed back:** The results of cross-checking were to be sent to the concerned laboratory by the 10th of the succeeding month. In case of high discrepancy rate i.e., 2% or above, the state programme officer and Regional



Director of each ROH & FW was to take the needful remedial action like supervision of the concerned laboratory reporting high discrepancy rate.

3.3 Need for strengthening the QA Programme

Over the years, the QA of malaria microscopy in the form of regular cross-checking of examined blood smears could not be sustained upto the desired extent due to various operational and technical reasons. One of the main reasons was/is vacant posts of laboratory technicians at each level that is at PHCs, malaria clinics, at State/Zone and ROH & FW. Besides, the quantity of the negative slides (10%) is too high. In this context, as well as due to increasing trend of *P. falciparum* cases, emergence of newer foci of drug resistance and high mortality due to malaria, an urgent need has been felt to revitalize the QA component of the laboratory services provided under the Dte. of NVBDCP.



Chapter 4

PROPOSED QUALITY ASSURANCE PROGRAMME

The plan of action for the proposed QA programme has been designed to be realistic, feasible and sustainable, based on a situational analysis. It is proposed to be implemented in phases, depending on the financial, organizational and human resources available in the country and compatible with the different needs and goals. Adequate capacity building as per Chapter 5 and strengthening of laboratories at regional and peripheral level would be an essential prerequisite for this programme.

4.1 QA Programme for laboratory diagnosis of malaria under NVBDCP

4.1.1 Malaria microscopy

As stated earlier, human factors as well as reagents and equipment affect the sensitivity and specificity of the technique. As the results of the tests are the basis for patient referral, the number of false positives projected may lead to an overburdening of the health service by unnecessary treatment and its cost. On the other hand, false negatives mean that some cases will not be identified or treated and in the worst scenario could lead to death. To aid clinicians in arriving at the correct diagnosis, laboratories must be efficient and reliable with results that are accurate, precise and comparable between different laboratories. Therefore, technical procedures require standardization to an extent that the test sensitivity and specificity are maintained within desirable limits.

4.2 Modalities

This can be met only through commitment to a well planned QA programme that ensures team work with trained, competent and motivated staff, handling the tests, supported by adequate supervision. QA also needs a logistic system that provides an adequate and continual supply of reagents and glassware, all essential functioning equipment including microscopes. This system will result in quick delivery of reliable reports and prompt & specific management of the disease.

4.3 Essential elements of the proposed QA programme

The essential elements of the present QA programme are

- i. Identification of nodal agencies and co-ordination at National, Regional and State level.
- ii. A realistic cost of action plan, developed according to a situational analysis
- iii. Strategy for training/ retraining that ensures a competent workforce of laboratory staff as per integrated training programme.
- iv. Standard Operating Procedures (SOPs) to be adopted at each level of health care.
- v. A support network that ensures that the performance is maintained at the required standards. This includes:
 - agreement for memorandum of understanding (MoU) for the activities for participating laboratory in the network.
 - strict maintenance of IQC in the laboratories
 - periodic consultative visits from higher level staff



- EQAS by cross-checking of slides and testing of competence level/proficiency by slide/blood panels
- an effective logistics system to supply and maintain the essential equipment, glassware and reagents.

4.4 Nodal agencies and networking for QA programme

4.4.1 Nodal Agency

Dte. of NVBDCP is the nodal agency for the QA programme on laboratory diagnosis of malaria. It is the.

- focal point for national and international contacts regarding any issue related to the National malaria QA programme.
- Responsibility of NVBDCP for establishing national standards for training courses and also for preparation of training materials and modules. Regions and states would translate these training materials and modules according to local situations and languages.
- in-charge of the QA division at the Dte would be the Nodal Coordinate for the QA programme on behalf of the Director, NVBDCP; who would be responsible for over all activities related to the QA across the country.

4.4.2. National Reference Laboratory (NRL)

Two National Reference Laboratories are being identified: one for QA of Microscopy and other for RDTs.

- (i) The National Institute of Malaria Research (NIMR), Delhi is the National Reference Laboratory (NRL) for QA of malaria microscopy. It would
 - provide technical support to the national QA programme, as per the criteria laid down by the Dte. of NVBDCP.
 - assign the responsibility for monitoring and evaluation of overall functioning of the regional and state level referral laboratories from time to time and provide feed back to the Dte of NVBDCP as and when required.
 - assess the competence and performance of Laboratory Technicians (LTs) as well as the relevant laboratory procedures including equipment, according to standards laid down in the SOPs.
- (ii) The National Institute of Biologicals (NIB), Noida U.P., the apex institute for diagnostics and biologicals is the second National Reference Laboratory (NRL) responsible for QA of malaria RDT.

4.4.3 Networking

4.4.3.1 Microscopy

The current network would have NRL (NIMR) and involvement of ROH & FWs, Gol, ZMO, NIMR field stations and Regional Medical Research Centres of ICMR. States where ZMOs are functional, they will carry out the cross checking activity in collaboration with ROH & FW, Gol. The states have been divided among identified laboratories as indicated in the Table 1 of manual of QA: networking.

4.5 Designing of a Quality Assurance Programme (QA Cycle)

Followings are the steps in a QA System:



4.5.1 Assessment of Human Resources

A well organized QA programme does not always guarantee improvement of laboratory work unless there are committed trained and disciplined staff, who understand the purpose of QA and do not ignore the results of QA. Training will be provided to all staff under the network according to national guidelines. Initial basic training must be supplemented by regular supervision and re-training by refresher courses. A trained staff should be designated as Quality Control (QC) officer.

4.5.2 Standard Operating Procedures (SOP).

This operational manual for SOP has been prepared to strengthen the laboratories engaged in diagnosis of malaria, in order to bring a qualitative improvement in sensitivity and specificity of the techniques being used under the programme. It aims to meet the norms and criteria laid by WHO for quality laboratory services for malaria and includes all aspects of IQC, EQAS and documentation. NVBDCP envisage that these procedures should be followed for each activity pertaining to malaria laboratory diagnosis and these should be on the laboratory table of the LT. These may also be used as troubleshooting guides for equipment, reagents and methods.

4.5.3 Internal Quality Control (IQC) :

- all testing laboratories should adhere to IQC procedures within each laboratory in the network with strict control of techniques and equipment as per the National SOP to ensure reproducibility and sensitivity of detection.
- periodic training and retraining of microscopists/laboratory staff should be ensured.
- availability of equipment in functioning state and good quality stains/kits should be ensured.
- in case of microscopy, the quality of each prepared slide is assessed at the time of microscopic examination. Whenever possible, any slide that is inadequately spread should be prepared again until a slide of an acceptable standard is produced.
- the Coordinator of each malaria Reference Laboratory at national/ regional/state level must ensure systematic compliance with the norms for IQC. In peripheral laboratories (PHC/CHC), the MO I/c / LT must assume this responsibility.
- troubleshooting guides for equipment, reagents and methods would be useful additions to the more isolated laboratories where instant help is not available.
- with a multitude of steps involved in processing of a specimen, errors can occur at any stage. Laboratory management needs to be aware where errors can happen to reduce the possibility of their occurrence and monitoring all stages from the preparation through the examination up to the results. In case of microscopy, whenever required, reference slides and coloured charts supplied by Dte of NVBDCP should be followed.
- frequency and magnitude of incorrect results may be determined: in case of microscopy by independent cross-checking of the results of a proportion of the routine slides by some senior staff in the laboratory, if present.

4.5.4 External Quality Assurance schemes (EQAS)

- **EQAS** should be carried out at all levels of the national laboratory network for checking of accuracy of results.
- results of each round of EQAS should result in prompt feedback and corrective action at all levels where problems were encountered.



- participation of each laboratory in periodic (annual) EQAS organized by the NRL will be mandatory. In turn, the NRL should be subjected to EQAs by some international laboratory.
- staff of all laboratories at Regional/state/ district and those at the peripheral level (PHC/CHC) for malaria microscopy will be subject to national assessment by three processes.

4.5.4.1. Performance evaluation:

4.5.4.1.1 Proficiency testing

This will be carried out through analysis of known but coded panel slides (high quality stained blood slides), representing all the species present in the region, different parasite densities, mixed infections and also negative slides. The NRL will prepare these according to standardized procedures and will send them for a fixed number of times per year, (not less than twice a year), to each participating laboratory where microscopists are to be assessed. These slides are examined by the same staff using the same procedures as normal specimens of the same type. The results of these tests will be dispatched to the National Reference Centre/ Institution concerned, within a specified time, for comparison with the national identities of each slide after decoding. This method checks the accuracy of the test results. Results from a laboratory might be highly reproducible but consistently incorrect. Feed back would be sent promptly to correct the results.

Slide banks of unimpeachable quality with their content validated at NIMR would be utilized for training as well as for support assessment of microscopists. Such coded slides prepared according to SOPs, would be acquired by NIMR through

its field stations, as they have access to the required range of *Plasmodium* species. NIMR should also be capable of providing coded and matching negative slides to make standardized and high-quality slide sets that can be used for EQAS. These slides must be cross-checked to ensure the accuracy of the original diagnosis. It should contain the slides of all the three human species of malaria parasites *Pf*, *Pv*, *Pm* (prevalent in India) in thick and thin smears with different parasitaemia level, including rare forms of *Pf*, mixed infections and negative slides as well.

4.5.4.1.2 Crosschecking

QC by cross checking of slides taken routinely by the laboratory services can be highly demanding on human and financial resources. The intermediate or National Reference Centers will handle the results of the indirect QC of slides prepared, stained and analysed by each laboratory. Earlier all positive and 10% of negative slides were sent to Zonal/ROH&FW laboratory for cross checking every month. **The proposed scheme would follow revised quantitative criteria of cross checking of all positive and 5% of negative slides.** Feed back of results is sent promptly by the supervisor in order to take corrective action (see SOP: M 8 for more details)

4.5.4.2 Supervision

Supervision of efficiency of laboratory is an important component of the programme. For this, a supervisory LT at district/zonal/state laboratories will be deputed to visit



every PHC at least once a month. It is also envisaged that based on the results of the EQA, staff from the higher level laboratories will visit the peripheral laboratories periodically to correct faults, check on the IQC and identify training and retraining needs. Supervision reports will be sent to the laboratories concerned and the NRL (for details see SOP: M 08)

4.5.4.3 Evaluation

Simple QIs listed below would be defined for monitoring by Nodal agencies in order to assess whether the standards laid down are being met.

Some of the proposed QIs would be - the number of laboratories:

- following IQC procedures
- reporting results within the turnaround time
- obtaining correct results
- participating in EQAS
- implementation of corrective action

This can be done in a number of ways, e.g.

- seeking the views of the competent authority and/or quality assurance team of the referral laboratories,
- collecting data on the quality indicators for laboratory functioning (whether the laboratory is meeting the required standards)
- instituting remedial actions which are necessary to improve the service.

In addition to IQC Standards, laboratories should be encouraged to engage in EQAS, sending batches of specimens for checking and comparison with designated Reference Laboratories of the medical colleges. Once the problems with quality in the laboratory service have been identified, the QA team should engage in the analysis of the problems and develop solutions. When a particular solution has been agreed, an Action Plan should be drawn up that identifies the action required to implement the chosen solution and clearly assigns responsibility for carrying out those actions in consultation with the RRLs and QA division of the Dte. of NVBDCP. The Action Plan should indicate a timetable for implementation and sets out the monitoring process that checks that the actions are being implemented.

After a sufficient period of time, post Quality Assurance scheme implementation, the Quality indicators would be measured to evaluate the success of the Action Plan by an expert team from national/regional/state team identified by the Dte. of NVBDCP.

4.5.5 Evaluating the quality improvements-

This can be done in a number of ways, e.g. seeking the views of the competent authority and/or QA team of the referral laboratories, collecting data on the quality indicators for laboratory functioning (whether the laboratory is meeting the required standards) and remedial actions which are necessary to improve the service.

4.6 Establishment of a supply chain



Establishment of an effective supply chain is essential to foresee and provide all the equipment and supplies that are needed to sustain an uninterrupted flow of reliable malaria diagnosis. To facilitate this, PHC wise standard establishment and

replenishment lists for glassware /reagent including equipment should be prepared by each district. However, in remote and inaccessible areas e.g., some areas in North East regions, if rapid replenishment of consumable items cannot be assured, buffer stocks equal to the operational requirements for at least 6 months should be maintained at all levels especially during the monsoon season. Logistics should be replenished as and when required.

4.7 Implementation of an effective QA Programme

This requires:

- motivated well trained LTs and supervisors
- adequate availability of funds
- good communication between LTs and supervisors
- an efficient postal system or a system to send the samples
- adherence to national safety guidelines

4.8 Measurable indicators of QA

- QA performed as per the criteria laid by the Dte. of NVBDCP.
- national nodal coordinator is identified and notified.
- state / Regional supervisory coordinating institutions are identified and notified.
- number of laboratories involved in QA scheme (per cent of public sector laboratories and per cent of private sector laboratories)
- number of visits made by national/regional coordinator/state level supervisors.
- implementation of a valid cross-checking system.



Chapter 5

TRAINING

A well organized QA Programme does not always guarantee improvement of laboratory work unless there are committed, trained and disciplined staff, who understand the purpose of QA and do not ignore the results of QA.

5.1 Integrated training under NVBDCP

Integrated training for capacity building of various levels of health functionaries across the country is one of the most important components of NVBDCP strategy. This is carried out by performance analysis of health workers, which help in identifying reasons for the constraints associated with the implementation of the NVBDCP strategies. After analysis, suitable curricula have been designed for different categories of workers including LTs.

LTs working at the PHC/CHC level are imparted practical training on collection, processing, staining, differential diagnosis, etc. of blood smears and preparation of stains at the well equipped training centres as in parasitology/ pathology/ microbiology department of medical colleges/ ICMR institutes or ROH & FW, GoI laboratory. There are two types of trainings for LTs: Induction level for newly appointed LTs and reorientation level as refresher training for those who are already in the job. Induction level training is for two weeks (10 working days) and the reorientation training is for 5 days. Each batch consist of around 20 participants. The microscopes used in these trainings are those used under the programme. Performance analyses of the technicians are referred to identify the training needs (Refer NVBDCP Operational guidelines on integrated training for more details).

5.2 Training manuals and Bench Aids

The NVBDCP training manual for Malaria Microscopy would be followed for training of the LTs. Moreover, WHO has produced bench aids for the diagnosis of malaria which contains 12 plasticized plates and is found to be suitable for day-to-day use in the laboratory. At present, these bench aids would be used at the peripheral laboratories till these are replaced by sets of bench aids on local languages. Besides, Dte. of NVBDCP envisages for providing electronic bench aids (Learn yourself type) upto district level, as all the districts are equipped with computer facility under NAMMIS. These electronic bench aids, with their potential for visual microscopy, would be a very useful adjunct to the training programme.

5.3 Refresher/ reorientation training (Corrective retraining)

If a LT's performance is found to be poor, either during supervisory visit or in the performance of EQAS, he/she should be referred for reorientation training. After the training, the performance of the LT/ microscopist should be strictly monitored. If the supervisory/ immediate officer is not satisfied with his performance, he should be again sent for reorientation training. Even then, if the performance of the LT/ microscopist is not improved he/she should not be allowed to examine blood smears any more. However, such decision should preferably be taken by observing proper administrative formalities.



