

MANUAL ON QUALITY ASSURANCE FOR LABORATORY DIAGNOSIS OF MALARIA : RAPID DIAGNOSTIC TESTS



STANDARD OPERATING PROCEDURES



सत्यमेव जयते

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FOREWORD

Malaria is one of the common public health problem in India. Early Diagnosis, appropriate treatment and effective vector control measures implemented diligently across the country will help to control and contain malaria. The Gold Standard for diagnosis of malaria is the microscopy of peripheral blood smear. However, it has certain limitations. To over come these alternative diagnostic techniques have an important role to play for diagnosis of malaria.

Recently, many rapid diagnostic tests have been developed which detect antigen of plasmodia. These have been evaluated and validated and found to be of useful for malaria diagnosis particularly in remote areas where both the technical expertise and the equipment to carry out malaria microscopy are not available.

Since it is an objective test governed by biological and environmental factors it is important to have a Quality Assurance Programme to ensure the reliability of the tests performed at peripheral level.

The manual on, “Quality Assurance for Laboratory Diagnosis of Malaria: Rapid Diagnostic Tests” has been developed to fulfill the above needs.

I congratulate the Director and faculty, Dte. NVBDCP for undertaking the development and preparation of this document, which will help to implement quality assurance programme for malaria diagnosis using Rapid diagnostic tests across the country.

Dr. R.K. Srivastava
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PREFACE

Malaria microscopy for diagnosis of malaria is a time tested “Gold Standard” test. However, it has certain constraints. A lot of research and development has been going on to develop alternative methods for laboratory diagnosis of malaria. Rapid diagnostic tests have been developed validated and field tested. These RDTs detect HRPII of *P. falciparum* and pLDH of *P. vivax* in patient’s blood by the principle of immunobromatography. Being a relatively new technology it is required that all laboratory personnel /field workers involved in malaria diagnosis be trained in RDTs. Further, there are many variables like quality of test kits, storage and transport, performance of test and internal quality control which can affect the test results. There is no proper quality assurance programme so far under the Directorate of NVBDCP to ensure the quality of the results produced.

This document entitled, **Manual on Quality Assurance of Laboratory Diagnosis of Malaria by Rapid Diagnostic Tests** has been developed to be used as a bench manual by laboratory personnel to perform RDTs and its quality assurance. The manual gives details of all aspects of RDTs, preparation of quality control panels and practice of quality assurance.

I am confident that this document will be very helpful in implementing the quality assurance for laboratory diagnosis of malaria using RDTs and training of the laboratory personnel to ensure accurate and reliable results.

Dr. G.P.S. Dhillon
Director, NVBDCP



ACKNOWLEDGEMENTS

There was an ardent need to establish quality assurance practices for diagnosis of malaria using rapid diagnostic tests. RDTs are relatively new and the workers in the field need to be made aware about use of these tests and to practice quality assurance to ensure that accurate results are produced. This manual is the first step towards fulfilling this aim.

The encouragement and technical guidance provided by Dr. P.L Joshi, former Director NVBDCP to take up the project is greatly acknowledged.

NVBDCP gratefully acknowledge the support, contributions and technical inputs of all the experts developing this manual specially Dr. Krishna Ray, Consultant Microbiology; Dr. Usha Kishan Baveja former consultant & HOD HIV, NICD and Dr. S.T.Pasha, ex. Joint Director & HOD Biotechnology NICD.

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This manual will go a long way to establish the accurate procedures for laboratory diagnosis of malaria using rapid diagnostic tests and provide training to the laboratory personnel.

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PROCEDURAL HISTORY

* For details refer back cover of this manual

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ACRONYMS USED

Ab	: Antibody
Ag	: Antigen
ASHA	: Accredited Social Health Activist
ATA	: Air Transport Association
AWW	: Angan Wari Worker
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
IATA	: International Air Transport Association
LT	: Laboratory Technician
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
RDT	: Rapid Diagnostic Test
RF	: Reporting Forms
ROH&FW	: Regional Office of Health & Family Welfare
RMRC	: Regional Medical Research Centre
RRL	: Regional Reference Laboratory
RBC	: Red Blood Cell
SOP	: Standard Operating Procedure
SRL	: State Reference Laboratory
ZMO	: Zonal Malaria Officer



Glossary

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

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 requisite.

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 parasitaemia 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. During the past one decade number of Rapid Diagnostic Test kits (RDTs) for malaria have been developed, evaluated and validated for improved sensitivity and specificity. These RDTs are based on the principle of immunochromatography, require finger prick blood and detect malaria specific antigen. Three different RDTs are available commercially, one of them is specific for detecting Pf antigen and the other two detects one or more of the three human malaria species prevalent in India. These RDTs provide quick results, require less skilled persons as compared to microscopic diagnosis, do not require electricity or any equipment and develops patient's confidence as well as health services. In India, under the National Vector Borne Disease Control Programme (NVBDCP) RDTs are being used for diagnosis of malaria (only for Pf) at peripheral level.