5.4 Procedures envisaged

- staff in laboratories should be technically competent. This means that they should have the skills to execute guidelines and standards in terms of dependability, accuracy, reliability and consistency.
- every staff member is to be assessed for his/her competence to perform all relevant tasks within the department. The assessor will generally be the Supervisor or Head of Department, but he/she may be any authorized person, who has been assessed as being competent for that particular task. Assessment can be based on past experience or active assessment.
- all relevant training completed is to be recorded for each member of the department.
- each member should have his/her competency re-assessed as required, at least annually. This may be through intra or inter laboratory comparisons.
- proficiency of laboratory / technical staff should be tested by conducting programmes like internal & external proficiency testing programme conducted by a competent agency.
- to begin the QA cycle, all malaria laboratory diagnostic staff and resources should be monitored by a senior laboratory technologist from the nodal Medical College (identified by the NVBDCP), who has experience to evaluate not only the skills of the personnel but also the quality of microscopes and other equipment. From this initial monitoring visit, the problem areas should be identified (staff skills/equipment/supplies).
- remedial steps should then be taken to correct equipment and supply deficiencies, followed by on-site staff training with pre and post training tests to evaluate the effectiveness of the training, as and when required.
- on-site monitoring and hands on training is better than conducting remote training workshops as locally encountered problems and deficiencies can be seen and corrected, rather than being reported. In addition, on-site visits are perceived as support that supplements the training aspects.
- ideally, these monitoring and retraining support visits should be conducted quarterly in the beginning, then at six months interval. Regular monitoring and training will ensure that supervisors know the staff and can evaluate each one and make appropriate recommendations.
- training should be based on SOPs i.e. there should be logical process, so that laboratory staff understand the importance of the SOPs (the basis of QA).
- the laboratory staff should also attend training / awareness programmes to understand the value and importance of GLP and use and maintenance of QI like SOPs and check lists etc.



Chapter 6

BIO-SAFETY IN LABORATORY AND SAFE DISPOSAL OF BIOMEDICAL WASTE



6.1 Introduction

Bio-safety, especially safety in laboratories is a key component of total quality control programme. There is definitely a potential risk of infection to Health care workers (HCWs), who provide direct or indirect health care to people and thus continuously come in contact with pathogenic organisms, (e.g. nurses, midwives, community health workers, hospital housekeepers and doctors) or handle samples of body fluids/tissues/ morbid specimens (Lab technicians, Microbiologists etc.), handle infected waste and transport potentially infected specimens (laboratory attendants, safai karamcharis etc.). They are exposed to certain infections by nature of their profession. These infections could be bacterial, viral, parasitic or fungal. Some of these are serious like plague, hepatitis, Human Immunodeficiency Virus (HIV) etc. and may even result in death, whereas, others are not serious and only cause morbidity.

6.2 Bio-hazards in a laboratory and practice of Bio safety

Laboratories, practicing microbiological work, are exposed to microbiological hazards, besides common hazards like fire, chemical and electrical, etc.

Safety is one aspect of quality, it minimizes the risks of injury, infection or other dangers related to laboratory services delivery. Safety involves the providers as well as the beneficiaries (patients), for example, safety is an important dimension of quality when collecting blood for making blood slides or for using Rapid Diagnostic tests for malaria to prevent transmission of infection such as Hepatitis B and C and HIV.

There are several ways HCWs engaged in malaria diagnosis can acquire blood borne infection from a patient or from his/her specimen either by:

- direct contact with blood/body fluids,
- accidental inoculation of infected blood/body fluids,
- accidental cuts with contaminated sharps,
- indirect contact with contaminated equipment or any other inanimate infected objects.

Before undertaking any QC programme in a microbiology laboratory, all biosafety measures should be ensured and HCWs must take all precautionary measures to protect themselves from accidental injury, while handling the blood (standard work precautions) and patients must also be protected from infection. The risk of acquiring HIV infection following sharp injuries from a patient or infected blood is extremely low i.e. 0.25 to 0.3 % but that of acquiring hepatitis B or C is higher.



6.3 Bio safety procedures

6.3.1 Adequate facilities

The laboratory should have adequate facilities, necessary equipment for undertaking the tests and following laboratory safety.

6.3.1.1 General laboratory specifications

- adequate space should be assigned for a particular laboratory work for the safe functioning.
- laboratory tables should be stable, impervious to water and resistant to disinfectants, chemicals and moderate heat.
- hand-washing basins, with running water, should be provided in each laboratory room. A dependable supply of good quality water is preferable.

6.3.1.2 Laboratory working place

- All tables must be kept clean, tidy and dry.
- Work surfaces must be decontaminated at the end of the working day.
- All chemicals, solutions and specimens must be properly labelled. Labels must include name, date prepared and expiry date, where applicable.
- Glassware and other materials for reuse must be rinsed properly with water after cleaning with detergent.
- Supplies and materials must be kept in designated drawers and lockers that are labeled with respective contents on the outside.
- Heavy equipment, glassware and chemicals are not to be stored above eye level.
- All equipments must be properly attached to electrical points in a way that prevents overloading and tripping hazards.
- Safety system should preferably have fire safety and electrical back up facilities for emergencies. All laboratory personnel should be trained for required awareness to use the facility in emergency.

6.3.2 Bio-safety practices in a health care setting These include:

6.3.2.1 Universal work precautions or standard precautions for blood and body fluids:

Attention should be paid towards the personal protection during handling of human specimens. e.g., care should be taken to prevent the entry of diseases pathogens like HIV 1 and 2 and Hepatitis B and C, by the routes mentioned above. Biological and safety hazards inherent in handling human specimen, eg. Contaminated blood and body fluids can be effectively prevented by diligent practice of **standard work precautions** by HCWs by presuming that all the specimens are infected or potentially infections.

Blood is the single most important source of HIV, HBV, HCV and other blood borne infections to HCWs.

Standard work precautions in a laboratory are:

- **Hand washing**



Hands must always be washed vigorously under running water using a skin disinfectant /antibacterial liquid (i.e. 4% chlorhexidine gluconate with added skin emollients) for at least 10 seconds and 70% alcohol before and after work and at any time before leaving the laboratory.

- **Barrier protection**

Laboratory gown, preferably wrap around gowns, disposable gloves and protective shoe covers must be worn at all times when working inside the laboratory and especially when handling human blood. Use gloves for all those procedures that may involve accidental, direct contact with blood or infectious materials. A generous supply of good quality gloves is required. Discard gloves whenever they are thought to have been contaminated or perforated, wash hands and put on new gloves. Gloves should be used in addition to hand washing. Laboratory clothings should be removed before leaving the laboratory.

- **Safe laboratory practices**

Besides the instructions mentioned above

- i. eating, drinking or storing food or drinks is strictly prohibited in the laboratory. Special personal lockers should be provided to the laboratory staff to keep all these items at the entry point of the laboratory area.
- ii. apply strict aseptic techniques throughout the procedure.
- iii. wash hands with soap and water immediately after any contamination and after work is finished. If gloves are worn, wash hands before and after gloves are removed.
- iv. all technical procedures must be performed in a way that minimizes the formation of aerosols and droplets. Work with human blood or serum requires the use of disposable equipment and supplies, whenever possible. Otherwise, all reusable materials must be autoclaved or placed in 1.0% hypochlorite solution for 24 hours before washing.
- v. ensure an effective insect and rodent control programme.

- **Safety procedure for malaria diagnostic tests**

- i) **Collection of blood by finger prick method**

Discard the lancet/ pricking needle after the finger prick straight in to a beaker containing 1% freshly prepared solution of sodium hypochlorite or any other appropriate disinfectant.

- ii) **Collection by venepuncture**

- wash hands before and after the collection of specimen.
- collect and place the specimen aseptically in an appropriate sterile, leak-proof, airtight container, whenever needed or follow SOP
- tightly close the lid of the container during transportation, if necessary
- completely fill the label on the specimen collection vial.
- collect the specimens by taking precautions to avoid unnecessary contamination of the material but also avoid self-infection, creation of aerosol or gross splashing (especially into eyes) or by injury such as syringe needle or contamination of damaged skin.

Similarly, after venepuncture, the syringe with attached needle may be disposed by different methods. **See under safe handling of sharps**

iii) Pipetting

use a rubber teat or automatic suction device properly, as outlined in SOP: G 6 for Pipetting Techniques. Mouth pipetting is strictly forbidden.

Biological safety cabinets, should be used whenever infectious materials are handled and there is an increased risk of aerosol production, which includes centrifugation, blending and mixing, etc.

- **Safe handling of sharps**

Sharps like disposable needles/ hypodermic needles or scalpels and broken glass pose the greatest risk of blood borne pathogen transmission in health care setting through per-cutaneous injury which occurs when needles are recapped, cleaned, improperly discarded or disposed off.

- i) limit use of hypodermic needles and syringes. They must not be used as substitutes for pipetting.
- ii) never recap, bend, break or remove disposable needles from disposable syringes.
- iii) always destroy needles and syringes by needle cutters, if available or the complete assembly should be placed in the puncture resistant disposal container after decontamination. In case of lancets or other sharps, dispose in the same container after decontamination. Puncture resistant disposal containers are specially labelled puncture-proof rigid containers fitted with covers. When the container is three-quarters full, it should be placed in an “infectious waste” container and incinerated, with prior autoclaving, if laboratory practice requires it.
- iv) do not dispose of sharp containers in landfills.

- **Management of accidental spill of blood**

- (i) any spilled biological material on floor/work surface must be covered with paper towel/ blotting paper/news paper/ absorbent cotton
- (ii) 1% hypochlorite solution is poured on and around the spill and left for 30 minutes before cleaning.
- (iii) all the waste removed with gloved hands and sent for incineration in yellow bags.

- **Management of accidental injury**

- i) **In the event of a puncture or penetrating injury** noticed during sample collection or any other hazardous procedure:
 - wash the affected part thoroughly with water and soap/disinfectant.
 - if the eye is splashed, rinse at once either with clean tap water or with irrigating solution held in the laboratory first aid kit or with sterile saline.
 - immediately seek medical attention and report to the designated nodal officer or laboratory supervisor.
 - document the incident / accident in respective register.



ii) Accident reporting

- date and time of accident.
- sequence of events leading to accident.
- the waste involved in accident.
- assessment of the effects of the accident on human health and the environment.
- emergency measures taken.
- steps taken to alleviate the effects of accidents.
- steps taken to prevent the re-occurrence of such an accident.

Date: _____

Signature: _____

Place: _____

Designation: _____

Bio-safety management

It is the responsibility of the laboratory supervisor (the person who has immediate responsibility for the laboratory) to ensure the development and adoption of a bio-safety management plan and a safety operations manual.

The laboratory supervisor should ensure that regular training in laboratory safety is provided. Personnel should be required to read the SOPM on safety and a copy of this manual should be available in the laboratory.

6.3.2.2 Effective sterilization and disinfection

- **Definitions**

- (i) **Sterilization:** Complete destruction of all living microorganisms including spores.
- (ii) **Disinfection:** Destruction of vegetative forms of organisms which might cause disease.
- (iii) **Disinfectant:** An effective all purpose disinfectant is sodium hypochlorite solution with concentration of at least 1.0%. There are other disinfectants also like lysol,

For purpose of disinfection, disposal and recycling, all the articles may be divided into three categories:

- i) **Disposables:** Soak the material overnight in a strong solution of disinfectant before disposing. 1% Sodium hypochlorite / 1% calcium hypochlorite, 10% solution of formalin or 3% lysol may be used as disinfectant.
- ii) **Reusable articles contaminated with morbid material:** Discard the material into a jar containing disinfectant solution. Let them remain in this solution overnight. Drain off the disinfectant. Transfer the material to a metal pot or tray with cover. Pour water and boil for 15 min. Cool and drain off the water. Pass on the articles for washing. Current procedures used for sterilisation, ie. Continuous boiling for 20-30 minutes or autoclaving are adequate. Autoclave monitoring is done by using chemical indicator strips. Syringes and needles should never be disinfected by chemical disinfectants.
- iii) **Material containing clinical specimen:** Direct on site incineration or autoclaving followed by incineration at a distant site.



6.3.2.3 Safe disposal of biomedical waste

- **Definitions**

Biomedical Waste is defined as unwanted trash generated during diagnosis, treatment or immunization of human beings, during research activities or testing of biologicals. Laboratories are major source of biomedical waste. These are:

- (i) **Biologicals / blood/ red cells / body fluids, etc.:** Blood samples collected and stored to use as red cell panel, serum and plasma.
- (ii) **Expired:** contaminated, deteriorated or any condition of infectious biological material generated for disposal.
- (iii) **Biotechnology waste:** Materials generated as waste from the kit like any reagent buffers, diluents, etc.
- (iv) **Sharp waste:** Glass slides, cover slips, needles, glass, Pasteur pipettes, test tubes, scalpels and blades, etc.
- (v) **Solid waste: other than waste sharps:-** Rapid strips, combs, cards, plastic vials, pipette tips, cotton, tissue paper and filter paper contaminated with blood during generated during QC testing.
- (vi) **Liquid waste:** Generated from laboratory during testing, from washing, cleaning and disinfecting activities.

- **Management**

Management of biomedical waste and disposal in any laboratory that deals with biological testing in terms of quality control and quality assurance applies to all who generate, collect, receive, store, dispose or handle biomedical waste in one or other form. It should be the duty of every person handling the bio-medical waste to ensure that such waste is handled without any adverse effect to human health and environment.

It is the responsibility of the laboratory personnel to:

- (i) recognize the type of biomedical waste generated in the laboratory, segregation, packing, storage and transportation (category 1-9) and follow treatment and disposal as prescribed in the schedule. [Ref. *The Gazette of India, Extraordinary, Part II-Sec.3 (ii)*].
- (ii) implement waste management in compliance with the prescribed standards.
- (iii) make sure that no waste is left untreated and it should not be kept stored *beyond a period of 48 hours*.

- **Treatment and disposal recommendations for management of bio-medical waste are**

- i. place all bio-hazardous waste (apart from sharps) in specially designated colour coded waste containers, separately from non-infectious waste.
- ii. all bio-medical waste containers / bags should bear biohazard symbol.
- iii. autoclave all infectious solid waste in leak-proof containers e.g. autoclavable, colour- coded plastic bags, before disposal in yellow bags and send to incinerator facility or incinerate within the laboratory, if feasible. Do not dispose infectious material in landfills.
- iv. collect all sharps in puncture proof containers and then in blue / white translucent bags.
- v. maintain documentation of waste generated during testing, separately for liquid and solid waste and treatment given and means of their disposal, regularly.



- vi. handle waste generated from the kits / during testing in such a way that infectious and non-infectious materials are discarded separately and treated accordingly before disposal.
 - vii. decontaminate potentially contaminated liquid waste eg. Blood before discharging to the community sanitary sewer system.
 - viii. frequently decontaminate the working area with disinfectant.
- Store all biohazardous waste separately from non infectious waste in leak-proof containers (autoclavable, colour- coded plastic bags with biohazard symbol), not more than 2 days and seal tightly when transported. In certain cases double bagging is required to prevent leaking. For disposal of sharps – see 6.3.2.1. The color coding of bags for biomedical waste disposal is shown in Table 2.

Table2: Colour coding of bags for bio medical waste disposal

Colour coding	Type of container	Waste category	Treatment and disposal options
YELLOW	Plastic bag	Human anatomical waste, animal waste, microbiology and biotechnology waste and solid waste.	Incineration / deep burial
RED	Disinfected container / plastic bag	Microbiology and biotechnology waste and solid waste.	Autoclaving / Microwaving / Chemical Treatment i.e. 1% hypochlorite solution
BLUE / WHITE TRANSLUCENT	Plastic bag / puncture proof container	Sharps waste & solid waste.	Autoclaving / Microwaving / chemical treatment i.e. 1% hypochlorite solution and destruction / shredding
BLACK	Plastic bag	Discarded medicines and cytotoxic Drugs, Incineration ash and chemical waste (solid)	Disposal in secured landfill.

• **Methods of disposal of waste**

The following are the methods of disposal

- i. Incineration – it is the best option as it renders the waste noninfectious and changes the form.
- ii. Autoclaving and disposal in general waste system at 121° C for 20 minutes.
- iii. Needle destroyer /cutter for destroying needle and part of the nozzle of syringe
- iv. Chemical – disinfection
- v. Deep burial - If incineration is not available, then all Red /Blue / White Translucent bags are collected for final disposal by deep burial. A pit should be dug about 2 mts. deep. It should be half filled with waste then covered with 50 cm of the surface before filling the rest of the pit with soil. It must be ensured that animals do not have any access to burial sites. Covers of galvanized iron wire meshes should be used to cover the waste burial pit.

On each occasion when waste is added to the pit, a layer of 10cm of soil should be added to cover the waste.

Records of all pits for deep burial should be maintained.

6.3.2.4 Immunization for Hepatitis B –All HCWs should be immunized against HBV.



Chapter 7

STANDARD OPERATING PROCEDURES: GENERAL FOR QUALITY ASSURANCE (SOP.G)

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	<i>GENERAL QUALITY ASSURANCE - MICROSCOPE</i>				
SOP No.	SOP G. 01	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP G. 01 – MICROSCOPE

7.1.1 Purpose

It is one of the most important components of QA on malaria microscopy. It is essential that the application of the different microscopes with specific reference to malaria microscopy should be known by each LT. The purpose of the microscope is to produce an enlarged, well defined image of objects, too small to be observed with the naked eye.

7.1.2 Principle

A microscope is an instrument designed to make fine details of the blood film visible.

7.1.3 Types of microscopes

Microscopes vary from an ordinary magnifying lens (magnifies 100 to 1000 times) to that of a sophisticated Electron microscope which magnifies a million times. The simple microscope is nothing but a magnifying lens consisting of two converging lenses fixed at two ends of a brass tube. The lens nearer to the object is called objective lens and the lens through which the final image is observed is called the eye piece or the ocular lens. The objective lens produces a real, inverted, intermediate image of the object which lies within the principal focus of the eye piece, while the eyepiece produces a magnified, virtual and inverted image. The final image is thus inverted, magnified and virtual. A compound light microscope has the capacity to increase an object by 1000 times so that an object of 0.1 micrometer or 100 nanometer is made visible.

Types of light microscopes

- bright field compound microscope
- phase contrast microscope
- dark ground microscope
- fluorescent microscope



7.1.4 Bright field compound microscope

The common microscope that is suited to see and study the microorganism routinely is the typical compound microscope, either monocular or binocular. Here the microscopic field or

area observed is brightly lit and the objects under study appear darker. Generally, these microscopes produce useful magnification of about upto 1000 times than the naked eye.

- **Monocular:** monocular microscopes have single eye piece and are convenient for use by beginners.
- **Binocular:** binocular microscopes have two eye pieces. They are recommended where much work has to be done, as this microscope causes less eye strain and fatigue.

7.1.5 Parts of a microscope

7.1.5.1. Mechanical

7.1.5.1.1 Stand

It forms the base of the microscope. It consists of a vertical pillar supported on a horse shoe shaped base or foot. It gives stability to the microscope. The stand is attached to the arm or limb by the hinged (inclination) joint, which can be adjusted to any convenient angle. The limb or arm carries the illuminating apparatus, the stage and the observation tube. It also serves as a handle.

7.1.5.1.2 Stage

It is a platform with a circular aperture in the center. Stages are usually of two types:

- a fixed stage in which the object is fixed by clips as in a monocular microscopy.
- a mechanical stage in which the object can be moved to the desirable distance, either sideways or forward and backward, as in binocular microscope. This type of stage is preferable for examination of a blood film or to locate a particular point in the object.

7.1.5.1.3 Focussing knobs

These are located on the side of the microscope; outermost is the course focus and innermost is the fine focus. On the binocular microscopes, these knobs control up/down movement of the stage.

7.1.5. 2. Magnifying parts

These are eyepieces and objectives. They are kept separated in a graduated tube.

7.1.5.2.1 Ocular lens or eyepiece

Usually of 6x or 10x magnification. Only one eyepiece is present in a monocular microscope and two in case of a binocular microscope.

7.1.5.2.2 Objectives

The objectives are screwed on to the rotating nosepiece which is attached to the lower end of the tube. They are usually 3-4 in number and designated according to their focal lengths.

- low power dry objective : 16 mm
- high Power dry objective: 4 mm
- oil Immersion objective : 1.6 mm

Their magnifying power and/or their numerical aperture may also be engraved on them. The eyepieces or the oculars are usually designated by their magnification eg. 10x.

7.1.5.2.2.1 Oil-immersion objective

This is the most frequently used objective because of the greater magnification and resolution which is required to study the morphology of parasites like malaria parasites. Some of the monocular and all the binocular compound microscopes, have 100x oil immersion lenses. These can be identified by a red band around the lens housing. At magnifications greater than about 500x, light is refracted too much, as it passes through air to yield good resolving power. Thus, optics for these higher magnifications are made to use with a high grade mineral oil as the medium for transmitting light. *It is imperative that only immersion oil is used and the lens is cleaned thoroughly with lens paper after each use everyday.*

7.1.5.2.3 Body tube

This contains mirrors and prisms which direct the image to the ocular lens/es.

7.1.5.2.4 Nosepiece

This holds the objective lenses and rotates with a positive click for each lens.

7.1.5.3 Illuminating parts

This consists of a condenser, an iris diaphragm, a mirror and the light source situated below the stage.

7.1.5.3.1 Condenser

The condenser is made up of a system of convex lenses. It concentrates the light rays reflected by the mirror to the object plane in the optical axis. The condenser can be raised or lowered. Lowering of the condenser diminishes illumination, whereas, raising it increases the illumination. While using oil-immersion objective, the condenser is completely raised as it requires more light. When the other objectives are used, it is lowered suitably. The condensers move up and down to focus the light beam.

7.1.5.3.2 Iris diaphragm

This helps to regulate the amount of light. It is opened widely when the oil immersion objective is used, as it requires maximum light and closed partially when the other objectives are in use. The diaphragm is located just below the stage and controls the amount of light which passes to the specimen and can drastically affect the focus of the image.

7.1.5.3.3 Mirror

This is a plano-concave mirror. It helps to reflect the light into the sub stage condenser. The plane mirror is used, whenever the oil-immersion objective is employed. The concave mirror is used with low and dry high power objectives.

Under the NVBDCP, both binocular and monocular compound microscopes are being used for malaria microscopy.

7.1.5.3.4 Light source

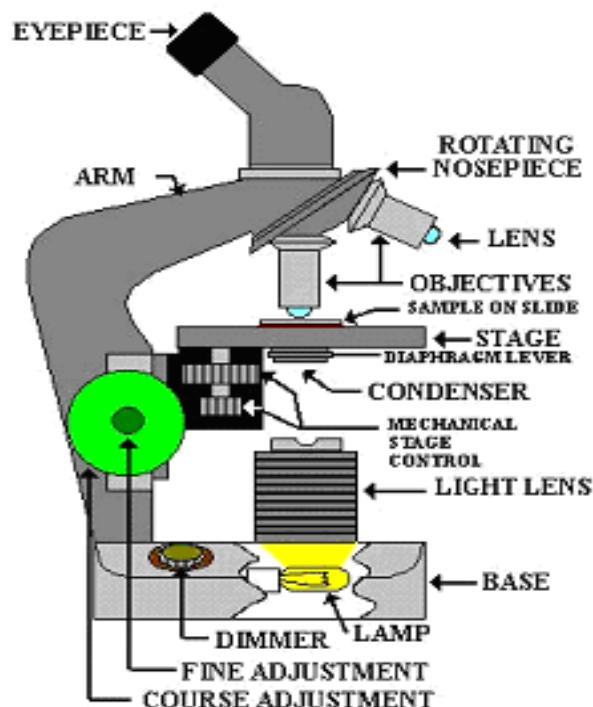
The microscope has either built in light sources as in binoculars or external light source as in monocular. The rheostat ON/OFF switch is located either on the microscope or on the external power supply and is used to regulate the intensity of light.

Various parts of a compound microscope are shown in Figure 3.

7.1.6. Magnification, resolution and working distance

7.1.6.1 Magnification is simply a function of making an object appear bigger. Magnification is produced at two stages, by the objective lenses and by the eye piece lenses.

Fig. 3 – Various parts of a compound microscope



The magnifying powers of both objectives and eye pieces are engraved on them and the overall magnification of the given microscope can be calculated by multiplying the magnifying power of the objective by that of the eyepiece.

Total magnification = ocular power x objective power.



The overall magnification achieved by the three objectives, 10x, 40x and 100x, when used with eye piece of 100x, 400 x and 1000 x, respectively.

7.1.6.2. Resolution:

Merely magnifying an object without a simultaneous increase in the amount of detail seen will not provide the viewer with a good image. The ability of a microscope (or eye) to see the details is a function of its **resolving power**. Resolving power is defined as the minimum distance between two objects at which the objects can just be distinguished as separate and is a function of the wavelength of light used and the quality of the optics. In general, the shorter the wavelength of the light source, the higher the resolution of the microscope.

Working distance is the distance between the objective lens and the specimen. At low magnification, the working distance is relatively long. As the magnification is increased, the working distance *decreases* dramatically. Oil immersion lenses practically touch the specimen. Be aware of this change in working distance with increasing magnification so as to prevent damage to your specimens.

7.1.7 Focussing procedure

7.1.7.1 Low / high power focussing

- turn on the light source.
- switch to the 10x objective lens.
- turn the coarse focus to raise the nose piece.
- place the specimen slide on the stage and secure in the proper position. Look at the slide and place it so that the specimen is over the light aperture in the stage.
- lower the objective lens to lower limit (close to slide). Raise the lens using the coarse focus knob until you see the image come into focus Adjust fine focus similarly.
- center the image and adjust the light using the diaphragm.
- readjust diaphragm if needed.
- now switch objectives to the 40x, if a higher magnification is needed. Readjust fine focus and light (diaphragm), as needed.

The microscopes should be **par focal** which means that when you switch from low (100x) to high (400x) power, a focused image at low power will remain more or less in focus at the higher power. Most likely the fine focus and diaphragm have to be readjusted slightly.

7.1.7.2 Procedure for using oil immersion lens

- locate the region of interest of the blood smear and center it with 40x objective.
- then the objective lens is raised to its limit (i.e., maximize the distance between stage and objectives) and swing the lens out of the way, about half way to the next position.
- place a small drop of immersion oil carefully, placed directly on the bloods smear over the center of the region of interest.
- rotate the oil immersion objective into position carefully and while looking from the side, lower it using the coarse focus knob until the lens *just* makes contact with the oil drop. The drop leaps up into a column as the contact is made.
- lower the lens a *smidgen* more and then using the fine focus and looking through the ocular lens, focus on the specimen.
- when done, clean lens with lens paper until no more oil comes off and clean slide if it is to be saved.



7.1.8 Handling of the compound microscope

The microscope is an instrument of precision and care must be taken to preserve its accuracy, So, precautions have to be taken to keep the microscope and lens system clean. There are only a few ABSOLUTE rules to observe in caring for the microscopes.

Please report any malfunctions immediately to your supervisor.

- Always use two hands to carry the scope -one on the arm and one under the base - no exceptions! Never carry the microscope upside down, for the ocular can and will fall out.
- Use lens paper to clean all the lenses before start of laboratory work and after using the oil immersion lens. Do not ever use anything other than lens paper to clean the lenses. Other papers are too impure and will scratch the optical coating on the lenses.
- Always remove oil from the oil-immersion objective after its use, with lens paper lightly moistened with alcohol.
- Always use the proper focussing technique to avoid ramming the objective lens into a slide. This can break the objective lens and/or ruin an precious slide.
- Always turn off the light when not using the scope.
- Always carefully place the electric wires out of harm's way. Wires looped in the leg spaces invite a major microscope disaster. Try sliding the wire down through the drawer handles by the side of your bench space.
- Avoid attack of dust and water to prevent fungal contamination.
- Never allow the objective lens to touch the cover glass or the slide. Never touch the lenses,
- Keep the stage of the microscope clean and dry.
- Do not tilt the microscope when working with oil immersion system.
- Never lower the body tube with the coarse adjustment while you are looking through the microscope.
- Never exchange the objective or oculars of different microscopes.

7.1.9 Care of the microscope

Provided normal care and common sense are exercised, the laboratory microscope will be useful for many years.

7.1.9.1 Removing dust and grease

- when not in use during the day, keep the microscope covered with a clean cloth or plastic cover to protect the lenses from dust that settles out of the air. Overnight, or if it is to remain unused for long periods, place the microscope inside it's box with the door tightly closed.
- to protect the objective lenses, rotate the 10x objective to line up with the ocular. Oil and grease from eyelashes and fingers are easily deposited on lenses and oculars as the microscope is used. Clean these parts with lens tissue or with very soft cotton cloth.
- clean the oil immersion objective after use. If it is not cleaned, the oil will harden and make the objective useless. A lens tissue or soft cotton cloth is usually sufficient for the purpose. However, never use this tissue or cloth to clean other objectives, the oculars or the mirror, otherwise oil will be transferred to these components.

7.1.9.2 Preventing the growth of fungus

In warm, humid climates, it is very easy for fungal growths to become established on lenses and prisms. These growths can create problems and may even become so bad that the microscope can no longer be used. The lenses may need to be re-polished by the manufacturer, which is very expensive and may take several months.

Fungus cannot grow on glass when the atmosphere is dry and therefore store the microscope in a dry atmosphere when it is not being used.

To avoid fungal growth, use a desiccant like silica gel, with the ability to absorb water vapour from the air. Self-indicating silica gel is blue when active but becomes pink when it has absorbed all the water. It can then be reactivated by heating.

7.1.10 Transporting the microscope

When the microscope is to be transported from one location to another, ensure that it is properly secured inside its box. The best way to do this is by means of the securing device, which screws into the base of the microscope.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	GENERAL QUALITY ASSURANCE – ELECTRONIC BALANCE				
SOP No.	SOP G. 02	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from “effective date”				

SOP G 02 – ELECTRONIC BALANCE

7.2.1 Purpose

This SOP describes the process for weighing ingredients of stains using the Analytical Balance.

7.2.2 Specifications

- microprocessor based single pan
- weighing capacity upto 100gm
- weigh upto 3rd decimal place
- autoself calibration
- auto zero setting
- liquid crystal display (LCD)
- high accuracy precision

7.2.3 Procedure

- switch on the balance by touching the ON/OFF key. The balance undergoes a brief test and is then ready for weighing.
- open the balance door.
- when using a weigh boat, reset the balance to zero by touching the TARE key.
- place the sample to be weighed on the weigh boat, and close the balance door.
- as soon as the stability detector symbol (the small ring to the left of the weight display) is seen, the reading is stable and the result can be recorded.

7.2.4 Maintenance

- clean the balance after every use.
- maintain the **LOG BOOK** for balance and record the data after every use.
- calibrate the balance at regular intervals and maintain the record of calibration in the laboratory.

FORM FOR RECORD OF BALANCE CALIBRATION

CALIBRATION RECORD OF BALANCE	
Laboratory Name:	Date of Calibration

<p>BALANCE DETAILS</p> <p>a.) Type of balance:.....</p> <p>b.) Nominal capacity (Weight in grams):</p> <p>c.) Balance number:.....</p>

<p>RESULTS OF THE TEST</p> <p>a.) Reference weight used :</p> <p>b.) Acceptable Range :</p>																						
<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th rowspan="2">REFERENCE WEIGHT (a)</th> <th colspan="2">ACTUAL WEIGHT (b)</th> <th rowspan="2">ACCEPTABLE RANGE</th> <th rowspan="2">REMARKKS</th> </tr> <tr> <th>(n)</th> <th>mean</th> </tr> </thead> <tbody> <tr> <td> </td> <td> </td> <td> </td> <td> </td> <td> </td> </tr> <tr> <td> </td> <td> </td> <td> </td> <td> </td> <td> </td> </tr> <tr> <td> </td> <td> </td> <td> </td> <td> </td> <td> </td> </tr> </tbody> </table>	REFERENCE WEIGHT (a)	ACTUAL WEIGHT (b)		ACCEPTABLE RANGE	REMARKKS	(n)	mean															
REFERENCE WEIGHT (a)		ACTUAL WEIGHT (b)				ACCEPTABLE RANGE	REMARKKS															
	(n)	mean																				
<p>STATUS OF BALANCE</p> <p>a.) Difference in actual and reference weight :</p> <p>b.) Difference in reference and test sample weight :</p> <p>c.) Balance status :</p>																						

<p>TEST PERFORMED BY :</p> <p>1.....</p> <p>2.</p> <p>CERTIFIED BY</p> <p>Name:.....</p> <p>Signature:.....</p>

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	GENERAL QUALITY ASSURANCE –PH METER				
SOP No.	SOP G. 03	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from “effective date”				

SOP G 03 –PH METER

7.3.1 Purpose

This SOP describes the method for using a pH meter required for determination of pH of buffers.