Microscopy being the "Gold standard" for malaria diagnosis has got its limitations.

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.

Like other diagnostic tests, various conditions of manufacture, transport, storage and the method of use may impair the accuracy of RDTs. Quality Assurance (QA) and adequate monitoring of laboratory services at the peripheral level have been perceived as important links in the programme. 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 a Standard Operating Procedure (SOP) for laboratory diagnosis of malaria RDT tests was felt imperative and the manual has since been developed.



Chapter 2

CURRENT STATUS OF MALARIA RAPID DIAGNOSTIC TESTS AND QUALITY ASSURANCE

New techniques have been developed and validated for use in laboratory diagnosis of malaria. The presence of malaria parasite in patient's blood can be detected either by detecting parasite antigens and or parasite DNA.

Parasite antigens are detected by immunochromatography and the test based on this principle is named the "Rapid Diagnostic Test" (RDT). It can detect malaria parasite antigens in lysed blood by an absorbent using monoclonal antibodies (*refer chapter 3 for details on RDT*).

The parasite DNA is detected by "Polymerase Chain Reaction" (PCR). PCR is not cost effective, require well established molecular biology laboratory and trained manpower.

RDTs are relatively easy to perform. The detection of parasite's antigen is an evidence of a current or recent infection. There is therefore, a distinct advantage in using a technique which determines whether antigens are present in a person's blood or not. RDTs have been introduced under NVBDCP in endemic areas which are inaccessible or where microscopic facilities are either poor or lacking (due to operational reasons). The sensitivity of malaria RDT is dependent on several factors, including the rate of flow of blood upto the nitrocellulose strip, the adherence of antibody (Ab) to the strip, ability of the Ab to bind antigen (Ag) and the integrity of the Ab-dye conjugate. All these factors are subject to deterioration in adverse transport and storage conditions. The rates of deterioration and their effect can vary between products.

Although, in India, under the NVBDCP cross checking of examined slides is existing for ensuring the quality of microscopy, there is no structured programme for QA of malaria RDT at present. It is important to develop, implement and establish a quality assurance programme for rapid diagnostic tests as in integrated part of malaria under NVBDCP.



Chapter-3

RAPID DIAGNOSTIC TEST FOR MALARIA DIAGNOSIS

Parasitic specific antigen is detected in a finger prick blood sample by RDTs which is an immunochromatographic test. At present there are 3 different RDT kits commercially available, one of the kits detects only *P.falciparum* form of malaria, the other 2 kits reacts to one or more of the 3 species of human malaria parasite i.e. *P.falciparum*, *P.vivax* and *P. malariae*. As per WHO recommendations the kits should have sensitivity more than 95% at parasite density count of 200 parasites per μl of blood. The RDTs have following advantages:

- Require less skilled persons as compared to microscopy
- Immediate result which gains patients confidence in diagnosis

3.1 Following types RDT kits available

- I. Histidine rich protein II (HRP II), a water soluble protein produced only by *P. falciparum* and specific to *P. falciparum*.
- II. Plasmodium lactate dehydrogenase (pLDH), currently used in products that include *P. falciparum* specific, pan specific and *P. vivax* specific pLDH.
- III. Aldolase (Pan-specific)

Target antigens of commercially-available malaria rapid diagnostic tests:

	HRP II	pLDH	Aldolase
<i>P. falciparum</i> -specific	+	+	
Pan-specific (all species)		+	+
<i>P. vivax</i> -specific		+	

3.2 Types of RDT formats

The kits come in the following formats:

- Plastic cassettes
- Card
- Dip stick
- Hybrid cassette/ dip sticks

3.3 Sensitivity and specificity

Since the sensitivity of a RDT detecting malaria parasites depends only on concentration of parasite antigen circulating in patient blood. It is recommended that the sensitivity of the kit should detect at least 95% of *P. falciparum* infection/cases in 200 parasites per μl of blood and higher at higher parasite density (WHO 2000, WHO 2003).

3.4 Stability

It has been noticed that commercially available RDT kits have shown low sensitivity in products that have performed well on previous occasions. Exposure to higher temperature



probably seems to be the major factor for poor performance. High humidity is the second important factor which degrades the RDT kits rapidly.