7.3.2 Principle

Before pH is measured, a one- or two-buffer calibration should be performed. The use of two buffers that cover the expected sample pH range is recommended, and calibration must be done every time the pH meter is used.

7.3.3 Specifications

- pH range 4-12
- combined pH electrodes
- liquid crystal display (LCD)
- temperature control facility

7.3.4 Reagents/equipment

- pH meter
- pH 4.0 or pH 10.0 buffer
- pH 7.0 buffer
- distilled H₂O
- beaker

7.3.5 Procedure

This procedure is specific for various makes of pH meter.

7.3.6 Measurement and auto calibration with two buffers

- select two buffers that cover the range of expected pH. One of the buffers should be near the isopotential point (pH 7.0) and the other near the expected sample pH (e.g. pH 4.0 or pH 10).
- rinse electrode with distilled water.
- place electrode on pH 7.0 buffer, then press MODE key. Calibration will be displayed on screen.
- press YES. P1 will show on the lower field of the screen.



- when the electrode is stable, Ready will appear on screen and the temperature-corrected pH of the buffer is displayed.
- press yes if the value shown on screen corresponds to the pH of the buffer. P2 will then appear on the lower field of the screen.
- rinse the electrode with distilled water, then place on the second buffer.
- when Ready appears, press yes.
- the pH meter automatically advances to the measure mode. Measure is displayed above the main field. Rinse electrode with distilled H₂O, then place on sample.
- once stable, record pH reading from meter display.

Note: Subject to change with the make of pH meter

7.3.7. Maintenance

- wash the electrode after every use thoroughly with distilled water.
- maintain the log book for pH meter and record the details after every use with remarks.
- calibrate the pH Meter at regular intervals and maintain the record for calibration in the laboratory.

CALIBRATION RECORD OF pH METER

Laboratory Name :

Date of Calibration:

pH METER DETAILS

a.) Type of pH meter:.....

b.) Name of manufacturer:

c.) pH Meter number:.....

RESULTS OF THE TEST

a.) Reference buffers used :

i. Name of manufacturer :

ii. Lot No. : pH 4.0
pH 7.0
pH 10.0

b.) Acceptable range :

REFERENCE pH (a)	ACTUAL pH (b)	ACCEPTABLE RANGE	REMARKS
	(n)		

STATUS OF pH METER

TEST PERFORMED BY

1.....

2.

CERTIFIED BY

Name:.....

SIGNATURE:.....



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	GENERAL QUALITY ASSURANCE - CLEANING AND MAINTENANCE OF GLASSWARE				
SOP No.	SOP G.04	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP G 04 - CLEANING AND MAINTENANCE OF GLASSWARE

7.4.1 Purpose

This SOP describes the method for cleaning and maintenance of the glassware used for malaria microscopy and different ways to accomplish it.

7.4.2 Principle

There has to be a minimum level of good laboratory practices which should be maintained at the laboratories under National QA Programme. Cleaning and maintenance of the glassware used for malaria microscopy is one of the basic requirements to achieve the quality laboratory results.

7.4.3 Materials required and procedures for cleaning of micro glass slides for cleaning micro glass slides the following materials are required

- a large plastic basin
- gauze or cotton wool
- a good quality detergent (powder or liquid)
- 2-4 clean, dry, lint-free cotton cloths
- clean water.

7.4.3.1 Micro glass slides

Microscope slides are usually supplied in boxes of 50 or 72. It may be described on the box as "washed" or "pre-cleaned", but the slides will still need to be properly washed, dried and wrapped. It is not possible to make good quality blood films on dirty microscope slides. Blood films made on dirty or greasy slides will wash off easily during staining. It is therefore best to discard slides that have an iridescent bloom or appear white or opaque and are not properly cleaned or slides from old stock with surface scratches or chipped edges.

7.4.4 Process for cleaning

7.4.4.1 New slides

Dip all new slides first in chromic acid overnight and then wash with detergent and clean water:

- after being soaked for a period between 30 minutes and 1 hour, rinse the slides under running tap water or in several changes of clean water.
- wipe each individual slide dry and polish with the clean, dry, lint-free clothes.
- handle cleaned slides by the edges only to avoid finger marks or grease being deposited on the surface.

7.4.4.2 Used slides

- soak used, dirty slides for a day or two in water containing detergent. Use warm water whenever possible.
- after soaking, clean the slides one by one with a small piece of gauze or cotton wool.
- remove all traces of the blood film and oil (used during microscopy) from the slides.
- do not leave the slides in the detergent for too long; soaking should be for a few days only, not weeks. If slides are left in the detergent solution for long periods, the water will evaporate, leaving a deposit on exposed slides that is impossible to remove.
- After cleaning, transfer the slides to a fresh solution of detergent and later rinse under running water or in several changes of clean water.
- Individually dry with the clean cotton clothes, as described previously.
- Separate slides that are slightly scratched and considered unsuitable for blood films during cleaning and discard them.

7.4.4.3 Wrapping cleaned slides

7.4.4.3.1 Materials required

Following materials are required to wrap cleaned slides correctly:

- sheets of thin, clean paper, about 11 cm X 15 cm in size
- empty cardboard slide boxes (of the type new slides are packed in)
- rubber bands or adhesive tape.

7.4.4.3.2 Method

- wrap clean slides with thin paper in packs of 10.
- secure each pack with adhesive tape or a rubber band.
- place pack in the cardboard slide boxes for later use or dispatch to the field.
- store slides in a dry place such as a warm-air cupboard. If stored at room temperature with high humidity, the slides will stick together after a few weeks. It will then not be possible to use them unless they are rewashed and dried.

7.4.5 Care of other glassware

Glassware such as measuring cylinders, pipettes and staining troughs must always be cleaned and dried before use. Rinse any glassware that has been used for preparation of stain in clean water immediately after use to remove as much of the stain as possible. It should then be soaked for some time, preferably overnight in a detergent solution.

- washing glassware in detergent gives satisfactory results, provided you rinse it thoroughly in clean water. Deposits of detergent left on glassware can upset the pH of buffered water and spoil the staining so always make sure that glassware is properly rinsed before being dried for future use.
- clean the staining jars at least once in a week. Any stain deposits that are allowed to dry on glassware will become difficult to remove and may spoil the staining of subsequent blood films. They can be removed by soaking the glassware in methanol and then washing it with detergent in the normal way.
- similarly, wash the beakers used for washing the stained slides once a week.

Chapter 8

STANDARD OPERATING PROCEDURES: MALARIA MICROSCOPY

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	<i>MALARIA MICROSCOPY- PREPARATION OF BLOOD SMEARS</i>				
SOP No.	SOP: M 01	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from “effective date”				

SOP: M 01- PREPARATION OF BLOOD SMEARS

8.1.1 Purpose

This Standard Operating Procedure describes the process for preparing blood smears for malaria microscopy

8.1.2 Principle

To diagnose whether a person is suffering from malaria or not, it is essential to examine the peripheral blood (thick & thin) film for malaria parasite. The thick film is made up of a large number of dehaemoglobinised red blood cells. The thin film consists of a single layer of red cells and is used to assist in the identification of malaria species, after the parasites have been detected in thick film. In a thick film, any parasites present are concentrated in a smaller area than in the thin film and are more quickly seen under the microscope. For QA of microscopy, quality of preparation of the blood smear is of vital importance.

8.1.3 Reagents, equipment required for slide preparation

8.1.3.1 Reagents/equipment and other essential items

- cleaned and wrapped slides
- spirit swab
- small bottle with cork for keeping spirit solution
- cotton
- clean cotton cloth
- slide box for 25-50 slides
- lead pencil
- register and MF-2 form
- carbon paper
- ball point pen

8.1.3.2 Specifications of glassware and other items required

8.1.3.2.1 Glass micro-slides



Glass slides used for blood smear should be clean, grease free, measuring 75mm length x 25mm width x 1.25 mm thickness and having smooth edges without any cuts. The glass should be glazed and not have any visual or chromatic aberrations or scratches.

8.1.3.2.2 Lancet/ pricking needle

Auto disposable pricking needles are best suited. However, under the NVBDCP, sterile lancets are being procured and supplied for use in malaria microscopy. **Syringe needles or other hollow needles should not be used** for collection of capillary blood. After use, the needles/lancets should be disposed/discarded in puncture proof containers after disinfecting them with 1 % hypochlorite solution.

8.1.4 Method of preparation of blood films

Standard precautions for handling and disposal of human blood should be followed (see chapter 6 on Bio safety). After the patient information has been recorded on the appropriate form, the blood films are made as under:

- take a clean glass slide free from grease and scratches
- clean the finger of the patient using a spirit swab



• Follow the following steps for preparation of the blood smear

	<p>i. select the second or third finger of the left hand</p>
	<p>ii. the site of the puncture is the side of the ball of the finger, not too close to the nail bed</p>
	<p>iii. allow the blood come up automatically. Do not squeeze the finger.</p>
	<p>iv. hold the slide by its edges</p>
	<p>v. the size of the blood drop is controlled better if the finger touches the slides from below</p>
	<p>vi. touch the drop of blood with a clean slide, three drops are collected for preparing the thick smear.</p>
	<p>vii. touch another new drop of blood with the edge of a clean slide for preparing the thin smear.</p>
	<p>viii. spread the drop of blood with the corner of another slide to make a circle or a square about 1 cm</p>
	<p>ix. bring the edge of the slide carrying the second drop of blood to the surface of the first slide, wait until the blood spreads along the whole edge</p>
	<p>x. holding it at an angle of about 45° push it forward with rapid but not too brisk movement</p>
	<p>xi. write with a pencil the slide number on the thin film, Wait until the thick film is dry</p>

While preparing the thick film remember the following points

- using the corner of the spreader, quickly join the drops of blood and spread than to make an even, thick film.
- do not excessively stir the blood but spread in circular or rectangular form with 3 to 6 movements.
- the circular thick film should be about 1 cm (1/5 inch) in diameter.

The thin film consists of a single layer of red blood cells and under the NVBDCP it is always used as a label to identify the patient.

After preparing the blood smear complete the following process :

- label the dry thin film with a soft lead pencil by writing the blood slide number and date of collection (in the thicker portion of the film).
- do not use a ball point pen for labeling the blood smears.
- allow the thick film to dry with the slide in the flat, level position protected from flies, dust and excessive heat.
- fill up the MF2 form for details of patient as indicated in the columns.
- when the thick film is dried / almost dry, put the slide in the slide box.
- dispatch it to the laboratory as soon as possible.
- second slide used for spreading the blood films may then be used for the next patient and another clean slide from the pack should be used as spreader.
- NEVER WRAP THE SLIDE IN MF2 FORM.

8.1.5 Drying the blood films

- Place the blood films properly in order to allow the thick film to dry evenly.
- Protect from flies and dust. The kind of box, good for both field and laboratory is shown in the Figure 4

Figure 4 – Box used in the field to dry blood smears



- Store the slides horizontally, which allows the thick film to dry and be at a level and with even thickness. There is a door to keep out flies and dust and a handle for carrying the box. When the thick film is completely dry, store the slides front to back in the cardboard slide box, previously used for the clean, wrapped slides.

8.1.6 Precautions to be taken while collecting blood (See Chapter 6)

8.1.7 Common faults in making blood films

Good quality thick and thin blood smears are the basic requirement for excellent microscopy. A number of faults are observed to be common in making blood films. These can affect the labeling, the staining or the examination, and sometimes more than one of these. Followings are few common faults encountered while making blood films.

8.1.7.1 Wrongly positioned blood films

Care should be taken that the blood films are correctly sited on the slide. If they are not, it may be difficult to examine the thick film. Also, portions of the films may even be rubbed off during the staining or drying process.

8.7.1.2 Excess blood

Thick films made with excess blood, after staining, will appear dark blue in their background. There will be too many white blood cells per thick film field and these could obscure or cover up malaria parasites that are present. If the thin film is too thick, Red Blood Cells (RBC) will be on top of one another and it will be impossible to examine them properly after fixation.



8.1.7.3 Too little blood

If too little blood is used to make the films, there will not be enough RBCs in the thick film and thus, the examiner will not have sufficient fields for standard examination. Besides, the thin films may also be too small for use as a label. If labeled on what so ever little blood is collected, there would not be enough blood film left for examination as well.



8.1.7.4 Blood films spread on a greasy slide

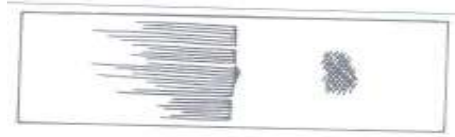
The blood films will spread unevenly on a greasy slide, which makes examination very difficult. Some of the thick film will probably come off the slide during the staining process.





8.1.7.5 Edge of spreader slide chipped

When the edge of the spreader slide is chipped, the thin film spreads unevenly, is streaky and has many “tails”. The spreading of the thick film may also be affected.



8.1.7.6 Wrongly prepared/ placed blood drops

If the thin film is too large, the thick film will be out of place and may be so near the edge of the slide that it cannot be seen through the microscope. During staining, some portion of the thick smear on the edge of the slide may wash off.



8.1.7.7 Rightly prepared / placed blood drops

A correctly made combination film should look like as given below note the size and thickness of both films. The patient's name and other identifying information can be written with an ordinary lead pencil in the thick end of the thin film.



8.1.7.8 Other common faults

Other faults that occur commonly in the preparation of blood films includes the following:

- flies, cockroaches or ants eat the dry blood and damage the films.
- blood films made on badly scratched slides (especially when old slides repeatedly reused after washing).
- the thick film is allowed to dry unevenly.
- auto fixation of the thick film occurs with the passage of time or through exposure to heat and staining becomes difficult or unsatisfactory.
- slides are wrapped together before all the thick films are properly dried and the slides stick to one another.

In warm humid climate, however, auto fixation of unstained slides occurs quite rapidly. Therefore, all the slides should be stained as soon as possible. When long storage is unavoidable, the slides can be kept in a dessicator to delay auto fixation. Besides, it is important to ensure that the slides are packed correctly and that they are not put into strong sunlight or near to any source of heat (e.g. exhaust pipe of a vehicle in the field).

Do not expose the smear to heat, sun light or alcohol as it fixes the thick smear and it would be difficult then to dehaemoglobinise it.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	PRESERVATION AND DISPATCH OF SAMPLES				
SOP No.	SOP: M 02	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: M 02 - PRESERVATION AND DISPATCH OF SAMPLES

8.2.1 Purpose

This SOP describes the correct procedure of collection, preservation and proper transportation of the blood smears to the laboratory under optimum conditions for malaria microscopy.

8.2.2 Principle

Effective diagnosis depends upon the correct procedure and the time of collection as regard to the stage of the disease, preservation and proper transportation of the clinical samples to the laboratory under optimum conditions.

8.2.3 Collection of blood smears for microscopy

See SOP M.1 for preparation of blood smears

8.2.4 Preservation of the blood films at peripheral level

- place the blood films properly in order to allow the thick film to dry evenly.
- protect from flies and dust. When the thick film is completely dry, store the slides in the cardboard/plastic slide box.
- when long storage is unavoidable, then keep the slides in a dry cool place away from direct sunlight or any source of heat.
- While storing, place the slides horizontally, which allows the thick film to dry with even thickness.

NOTE: The common practice at present is that the slides are wrapped in MF2. It is wrong practice, many a times the MF2 are torn, resulting in loss of patients details in many times. Therefore, under QA it is envisaged that if required, slides along with the MF2 should be wrapped in a separate clean piece of paper.

8.2.5 Dispatch of the blood smear from the periphery

- keep the blood smears collected in the field in the slide box till these are deposited to the malaria laboratory.
- wrap these dry slides and MF2 in a clean paper and in the same packet to avoid mixing up of the slides with those submitted by others and deposit in the laboratory.



- care should be taken while wrapping by avoiding 2 smears keeping together. As both the smears would get attached and both the smears would be lost / damaged.

8.2.6 Receipt of the blood smears in the malaria laboratory

- the LT will receive these slides and the MF2 form.
- after the smears are stained, examined and results handed over to the field/health worker, do not leave the examined slides as such.
- from these slides, remove the oils by gently rubbing with a tissue paper or soft cotton cloth.
- separate the positive and negative slides and pack separately.
- send these slides for cross checking as per instructions, to the competent authority.
- do not discard the batch of slides till the cross checking results are received, as some slides may be required to be re-examined after getting the cross checking results.
- the MO PHC will ensure that all these procedures are observed in malaria laboratory.
- where regular MO is not posted, the malaria inspector should be assigned these supervisory activities.

Note: In warm humid climate, auto fixation of unstained slides occurs quite rapidly. Therefore, stain all the slides as soon as possible.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	MALARIA MICROSCOPY - STAINING AND EXAMINATION OF BLOOD SMEARS				
SOP No.	SOP: M 03	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: M 03 - STAINING AND EXAMINATION OF BLOOD SMEARS

8.3.1 Purpose

This SOP describes the process of staining and microscopic examination of thin and thick smears prepared under SOP-M 01 for malaria parasites.

8.3.2 Principle

For accurate laboratory diagnosis, correct staining and careful examination of the blood smear is of vital importance.

8.3.3 Equipment/glassware/reagents required

8.3.3.1 Equipment

- compound microscope with 100x oil immersion objective (see SOP :G.01)
- chemical balance (see SOP: G 02 for more details)
- tally counter

8.3.3.2 Glassware

- flat bottom round flask (1000ml)
- reflux condenser
- pipette
- staining Jar
- beaker

8.3.3.3 Reagents

- J.S.B. Stain (Jaswant Singh & Bhattacharjee stain)
- Immersion oil

Constituents

The J.S.B. stain comprises of two solutions, J.S.B.I and J.S.B.II. The composition of each staining solution is as follows:

J.S.B. Solution I

- | | |
|------------------------------------------------------------------------------------------------|--------|
| • methylene blue (Medicinal) | 0.5 gm |
| • sulphuric acid (H ₂ SO ₄) 1% | 3.0 ml |
| • potassium dichromate (K ₂ Cr ₂ O ₇) | 0.5 gm |
| • disodium hydrogen phosphate dihydrate (Na ₂ H PO ₄ 2 H ₂ O) | 3.5 gm |
| • distilled water | 500 cc |

J.S.B. solution II

- | | |
|-------------------|--------|
| • eosine | 1.0 gm |
| • distilled water | 500 cc |

Buffered water

- | | |
|------------------------------------------------------------------------------------------------|---------|
| • disodium hydrogen phosphate dihydrate (Na ₂ H PO ₄ 2 H ₂ O) | 0.22 gm |
| • potassium acid phosphate (KH ₂ PO ₄) | 0.74 gm |
| • distilled water | 1000 ml |

8.3.3.4 Others

- enamel tray (for keeping the staining kits)
- heating mantle/stove for boiling stain
- filter paper for filtering the stains for day to day use
- soft cloth

8.3.4 Method of preparation of JSB stain

8.3.4.1 JSB I

- take 500 ml of distilled water in a flat bottom round flask (1000 ml), add 0.5 gm methylene blue and stir the whole solution in order to get it dissolved.
- further, add 3 ml of 1% sulphuric acid in three equal parts with stirring to ensure thorough mixing.
- add potassium dichromate (0.5 gm), which forms a purple precipitate of methylene blue chromate by oxidation of dye in acid medium.
- subsequently, add 3.5 gm of disodium hydrogen phosphate dihydrate and again stir the whole solution thoroughly, ensuring that the precipitate dissolves.
- then boil the solution with reflux condenser for one hour.
- keep the solution thus prepared overnight at room temperature for 24 hours for maturation before use.

8.3.4.2 JSB II

- measure 500 ml of distilled water into a flask and add 1.0 gm of eosin and stir the whole solution.
- leave this solution for 48 hours at room temperature for maturation before use.

8.3.4.3 Buffer water (pH 6.2 - 6.8)

- the ideal pH of water desirable for staining in JSB solution should range between 6.2 to 6.8.
- to prepare buffer wash water, in 1000 ml of distilled water in a flask, add di-sodium hydrogen phosphate (0.22 gm) and potassium acid phosphate (0.74 gm).
- thoroughly stir the solution in order to dissolve the ingredients.

8.3.5 Staining

8.3.5.1 Preparation for staining

- before staining, filter both the JSB I & II. Keep used filter papers daily till the supervisory visit by a senior officer or supervisory technician. All these filter papers must contain dates.
- change buffer water daily.

8.3.5.2 Method of staining

- dip the thin smear in methyl alcohol for a second or two for fixation.
- thick smear contains more blood, therefore, if not stained immediately smears are over dried and has to be dehaemoglobinised before staining.
- dry thoroughly in the air.
- immerse the thick & thin smears in solution II for a second or two.
- wash twice or thrice in a jar containing buffer water (pH 6.2-6.8).
- then immerse in solution I for 45 seconds.
- wash 3 or 4 times in buffered water.
- dry in air.

8.3.5.2.1 Dehaemoglobinisation of thick smear

- dip dried thick blood smears once into a beaker containing normal water and take out immediately.
- care should be taken during the process as sometimes the thick smear is washed off.
- do not touch or wipe the wet smear.
- however, in freshly prepared slides, dehaemoglobinisation is not required.
- alternatively, keep the dried smears flat and add 2/3 drops of normal water on the dry smears.
- after 30 seconds, the slide is ready for staining.
- care should be taken not to wet the thin smears during the process of dehaemoglobinisation.



8.3.5.3 Re-staining of old stained blood smears

Sometimes, need arises for re-examination of an old and stained blood smear which is already being examined. Even nicely stained smears in first instance usually fade when kept for a long time. The need of re-examination of old slides also arises due to poor staining in the first instance. In such condition the stained and oiled slide is immersed in xylene for a while, followed by a gentle wipe by a soft cloth which removes the old stains as well as the microscopic oils. The slide is then ready for re-staining following the same procedures as being done in case of fresh smears. In case, the thick smear is too thick or having more blood, the smears should be dehaemoglobinised (*as mentioned at 3.5.2.1 above*) before staining.

8.3.6 Examination of the stained blood smears

8.3.6.1 Thick film

Routinely, thick films are being examined under the NVBDCP. Routine examination of a thick film is based on examination of 100 good fields. In case of any doubt for identification of parasite species, further 100 fields should be examined before a final conclusion is made. This ensures that there is little possibility of a mixed infection (more than one species present in the blood film) being overlooked. A slide should be declared negative only after no parasites have been found in 100 fields of the blood film. A thick blood film consists of many dehaemoglobinized red blood cells packed together in a thick mass.

8.3.6.2 Thin film

Under NVBDCP, routine examination of thin film is not recommended, as it takes almost ten times longer to examine a thin film in comparison to examine a standard thick film. Very low parasitaemia could also be missed in the thin film. Examination of thin film is recommended under NVBDCP in the following circumstances

- when no thick film has been provided
- when the thick film is fixed or unreadable
- when it is necessary to confirm the identification of a species

When a thin film has to be examined, this should be done in a systematic way as given below:

8.3.6.2.1 Method

- place the slide on the mechanical stage of the microscope.
- position the 100x oil immersion objective over the edge of the film (where the red cells are the thinnest).
- place a drop of immersion oil on the edge of the middle of the film.
- lower the oil immersion objective until it touches the immersion oil.
- examine the blood film by moving along the edge of thin film then moving the slide inwards by one field, returning in a lateral movement and so on
- examine a minimum of 200 fields in a thin film.

8.3.7 Results

While examining thick and thin smears, along with the malaria parasite, normal components of the blood is also seen as described at 3. 7.4.

8.3.7.1 Malaria parasites

Malaria parasites take up stain in a special way in both thick and thin blood films that enables to distinguish the various parts of the parasite. They pass through a number of developmental stages, However, in all stages, the same parts of the parasite will stain the same colour.

- Chromatin (part of the parasite nucleus) is usually round in shape and stains deep red.
- Cytoplasm occurs in a number of forms, from a ring shape to a totally irregular shape. It always stains blue, although the shade of blue may vary between the malaria species.

8.3.7.1.1 Stages of the malaria parasite

Stages of the malaria parasite that are seen in peripheral blood films are described below:

Trophozoite stage

This stage is most commonly seen; it is often called the ring stage, as it mostly takes the form of an incomplete ring. The trophozoite stage is a growing stage, the parasite within the red blood cell may vary in size from small to quite large. Pigment appears as the parasite grows. Malaria pigment is a by-product of the growth or metabolism of the parasite. It does not stain but has a colour of its own, which may range from pale yellow to dark brown or black.

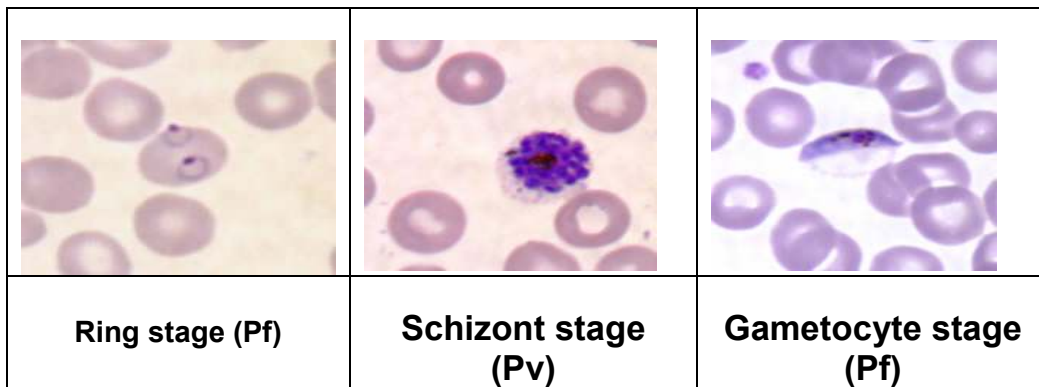
Schizont stage

During the schizont stage the malaria parasite starts to reproduce. This reproduction is referred to as asexual because the parasite is neither male nor female but reproduces itself by simple division. There are several obvious phases in this stage, ranging from parasites with two chromatin pieces to parasites with a number of chromatin dots and definite cytoplasm.

Gametocyte stage

The gametocyte is the sexual stage in which the parasites become either male or female in preparation for the next stage, the extrinsic phase which takes place in the stomach of the female anopheline mosquito. Gametocytes may be either round or banana-shaped, depending on the species. The way in which the parasite takes up the stain also helps to identify male (microgametocyte) or female (macrogametocyte). Some stages of the malaria parasite are shown in Figure 5.

Fig. 5 – Various stages of malaria parasite



(Photo source: RMRC, ICMR, Diburgarh)

8.3.7.1.2 Species of malaria parasite

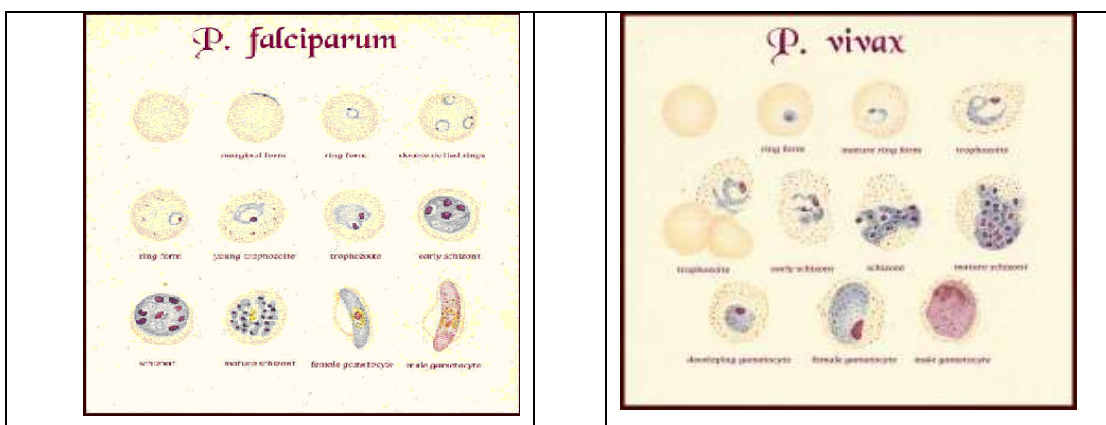
The effect of the parasite on red blood cells is also important because it helps to identify the malaria species. There are four species of malaria parasites that affect humans:

1. *P.vivax* is the commonest species in India. It is the largest of the malaria parasites found in humans.
2. *Plasmodium falciparum*, which is also the common species in India and responsible for conditions like cerebral malaria and even death.
3. *P.malariae* is a less common species in India, prevalent in some parts of Orissa, Madhya Pradesh and Chhattisgarh.
4. *P.ovale* is not prevalent in India but reported from many countries, especially from Africa.

8.3.7.2 Appearance of parasite species in thin blood films

The simplest guide to distinguish between the four species of malaria is the effect the parasite has on infected red blood cells. Features to concentrate on include the size of the red blood cell (whether it is enlarged or not) and whether or not staining reveals Schuffner's dots or Maurer's dots (also known as Maurer's clefts) within the cell as shown in the Figure 6.

Fig. 6 - *P. falciparum* and *P. vivax* as seen in the thin smears

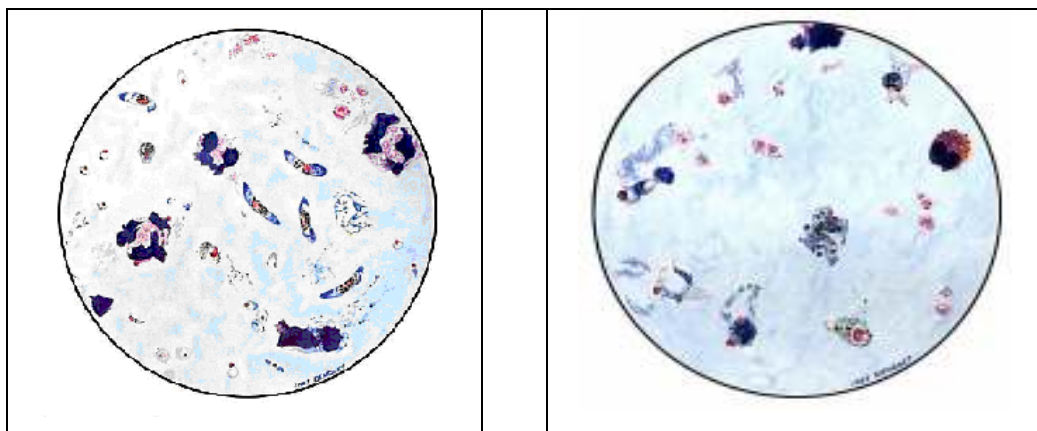


(Photo source WHO)

8.3.7.3 Appearance of parasite species in thick blood films

In a thick blood film with 100x oil immersion objective and x 7 ocular, no red blood cells are seen. The malaria parasites are seen along with the white blood cells as shown in the Figure 7. However, the parasites appear to be smaller in the thick film than in the thin blood films. The fine rings of cytoplasm of the trophozoites may appear incomplete or broken in thick blood films. Absence of red blood cells may make Schuffner's dots difficult to see. In fact, in the thicker parts of the film it may not be possible to see the stippling at all. Red cells can usually be seen surrounding parasites in the thinner parts of the films often towards the edge.