3.5 Storage

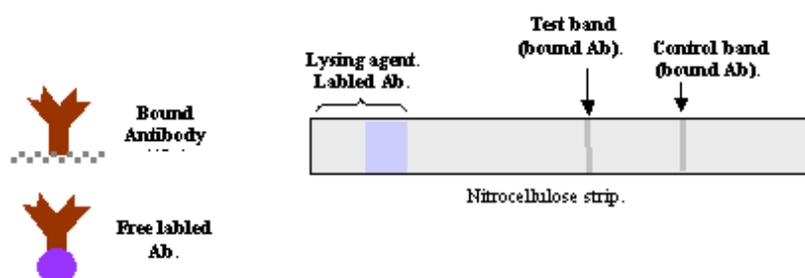
Temperature control, storage, shelf life are important factors for consideration for using RDT kits in remote areas by health workers. Kits stored at higher temperatures (> 40° C) are likely to lose sensitivity prior to expiry date. Therefore, it is recommended that the sensitivity of RDT kits may be monitored at regular intervals. Out of the 3 kits, HRP II is the most stable as compared to pLDH and Aldolase.

3.6 Mechanism of action of malaria rapid diagnostic tests

RDTs are lateral flow 'immuno-chromatographic' antigen-detection tests, where the dye-labeled antibody first binds to a parasite antigen and the resultant complex is captured on the strip by a band of bound antibody, forming a visible line (test line) on a nitrocellulose strip. A control line gives information on the integrity of the antibody-dye conjugate, but does not confirm the ability to detect parasite antigen. The various steps involved are:

- mixing the patient's blood with a lysing agent in a test strip or well, which ruptures the red blood cells, releasing more parasite protein. Labeled antibody, binds with the target protein (antigen).
- the antigen-labeled antibody complex then passes along the nitro cellulose strip by capillary action and passes over the test and control bands.
- the free, labeled antibody will capture the parasite antigen if present which will in turn be captured by the test band antibody. The dye particles on the thin band produce a visible line if sufficient antigen labeled antibody complex is present.
- the control band will become visible as sufficient labeled antibody accumulates on the line. Antibodies (or antigen) binds to the strip captures labeled antibodies which failed to bind to antigen from the patients's blood. A visible control line indicates that labeled antibody has traversed the full length of the strip, past the test line.
- the intensity of the test band will vary with the amount of antigen present, at least at low parasite densities (antigen concentration), as this will determine the amount of dye particles which will accumulate on the line. The control band intensity may decrease at higher parasite densities, as much of the labeled antibody will have been captured by the test band before reaching the control.
- mode of action of common malaria RDT format
 - i) dye-labeled antibody, specific for target antigen is present on the lower end of nitrocellulose strip or in a plastic well provided with the strip. Antibody, also specific for the target antigen, is bound to the strip in a thin (test) line and either antibody specific for the labeled antibody or antigen, is bound at the control line (**Figure 1**).

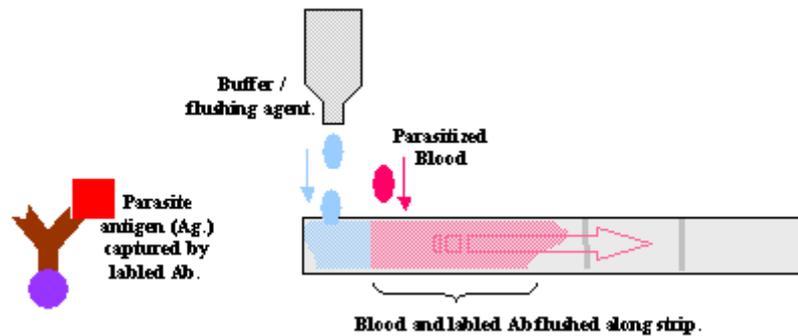
Fig 1. Nitrocellulose strip with bound Ab. (test and control lines)





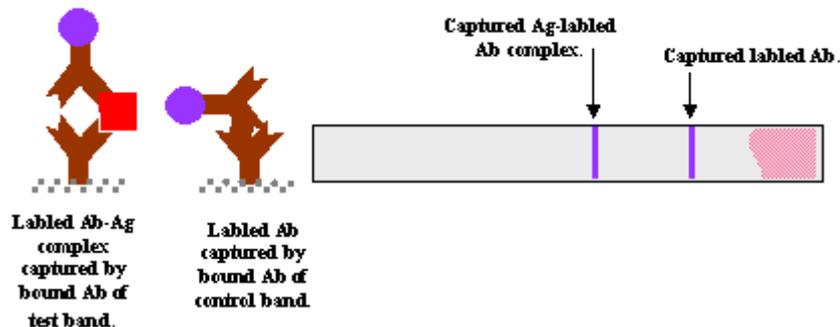
- ii) Blood and buffer, which have been placed on strip or in the well, are mixed with labeled antibody and are drawn up the strip across the lines of bound antibody (**Figure 2**).

Fig 2. Blood and labeled Ab flushed along strip



- iii) If antigen is present, some labeled antibody will be trapped on the test line. Excess-labeled antibody is trapped on the control line (**Figure 3**).

Fig 3. Captured Ag-labeled Ab complex and captured labeled Ab.