Fig. 7 - *P. falciparum* and *P. vivax* as seen in the thick smears



(Photograph source WHO)

8.3.7.4 Red blood cells

The shape of red blood cells or erythrocytes is described as a biconcave disc. Erythrocyte is the commonest cell that is seen in thin blood film. There are about 5,000,000 red blood cells in each micro litre of blood. After staining, the RBCs appear pale greyish pink. It measures about 7.5 micrometers in diameter. Red cells do not have a nucleus. Some cells may contain material that has stained differently. Such cells may appear larger than normal cells.

8.3.7.5 White blood cells

The total number of white blood cells in a microlitre of blood is about 6000-8000, which is much lower than the number of red blood cells. There are several different types of leukocytes as shown in Figure 8 which stain differently.

Typical white blood cells are described below:

8.3.7.5.1 Group – 1 Multi lobed leukocytes

Neutrophils

Neutrophils make up about 50-70% of the total white cell count in the blood of healthy persons. They have well defined granules in the cytoplasm and nucleus that stain deep purple.

Eosinophils

Eosinophils make up about 1-4% of the total white cell count in the blood of a healthy person. The granular nature of the cytoplasm is very distinctive, with the granules taking on the pinkish colour of eosin.

Basophils

Basophils are rare leukocytes, usually making up less than 1% of the total was population. Large blue or mauve granules can be seen in the cytoplasm after staining.

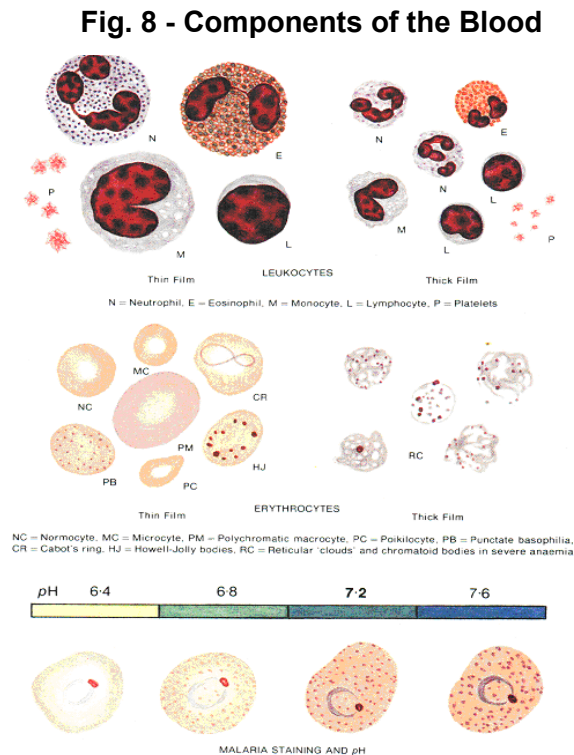
8.3. 7.5.2 Group – 2 Non-multilobed leukocytes

Monocytes

Monocytes usually make up to less than 1% of the total leukocytes. These are the largest of the white blood cells, about 12-18 um in diameter, having a large nucleus, kidney or bean shaped. The cytoplasm may contain a few granules that stain pinkish or red.

Lymphocytes

The two types of lymphocytes: large and small, make up 20-45% of the total white cells. The nucleus of the large lymphocyte is round and appears deep mauve in colour in well stained blood films. The large amount of cytoplasm stains clear water blue and may contain a few mauve staining granules. While the small lymphocyte is slightly larger than a normal red blood cell, it has very little cytoplasm and its nucleus stains a dark blue-black colour.



(Photograph source: WHO)

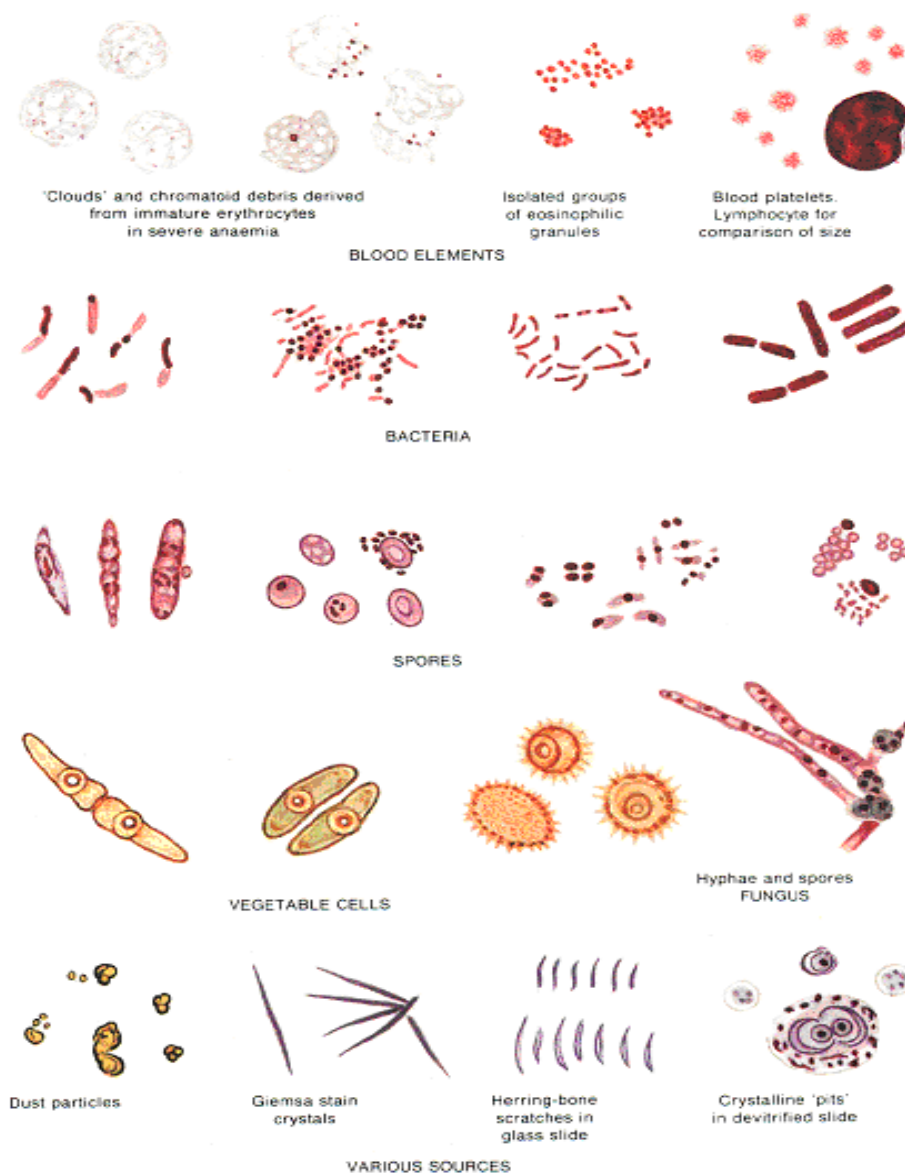
8.3.7.6 Platelets

Platelets are small, red-staining bodies of irregular shape and without nuclei they number about 1,00,000 per microlitre of blood. They often appear in groups of 5-10 but may clump together in larger numbers, if a blood film has been poorly made. It is important to be able to identify them as they may be confused with malaria parasites by inexperienced microscopists.

8.3.7.7 Artifacts in blood films

Blood films may contain many features that can cause confusion and problems in diagnosis and problems. Such features are known as artifacts. Some artifacts are depicted in Figure 9.

Fig. 9 - Artifacts that may cause confusion in diagnosis



(Photograph source: WHO)

Fungus

Will show up as artifacts on blood film. The best way to prevent fungal growths on slides is to stain blood films as soon as possible after preparation and drying them- within 48 hours at most.

Other contaminants

Dust particles floating in the air will settle on blood films while they are drying either before or after staining. Specks of dirt may be transferred from a patient's finger when a blood sample is taken or the original slide may not be perfectly clean. Some artifacts are shown in Figure 9.

8.3.8 Estimation of parasite density

8.3.8.1 Determination of parasite density in thick film

- using the 40x objective, select a part of the film that is well stained, free of staining debris and well populated with white blood cells. A well made film of even thickness is ideal for determining parasite density.
- place the immersion oil on the thick film.
- swivel the 100x oil immersion objective over the selected portion of blood film.
- confirm that the portion of film selected is acceptable and continue to examine the slide for 100 oil immersion fields. Move the blood film by one oil immersion field each time, following the pattern described by thin film.
- use a tally counter to count the fields as they are examined. For counting of parasite density by following the methods described below:

8.3.8.2 Counting of the parasite

It is necessary to establish a parasite count for the blood film for the following reasons:

- parasite counts are especially important in *P. falciparum* infections which are potentially fatal:
 - to know the severity of malaria.
 - for special purposes, such as testing the sensitivity of parasites to antimalarial drugs.
 - to know the response of the malaria parasites to the anti malarial treatment being given. This can be monitored over time by plotting the parasite count on the day of treatment and comparing it with the count in a blood film made at some specified time later.
- Besides, parasite count is very essential for preparation of QC/EQA samples to be used for testing malaria RDTs. Prior to preparation of QC/EQA samples, it is recommended to pre-qualify LTs to be utilized in the field to ensure accuracy of subsequent parasite count.

Parasite count should not start until examination of 100-field is completed and the parasite species and stages present are identified. Two methods are used to establish the parasite count as earlier.

8.3.8.2.1 Method 1: Parasites per microlitre of blood

This is a practical method of reasonable and acceptable accuracy. The number of parasites per microlitre of blood in a thick film is counted in relation to a standard number of leukocytes (8000). Although there are variations in the number of leukocytes between healthy individuals and even greater variations between individuals in ill health, this standard allows for reasonable comparisons. For this two tally counters are required, one to count parasites and the other to count leukocytes.

☞ Step 1

(a) If 10 or more parasites have been identified and counted, against 200 leukocytes, record the results on the record form in terms of the number of parasites per 200 leukocytes.

(b) In case, if only 9 or fewer parasites have been counted against 200 leukocytes counted, then continue counting until 500 leukocytes are counted; then record the number of parasites per 500 leukocytes.

☞ Step 2

In each case, the number of parasites relative to the leukocyte count can be converted to parasites per microlitre of blood by the simple mathematical formula:

$$\frac{\text{Number of parasites} \times 8000}{\text{Number of leukocytes}} = \text{parasites per microlitre}$$

In effect, this means that if 200 leukocytes are counted, the number of parasites is multiplied by 40 and if 500 leukocytes are counted the number of parasites is multiplied by 10.

8.3.8.2.2 Method 2: the plus system

A simpler method of counting parasites in thick blood film is to use the plus system. This system is less satisfactory and should not be used when parasite count is done for preparation of QC/EQA samples for use with malaria RDTs. However, in the peripheral laboratory under QA/EQA of malaria microscopy for competency/ proficiency testing of the laboratory technicians, this method would be suitable. The system entails using a code between one and four plus signs, as follows:

+	=	1-10 parasites per 100 (thick film) fields
++	=	11-100 parasites per 100 (thick film) fields
+++	=	1-10 parasites per single (thick film) fields
++++	=	more than 10 parasites per single (thick film) field



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
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SOP: M 04- REPORTING AND DOCUMENTATION OF DATA

8.4.1 Purpose

To ensure that the patients can be traced easily for providing radical treatment/follow up, it is important to record all the required information, whether the blood smears are collected during door to door visit (active surveillance) or when the patients attend the clinic (passive surveillance).

8.4.2 Principle

For ensuring uniformity in data across the country and ease in analysis and interpretation, information is usually recorded on specially designed proformae. Under NVBDCP, Malaria Forms (MFs) are used for recording these informations. These MFs are in consonance with the parameters used in the malaria programme.

8.4.3 Reporting format for EQA

Same MFs would be utilized under EQA/QA programme, as a new reporting format may create confusion among the LTs. Besides, it would increase the paper work for the LTs and there would always be a chance for discrepancy while using the same data in separate formats. Therefore, the following MFs that are crucial in providing information regarding malaria laboratory-service would be vital for EQA programme as well, which are as under (Form 01 to 08):

- Form 01 (MF 2) – Normally used by the MPWs for collecting details of the fever cases e.g., patient's name, age, sex and village, etc. A code number is given to each patient in terms of blood smear number. This will help in identification of each fever case screened, for tracing out to provide radical treatment and also for follow up, if necessary.
- Form 02 (MF 4) – Used in the PHC for monthly reporting, it provides details of the agency wise blood smears received and their results.
- Form 03 (MF 5) – Used in the PHC for monthly reporting, it provides species wise details of the positive cases and radical treatment provided.
- Form 04 (MF 7) – Maintained in the PHC, it provides age/sex wise details of each blood smear received (name and address), its result, date of radical treatment, focal spray if conducted in and around the houses reporting *Pf* cases and its detail and also the details of the slides collected from mass and contact of the *Pf* case including follow ups. It is useful for supervisors to see the time lag between blood smear collection and administering RT and action taken to locate the transmission foci and also to eliminate it.



- Form 05 (MF 8) – Maintained in the PHC, it provides agency wise details of blood smears collected (by individual MPW, FTD, etc), date of collection and submission at the laboratory and date of receipt of result. It is usually referred for cross checking the activities of the MPWs and time taken by the LT to provide the results. It is useful for the supervisors to see the performance of individual field worker.
- Form 06 (MF 9) – Used for village/sub-centre wise monthly analysis of epidemiological situation.
- Form 07 (MF 10) – Provides details of the institutional surveillance
- Form 08 (MF 16) – Provides details of the services provided by community volunteers and by malaria clinics

Data on these forms are considered as the minimum essential under the NVBDCP.

The Medical officer I/C PHC should check all data forms on a daily basis not only for completeness but also to ensure that they are being filled clearly and information collected make sense, specially regarding the services provided to the patients.

Care should also be taken to ensure that samples are correctly labeled and that all laboratory results are reported correctly and properly, as many of these would be used for preparation of test panels for EQA of RDTs.

These MFs would be filled/maintained at PHC level. However in the district level, data entry MUST BE carried out in the web based NAMMIS. As mentioned earlier, all the districts across the country are networked with computerized reporting system (For more details refer www.nvbdc.gov.in).

As the staff associated with malaria control programme is acquainted with these MFs, Dte. of NVBDCP envisages that no change would be made in these forms and these would be used, as such, for EQA programme as well.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	CROSS CHECKING OF ROUTINE SLIDES FOR QA				
SOP No.	SOP: M 05	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: M 05- CROSS CHECKING OF ROUTINE SLIDES FOR EQA

8.5.1 Purpose

It is one of the important components of EQA, widely used in malaria microscopy, used for evaluation of a LT's performance by some external agency.

8.5.2 Principle

This method of QA in malaria microscopy is the current practice in most countries. All positive and 5% of negative slides are sent to Reference laboratory for cross checking by another superior agency. Feed back is sent promptly to correct the results and also to take remedial action to improve the capacity of an individual LT.

8.5.3 Process

As mentioned in the manual on networking and capacity building, networking of laboratories has been proposed (Refer Figure 1, Manual on malaria Networking of laboratory) and the states/UT's are distributed among these Reference laboratories in the network (Table 1, Manual on malaria Networking of laboratory).

8.5.3.1 Primary level laboratory (PHC)

- the respective ROH & FWs or ICMR institutes (as mentioned in the chapter 2, Manual on malaria networking of laboratory) would convey a code number (digit) to the states and districts using the NAMMIS (all the districts in the country have access to computerized National Anti Malaria Management Information System or NAMMIS).
- however, if NAMMIS is not working due to some reason, code number would be sent by telegrams/fax etc.
- the code shall be issued on 10th day of the month and the same should be forwarded to the Primary Health Centres on the same day or latest by 11th day of the month.
- on day 12th, the slides would be dispatched from the PHC to their respective District Malaria Offices (DMOs).
- the DMO would send these slides on 13th day to the cross-checking laboratory under intimation to the State Programme Officer. The DMO would be responsible for ensuring that each PHC sends the slides in time for cross-checking.

8.5.3.1.1 Results

- the results of cross-checking by the Reference Laboratories in the Form 09 should be sent to the concerned DMOs by the 15th of the succeeding month with a copy to the state and Dte. of NVBDCP. Dte. of NVBDCP envisages use of NAMMIS for transmission of cross- checking of results as well.
- the district would pass on the results to the PHCs during the monthly review meeting which is held in each district every month.
- the states would compile the data of each district and send to the Dte. of NVBDCP.
- in case of high discrepancy rate i.e., 2% or above, the cross-checking laboratories would take the needful remedial action.
- there will be supervision of the concerned laboratory to find the condition of the microscope and to provide hands on training to the concerned LT (s).
- these remedial actions would be taken in consultation with the state programme officer.

Proforma given at Form No 09 would be used for cross-checking of slides by the designated reference laboratories.

8.5.3.1 Regional Referral Laboratories

The slides cross checked at Regional Referral Laboratories (ROH&FW/ZMO/ICMR insts) shall be cross checked by National Referral Laboratory to maintain the quality of cross checking.

- This process shall be twice in a year. Once slides examined in the PHCs during transmission period and subsequently cross checked at RRLs. Another during pre/post transmission period.
- Every fourth positive slides and tenth negative slides received from the PHC shall be send by RRLs after cross checking for re-cross checking by NIMR.
- NIMR shall convey the months to the RRLs for sending the slides cross checked by them as the transmission period is not uniform across the country.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	PREPARATION OF QA PANEL SLIDES FOR EQAS				
SOP No.	SOP: M 06	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: M 06- PREPARATION OF QA PANEL SLIDES FOR EQAS

8.6.1 Purpose

This SOP describes the preparation of Malaria positive blood smears of known diagnostic composition, for conducting EQA.

8.6.2 Objectives

The validated and coded blood smears for malaria microscopy will be prepared by the identified external institution for testing the proficiency of the LTs and accuracy in expressing results.

8.6.3 Procedure

One of the important responsibilities of the National Reference Laboratory (National Institute of Malaria Research) is to prepare the QA panel slides for conducting EQAS. The slides will be prepared by the following steps:

- prepare blood smears from malaria positive patients, following the method already described in SOP M. 01.
- collect 5ml blood by venepuncture from patients found to be malaria positive (donor's venepuncture: patients found to be malaria positive collect 5 ml blood after obtaining consent).
- on an average, prepare 50 slides from each donor's blood and stain in separate batches to avoid cross contamination during the preparation, following the procedures at SOP M. 03.
- each slide will have of both thick and thin smear.
- stain slides with JSB stain and protect by permanent mounting with cover slip.
- take proper care to mount the slides free from air bubbles.
- label the slides with a code and ID number.
- determine the consensus diagnosis by experienced microscopists from NIMR for each patient/donor and record/report the results as positive or negative for malaria, the species and the parasite density for upper and lower limit.
- fifty (50) sets of twenty (20) slides, i.e. one thousand (1000); each set containing 8 *P. falciparum*, 4 *P. vivax*, 4 *P. falciparum* + *P. vivax* mixed infection and 4 malaria negative slides will be provided for assessing the competence and proficiency of LTs in malaria diagnosis, species identification and density determination.
- to accommodate the anticipated high demand, the distribution of these sets of slides will be on a time-restricted manner twice a year.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	EQAS FOR MALARIA MICROSCOPY				
SOP No.	SOP: M 07	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: M 07- EQAS FOR MALARIA MICROSCOPY

8.7.1 Purpose

This SOP describes a structural set up for the assessment of the performance of laboratories conducting and participating in malaria microscopy EQAS by cross checking.

8.7.2 Principle and objectives

It is one of the important components of EQA for malaria microscopy for evaluation of a LT's performance and is to be introduced in India. Feed back is sent promptly after receiving the results from PHC for correction, if necessary. The objectives of this SOP are to provide guidelines to every laboratory conducting/participating in EQAS for successful performance of the programme by strictly following all stages described in the SOPM.

8.7.3 Process

8.7.3.1 Identification of members of the team

As mentioned in the manual on "Networking and Capacity Building" EQA is a team activity involving National, Regional, State, Zonal, District and PHC level members. Therefore, team members have to be identified to carry out the activities as defined for each echelon.

- **Team members at NVBDCP set up**

PHC level	-	Medical Officer I/C and LT
District level	-	District Malaria Officer
State level	-	State Programme Officer
ZMO	-	Zonal Programme Officer
ROH & FW	-	Medical Officer in charge of laboratory (wherever present), Regional Director and LTs
National level	-	QA division Dte. NVBDCP

- **Team members at NIMR set up**

Field stations	-	In charge of Parasitology Lab (wherever present) or I/c Field Station and Parasitology Lab Staff
Head Quarters	-	Coordinator, QA



- **Team members at Medical College set up**

Department of Microbiology and/or Pathology - HOD and his/her identified laboratory staff for QA

8.7.4 Setting standards and targets for EQAS

8.7.4.1 Slide bank and its role in EQAS

- preparation of QC panel slides to conduct EQA as mentioned in SOP : M 6
- maintenance of these slides in the slide bank
- in the current QA programme, the slide bank is located at the NIMR, Delhi
- NIMR would be conducting EQAS of the Regional Reference Laboratories (ROH & FWs/ZMO and NIMR Field Stations)
- NIMR would send the EQA panel slides to the ROH & FWs in the set of 5: 2 Pf, 1Pv, 1 mixed (Pf and Pv) and 1 negative
- each ROH & FW would receive 50 such sets every year

8.7.4.2 Steps of EQAS

8.7.4.2.1 At National Reference Laboratory (NRL) : (NIMR)

Twenty coded positive or negative blood smears along with the proformae properly packed (form 10) will be sent by post to Regional Referral Laboratories (ROH&FWs and NIMR field stations), every 6 months.

8.7.4.2.2 At Regional Reference Laboratory (RRL) : (ROH & FWs)

Pack 20 coded positive *P. falciparum*, *P. vivax* and mixed infection with different parasite density and negative blood smears (see SOP m6:6.3 for more details) along with proformae (form 10) will be properly packed and sent by post to PHC level laboratory, every 6 months for external quality assessment.

Though both the ROH&FWS and NIMR field stations are involved in cross checking of blood smears, for this activity only ROH & FWs would be given the responsibility. The NRL (NIMR, Delhi) would be sending the EQAS panel slides to the ROH & FWs. The ROH & FWs would be sending these slides to the PHCs either directly in the States under their jurisdiction or through ZMO. The PHCs would send the results to the ROH & FWs in time, who will compile all the reports and send quarterly to the NIMR, Delhi, where the designated official will decode, assess and compare these results and send the report to the Dte. of NVBDCP with a copy endorsed to respective RRL.

8.7.4.2.3 At PHC laboratory

Slides are to be examined at the earliest by the Lab. technician, proforma duly filled, counter signed by the medical officer and sent back to respective ROH & FWs for analysis and feed back.

8.7.4.3 Analysis of results and problems



At each level, based on the results received in the proforma given at Form 10 following actions have to be taken

8.7.4.3.1 Regional Reference Laboratories (ROH & FW) :-

- compare results of malaria microscopy of PHC laboratories with the known key.
- analyse results and identify the quality problems.
- analyse problems and provide feedback to respective laboratory.
- send quarterly reports to NIMR.

8.7.4.3.2 National Reference Laboratory (NIMR):-

- compare results of EQAS panel of slides received from RRLs.
- analyse EQAS results of RRLs and the PHC level, received quarterly from the ROH & FW and identify the quality problems.
- analysis of problems and feed back.
- quarterly report to Dte. of NVBDCP.

8.7.4.3.3 In collaboration with NVBDCP the NRL would undertake the following

- develop solution to the problems for improving quality.
- evaluate the quality improvements after EQAS implementation.
- dispatch the feed back and suggest solutions to PHC laboratory through ROH & FWs and respective SPOs/DMOs.

8.7.5 The EQAS results would be referred to for accreditation of the LTs.

8.7.6 Confidentiality

Do not, under any circumstances, divulge the results of the tests to unauthorized individuals and results of one laboratory to another.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	<i>SUPERVISION FOR ENSURING COMPETENCY AND PERFORMANCE OF LABORATORY TECHNICIANS</i>				
SOP No.	SOP: M 08	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: M 08- SUPERVISION FOR ENSURING COMPETENCY AND PERFORMANCE OF LABORATORY TECHNICIANS

8.8.1 Purpose

This SOP describes the need for supervision in malaria microscopy and the different ways to accomplish it.

8.8.2 Principle

There has to be a minimum level of competency and performance that should be achieved at the four levels of a national QA programme. The actual levels within a programme will vary according to the programme needs and the resources available.

8.8.3 Need for supervision

Supervision is necessary for a number of reasons as given below:

- to confirm that the LTs are doing their jobs according to their training.
- to enable them to make minor but necessary corrections to their work as per the local requirement.
- to assess whether they need re-orientation training or whether their performance is satisfactory.
- to enable the superiors to find whether the performance of the LT is being affected due to faulty equipment or poor quality of logistics supplied.
- to provide a good opportunity to LTs to discuss with the supervisor any difficulties they may be having which could be rectified locally.

8.8.4 Types of supervision

There are two basic types of supervision – direct and indirect.

8.8.4.1 Direct supervision

In direct supervision, the supervisor is in constant touch with the LT over a period of time. The supervisor is able to see the work place of the LT, how he/she performs. The LTs have the opportunity to discuss important aspects of work and any problem faced by them and this is helpful to both the supervisor and the assessee. LT should take corrective measures, locally.



Microscopy skill	PHC	District	ROH & FW/ZMO	National
Sensitivity: trophozoite detection	85%	90%	95%	99%
Specificity: trophozoite detection	85%	90%	95%	99%
Accuracy of reporting <i>Pf</i> when present	90%	90%	99%	99%
Quantitation-accurately distinguishing <i>Pf</i> at <10/field and > 10/field	85%	90%	99%	99%

8.8.4.2 Indirect supervision

In indirect supervision, a supervisor can judge how well the LTs are working only from the records that are being submitted regularly. However, the supervisor also needs to see that the LTs are performing in terms of providing diagnostic services. The supervisor will be assessing the quality of staining, check the filter papers and also see the microscopes, whether these are in working order or not. This will help him/her to assess how accurately slides are being examined. For this purpose, the supervisor will need to re-examine or cross-check a number of slides already examined.

8.8.5 Frequency

For this, a supervisory LT from District/Zonal/State laboratories should visit every PHC at least twice in a year.

8.8.6 Competence and performance

8.8.6.1 Competence

In microscopy, competence is the skill of a LT for performing an accurate examination and reporting of a malaria blood film.

8.8.6.2.1 Assessment of competence

Competency assessment is required at each level of the laboratories in the QA chain. A LT at PHC (primary level), District (secondary level) or ROH & FW (Regional level) would be assessed by specific tasks required at each level of the services as given in the Form no 11.

The supervisor must carry the above form during the visit, fill it and send it to the respective laboratory superior to the assessed laboratory (e.g. if PHC is assessed then to the DMO, if at District to ROH & FW, and ROH & FW is assessed, then the result is sent to NIMR). Besides, a copy would be endorsed to the Dte of NVBDCP.

8.8.6.1.2 Setting competence standards

For measuring competency standard, training courses/ materials and assessments at the end of training are essential. After assessing the competence level by above criteria there is



a need for setting standards of competence at various levels. Under the National QA programme Dte. of NVBDCP envisages that the following minimum competence levels should be achieved at the four levels of health care

National = Nodal referral laboratory: NIMR; Pf- *Plasmodium falciparum*

8.8.6.2 Performance of a laboratory technician

This refers to the accuracy of a LT examining malaria slides in routine practice. For assessment of the performance of a LT, setting standards of performance is a requisite. Under the NVBDCP, EQAS is proposed in terms of standardizing unbiased cross-checking of slides routinely examined by the LTs at peripheral laboratories (Chapter 4) as well as by assessing the LT by allowing them to examine coded panel slides. Besides, performance can be improved through effective response to problems locally by consultation visits by supervisors, re-orientation training, etc.

8.8.6.2.1 Assessment of performance

The minimum level of performance that has to be achieved at the four levels of malaria microscopy after implementing QA programme as shown in the table given below:

Microscopy Skill	PHC	Dist	ROH & FW/ZMO	National
Sensitivity- trophozoite detection	80%	90%	>95%	>99%
Accuracy of reporting Pf when present	85%	90%	>95%	99%
Quantitation- accurately distinguishing Pf at <10/field and 10/field	85%	90%	>95%	99%

8.8.6.3 Log book

- each laboratory should have a log book to record all activities carried out. The remarks and assessment of supervisor should be entered in the log book.
- the visiting supervisor/ consultant should enter details of the visit and comments in this log.
- the log book will contain duplicate pages containing the details of the checklist, as above. Besides, there would be a column for the over all remark of the supervisor.

8.8.6.4 Consultative visits

8.8.6.4.1 Importance

Staff competence is only one of many factors that can affect performance. For example, the majority of poor examination results do not always relate directly to the diagnostic capability of the LT. Rather, poor results are often due to the following:



- personal problems of the LT (e.g family emergency, sickness, etc.)
- a defectively maintained or poor quality microscope
- poor supply chain
- badly prepared, stored or transported blood slides
- poor quality of stains supplied
- reuse of slides for several time after washing
- badly stained blood slides and an unsustainable workload
- poor motivation for a variety of reasons

These deficiencies have to be dealt with “at source”. Although “on site” evaluations are time consuming and costly, they are essential for the operation of all EQA programmes, because they enable the supervisor to:

- correct all incorrect procedures “on site”
- relate the conditions of work to the performance of the staff that have been assessed by external cross- checking of slides
- assess the IQC procedures and the logistic procedures for maintaining equipment and supplies
- discuss with the LTs and the laboratory management the problems encountered by the laboratory and suggest improvements on the spot
- make decisions on training need and
- establish communication with the staff in the routine laboratories

While consultative visits are considered the most effective form of continuous monitoring of performance, some issues can limit their optimal frequency and/ or their effectiveness including:

- availability of an experienced supervisor at all level.
- cost
- inaccessibility of certain laboratories (e.g. in some remote areas of NE)
- the skills of the person conducting the consultative visit, this can produce different levels of effectiveness, as not all people have the same interpersonal skills and
- other factors that can interfere include gender and age of the consultant.

8.8.6.4.2 Consultants

Staff from the ROH&FW, NRL(NIMR) and from the Dte. of NVBDCP would perform these visits. Also from some Medical Colleges (Microbiology Division) and other research institutions would be requested by the Dte. of NVBDCP for consultancy as and when required.

8.8.6.4.3 Frequency

Consultative visits should be:

- conducted at least twice a year
- supplemented by special visits from Nodal Agency as soon as possible, if any major problem arises
- pre-informed (to ensure staff availability)

8.8.6.4.4 Tasks during the visits

At a minimum:

- the supervisor should complete the check list at form 11.
- corrective measures (e.g. training) should be undertaken, as appropriate.
- the supervisor should provide verbal feedback to the staff for improvement in the skill before leaving the laboratory (without personal and emotional hurting).
- after completion of entry in the log book, the supervisor should tear off the first copy and send this report to appropriate authorities, as soon as possible after the visit.
- each LT should be instructed to keep all slides for at least 1 month after examination. This allows the supervisor to look at previous slides.
- check whether the stain is filtered every day before use (filter paper used daily to be kept for cross-checking. Each filter paper should contain the date of use).

8.8.7 Implementation of good EQA also requires that

- LTs and the supervisors are motivated, well-organized and well-trained;
- LTs send the slides to the supervisory laboratory at the designated times and understand the reasons for sending them.
- maintenance of supply chain for uninterrupted availability of logistics.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	QUALITY AUDIT				
SOP No.	SOP: M 09	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: M 09- QUALITY AUDIT

8.9.1 Purpose

This SOP describes a process of structured internal assessment of the performance of laboratories conducting QA.

8.9.2 Principle and objective

Quality audit is the process of critical review of the laboratory to establish whether all activities that can affect quality are being carried out properly. These audits are also called first party audits i.e those audits which are performed by the staff of laboratories themselves to inspect their own system. Once a QA programme has been developed and implemented the only possible way a laboratory can verify its effectiveness is to carry out regular audits.

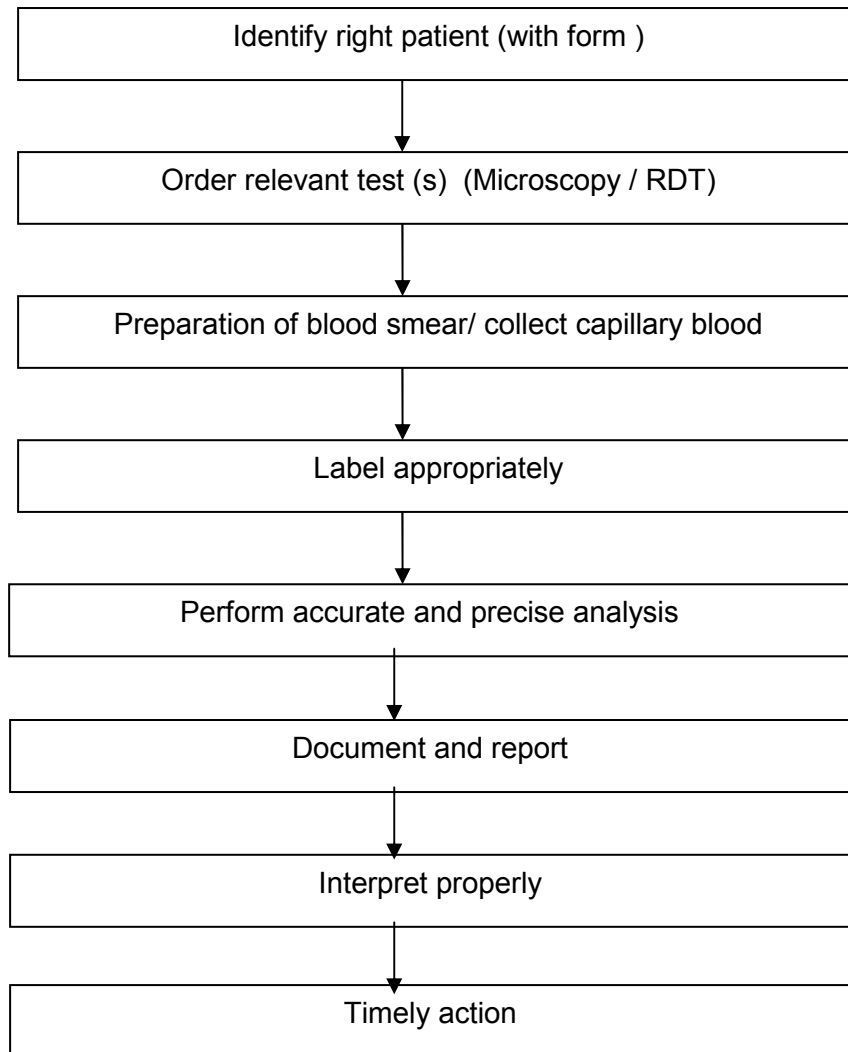
8.9.3 Process

Internal Quality Control (IQC) should be an on-going process in all laboratories, including structured review by routine staff members and formal auditing at set intervals. A person or persons should be designated to oversee IQC procedures.

8.9.4 Procedure

- Quality audits should ideally be performed at least 6 monthly, and at an interval after new staff commence work in the laboratory.
- the evaluation should be carried out by means of a formal check list, based on the external evaluation process (RF11).
- the checklists should cover the areas namely administration, safety and quality control.
- the same check list should be used each year to assess improvement in performance over time.
- during and after the evaluation, the checklists are discussed between staff of the laboratory. The check list should include recommended corrective actions wherever needed.
- completed checklists should be retained for EQAS purposes.

A simplified algorithm for quality audit of malaria diagnostic test is as follows:





Chapter 9

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Reporting form for QA of microscopy

Form 01 (MF 2) : FOR REPORTING OF BLOOD SMEARS BY SURVEILLANCE WORKER/ MULTIPURPOSE WORKER/PASSIVE AGENCY/HI/MI

Name of Subcentre : _____ Name of PHC: _____ Code Number: _____

Population : _____ Headquarters: _____

Village	No. of house	Name of the head of family	Name of patient/person	Gender	Sl. No. of blood smear	Treatment No. of tablets given Chloroquine Any other (name & Nos.)	Date of collection	Results				If +ve progressive case no.	
								P.f.		P.v	P.m.		Mixed indicate stage
								R	RG				
1	2	3	4	5	6	7	8	9	10	11	12	13	14

Note: This proforma should be in triplicate and three copies forwarded to PHC laboratory Technician who will retain one copy and send the other two to the Surveillance Inspector/Malaria Inspector.

Signature of Laboratory Technician with date of examination

Signature of field Worker/MPW/SI/MI/Passive agency

Reporting form for microscopy

Form 02 (MF 4) MONTHLY REPORT OF MALARIA PROGRAMME OF PRIMARY HEALTH CENTRE

Name of the State : _____

Name of the Distt. : _____

Total Population : _____

Sl. No	Name of PHC/Sub-centre	Total population	Active			Mass and contact			Passive			Total			Agewise Positives				RT Done				Death due to mal.	
			Blood Slides			Blood Slides			Blood Slides			Blood Slides			Under 1 year	1-4	5-15	15 +	Under 1 year	1-4	5-15	15 +	Pf Microscopically Confirmed	Only Clinically Diagnosed
			Collected	Examined	Positive	Collected	Examined	Positive	Collected	Examined	Positive	Collected	Examined	Positive										
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25

Signature :

Name : MO I/C:

Reporting form for microscopy

Form 03 (MF 5) : MONTHLY EPIDEMIOLOGICAL REPORT OF MALARIA PROGRAMME OF PRIMARY HEALTH CENTRE

Name of the State: _____

Name of the Distt: _____

Sl. No.	Name of PHC	No. Positive			Species				RT Given	Total focal spray of rooms	Total fever cases treated with 4 x AQ tabs single dose w/o B.S	Mass therapeutic measures				Balance Tablets				
		Male	Female	Total	PV	PI		others				Total	Single dose 4A.Q.+ 8 A.Q. 600 mg + 45 mg	Single dose Sulpha + Pyri	RT 5 days	Total	4 AQ	8 AQ	Sulpha + Pyri	Qin -ine
						R	RG													
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Total																				

Signature :.....

Name : MO I/C:.....

Reporting form for microscopy

Form 04 (M F 7): DETAILS OF POSITIVES AND REMEDIAL MEASURES

Subcentre: _____

District/PHC: _____

Population: _____

Code No.: _____

SI. No.	P.C. No.	Source	Group No.	Village	Name of Head of family	Name of Patient	Age	Gender	Code B.S. No.	Date of Collection	Date of examination	Species	Date of receipt of results by MPW	Radical Treatment		If died date of death and species	
														From	To		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	

B.S. Collected				FOCAL SPRAY				TIMELAG BETWEEN		Follow-up smear number and date
Contact		Mass		Date	Targeted Rooms	Sprayed Rooms	% of coverage Rooms	Collection & RT	Collection and focal spray	
No	Result	No.	Result							
18	19	20	21	22	23	24	25	26	27	28

Sign: _____

Name : _____

Designation: _____

Reporting form for microscopy

**Form 05 (MF 8) : REGISTER OF BLOOD SMEARS RECEIVED AND EXAMINED
(SUBCENTRE-WISE)**

Name of Subcentre : _____

Name of PHC : _____

Population : _____

Year : _____ Code No. : _____

Date of receipt	Name of MPW or other agency including FTD etc.	Fever treated w/o B.S. M.S.T. done	Active (A)			Passive (P) FTD			Mass & Contact (M&C)			Period of collection	Date of examination	*Number of B.S. Examined-Positive			POSITIVE SPECIES				Sl.No. of positive cases	Date of despatch of report	
			Sl.No.		Total B.S.	Sl.No.		Total B.S.	Sl.No.		Total B.S.			A	P	M&C	Pv.	Pf.	Pm.	Mixed			
			From	To		From	To		From	To													
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Total for the week																							

*In col. No. 15, 16, 17 - the no. of B.S. examined from Active (A), Passive (P) and Mass & Contact (M&C) are posted. Below these the positives among them are posted in a circle.

Sign: _____

Name: _____

Designation: _____

Reporting form for microscopy

**Form 06 (MF 9) : EPIDEMIOLOGICAL EVALUATION MASTER REGISTER
(SUBCENTRE-WISE, VILLAGE-WISE and MONTH-WISE)**

Name of State : _____ Name of Distt. : _____

Name of PHC : _____ Name of Sub-Centre : _____ Code No : _____

Sl No	Name of village	Population	Target B.S.	Fortnight	B.S. Active	AGENCY-WISE, SEX-WISE POSITIVE						AGE-WISE POSITIVE								Pf. rings only	Total Positive	API			
						ACTIVE		PAS SIV E	MASS & CONTACT		TOTAL		Pf. & MIXED				Pv. & OTHERS								
						Male	Female	Male	Female	Male	Female	Male	Female	Under 1-Yr.	1-4	5-14	15+	Under 1-Yr.	1-4				5-14	15+	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
1	A			1																					
				2																					
2	B			1																					
				2																					
Total for the month (Blue ink) (Monthly Report)																									
Total in the next month (Red ink) (supplementary report)																									

*Separate page for each month from columns 5-23 though the list of villages remains common on first page.

Sign: _____
Name : _____
Designation: _____

Reporting form for microscopy

Form 07 (MF 10) : PASSIVE AGENCIES INCLUDING FEVER TREATMENT DEPOTS REPORT
For the Month of _____

Name of PHC : _____

Name of the District : _____

Sl. No.	Name of agency/FTD	OPD-New cases	No. of fever cases	Fever cases treated with 4-AQ without B.S.	B.S. Collected	Number positive	4-AQ consumed	Number R.T. given	8-AQ consumed	Balance of drug	
										4-AQ	8-AQ
1	2	3	4	5	6	7	8	9	10	11	12
2											
3											
4											
5											

Sign: _____

Name : _____

Designation: _____

Reporting form for microscopy

**Form 08 (MF 16) : FOR REPORTING DRUG DISTRIBUTIOIN CENTRES,
FEVER TREATMENT DEPOTS AND MALARIA CLINICS**

Name of the State : _____

Report of the month of : _____

Sl. No.	District	DRUG DISTRIBUTION CENTRES				FEVER TREATMENT DEPOTS				MALARIA CLINICS			
		No.req- uired	No.esta- blished	No. of cases attended during the month	Cumulative No. of cases attended up to date	No.req- uired	No.esta- blished	No. of cases attended during the month	Cumulative No. of cases attended up to date	No. required	No. esta- blished	No. of cases attended during the month	Cumulati ve number of cases attende d up to date
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1													
2													
3													
4													
5													
Total													

Sign: _____

Name & Designation: _____

REPORTING FORM FOR EQA OF MICROSCOPY

Form 09: Proforma for cross-checking by reference laboratory

Name of the Reference laboratory _____

Total No. of blood slides received -----

Name of PHC _____

Name of District _____

Date of Receipt _____

Name of the State _____

Date of cross checking _____

Month of B.S.C. _____

S.No.	Blood Smear No.	Quality		Result						Remarks
		Smear	Stain	As Examined at PHC			Cross-Checking Organization			
				Neg	Pf	Pv	Neg	Pf	Pv	Quality of slide
1										
2										
3										
4										
5										

Internal Control slide No. used

Quality of slide:-

Preparation:-Good(G)/Average(A)/Poor(P)

Staining:- Good(G)/Average(A)/Poor(P)

Diagnosis:-Correct (c) /Incorrect(IC)

Species-Correct©/Incorrect(IC)

Signature of cross checker with date

(COUNTER SIGNED BY I/C REFERENCE LABORATORY)



REPORTING FORM FOR QA OF MICROSCOPY

Form 10: PROFORMA FOR EQAS ON PANEL SLIDES----- EQAS Year.....Round-No.

Name of the Reference laboratory _____

Name of PHC _____

Name of District _____

Date of EQAS performed. _____

Name of the State _____

S.No.	BS No.	Quality		Result							Remarks	
		Smear	Stain	species			Stages seen					
				Neg.	PV	PF	Ring	Troph	schizont	gametocyte		
M1.												
M2.												
M3.												
M4.												
M5.												

Internal Control slide No. used

Quality of slide:- Preparation:-Good(G)/Average(A)/Poor(P)

Staining:- Good(G)/Average(A)/Poor(P)

Remark Column:- Signature of Lab. Tech. with date



REPORTING FORM FOR EQA OF MICROSCOPY

Form 11. Assessment of lab facilities and competence of Laboratory Technicians

Date of visit

Activity	PHC	District	ROH&FW	Medical Research	College/ Institutes
1. Workplace					
Dedicated work area					
Efficient workflow					
Cleanliness, tidiness					
Water, electricity services					
2. Human resource					
Total No of laboratory staff					
No of trained staff					
Last training attended (Date/ Place)					
3. Standard operating procedures					
Aware about the operational guide					
Operational guide available in the laboratory					
Operational guide being followed strictly					
4. Microscope					
No of microscopes available (Monocular/Binocular)					
No of microscopes in working condition (Monocular/ Binocular)					
Proper set up of the microscope (correct illumination)					
Correct use of the microscope					
Daily cleaning/ maintenance					
Troubleshoot microscope problems					
Maintenance records					
5. Use of slides					
New slides – quality of slides					
Clarity					
Washed before use					
Used slides – no. of times used					
Washed properly					
Criteria for discarding old slides					
6. Preparation of blood smears					
Use of needle/lancet					
Quality of thick smear					
Quality of thin smear					
7. Staining					
Daily filter of J.S.B I & II before use					
Quality of stain					
Whether QC slides available and referred to regularly					
Troubleshoot staining problems					
8. Examination of stained blood smears					
Accurately identify trophozoites					



Accurately identify schizonts				
Accurately identify gametocytes				
Can differentiate between Pf and Pv				
Identify mixed infection				
Identify WBC				
Recognize the artifacts				
Whether QC slides referred to regularly				
Can identify other pathogen (e.g. mf)				
Perform parasite count				
Use of referral system				
9. Maintenance of laboratory results/ records				
Record results in a lab register & MF forms (4,7 & 8) regularly				
Total no of registers being maintained				
Results register available in the laboratory				
Register updated				
Certified by supervisor				
Data analysis				
10. Computer skill (not applicable to PHC)				
All documentation computerized				
Maintenance				
11. Training				
Training (induction and / or reorientation)				
Training on QA				
Last training attended (place)				
12. Bio-safety and waste management (Medical College & research institutes only)				
Adherence to standard practices				
Protective clothing worn in laboratory				
Appropriate biohazard waste disposal				
If needles are used then needle destroyer used or not				
Use of puncture proof container				
Sufficient sharp bins				
Availability of disinfectant				
Name of disinfectant and concentration				
13. Logistics				
Adequate supply of laboratory logistics				
Continuity of logistics				
Any problems or discontinuity in supply chain				
Laboratory inventory maintained				
14. External Quality Assessment				
"Quality Assurance" manual available in the laboratory				
Participation in Cross checking				
Number of times in a year				
Participation in National EQAS				
Results of last EQAS				



- Date of previous supervision, suggestion given by the supervisor and actions taken on suggestions
- Remarks of the supervisor, if any

Name and Designation of the supervisor:

Signature:

Note: A copy of this form should be send to Dte. of NVBDCP