3.7 Advantages of RDTs in emergency situations

- quick to perform (in practice, about 20 minutes for most tests)
- sensitive in detecting *P.falciparum*, which causes severe malaria
- does not require skilled laboratory technicians and can be used by health workers and non health personnel after a few hours of training
- does not require electricity or laboratory equipment

3.8 Misleading results by RDT

Sometimes the result of RDTs may mislead due to certain reasons, which are as follows:

- i) **A negative test result does not always exclude malaria with certainty as:**
- there may be insufficient parasites to register a positive result
 - the RDT may have been damaged, reducing its sensitivity



- illness may be caused by another species of malaria parasite which the RDT is not designed to detect.

ii) **A positive result does not always signify malaria illness because:**

- antigen may sometimes be detected even after the infecting parasite have died (i.e., after treatment) or due to the persistence of malaria gametocytes which do not cause illness.
- the presence of other substances in the blood may occasionally produce a false positive result.
- the presence of parasites does not always signify malaria illness in individuals with high immunity as there may be other causes of fever.

3.9 Use of Rapid Diagnostic test kit under NVBDCP:

Currently under NVBDCP, HRP II based RDTs (only for Pf) is being procured and supplied to endemic peripheral areas as per the guidelines. However, RDT is not an alternative to microscopy for the diagnosis of malaria. It is implemented in the areas where microscopic facilities are not available.

It envisages that the diagnosis is taken into account by both RDT results and clinical assessment, including history and examination. Where skilled clinical assessment is not available like in the periphery (ASHA/AWW/FTD) NVBDCP guidelines as per National Drug Policy should be followed for management of malaria.

3.10 Guidelines for receiving /dispatching and storing of RDTs at periphery

RDT kits are an expensive resource and care should be taken regarding their storage to prevent losses, in inventory management. The following principles should be followed at the district/PHC level for receiving /dispatching and storing of RDTs :

- i. Before receiving the consignment, the DMO should prepare a consignee list upto village level which will be later on referred for Quality Assurance of RDTs (for details please refer SOP R - 02).
- ii. On receipt of RDTs consignment:
 - the DMO or MO PHC should undertake physical verification of the stock (or depute a responsible person for this purpose) and check for any signs of damage or tampering.
 - any damage or tampering if noticed should be immediately notified to the transporting person/ authority and the kits should not be accepted.
 - should check the Date of Expiry, if the consignment has less than 6 months expiry should not be accepted and inform the SPO/NVBDCP immediately.
 - the numbers of RDTs received should be ascertained, enter the quantity in the stock register.
- iii. from the district level immediately the RDTs should be dispatched to the PHC with instruction to distribute upto village level as per the consignee list made by



the DMO. A copy of the consignee list should be provided by the DMO to each PHC.

- iv. the principle of First Expiry First Out (FEFO) should be followed in utilizing the Kits. Those which are closer to expiry should be placed in the front and utilized ahead of others.
- v. not more than 10% of the RDT should be utilized at district hospital/ PHCs/ CHCs during emergency hours when LT is not available.
- vi. sufficient stock of RDT should be maintained in remote and inaccessible high risk areas.

RDTs supplied by NVBDCP though stable at temperatures up to 40°C, should however be kept in a cool, dry place away from direct sunlight. Care should be taken that the kits are at a considerable height from the ground away from dampness. The storage should be protected from rodents, fire, water and high temperature.

Things to remember

- prior instruction in the use and interpretation of the particular product is vital
- blood safety precaution should be strictly followed.
- product instructions should be strictly followed
- the RDT should be discarded if the envelope is punctured or badly damaged
- the result should be read within the time specified by the manufacturer

Note: An RDT can not be reused.



Chapter 4

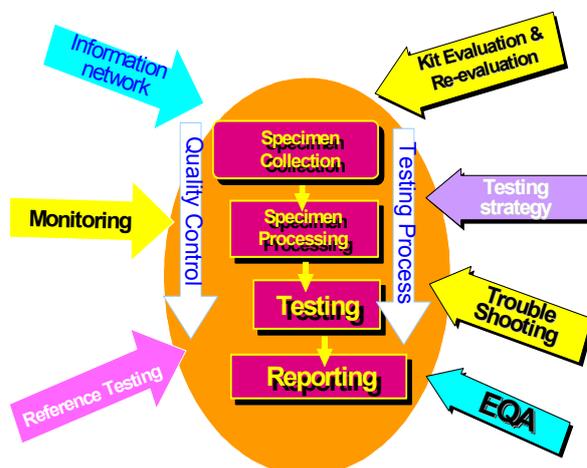
QUALITY ASSURANCE OF MALARIA DIAGNOSTIC TESTS

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).

4.1 Quality Assurance Programme

A Quality Assurance Programme (QAP) 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 of QAP are as shown in Figure 4.

Fig 4. Essential Components of QA



4.1.1 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
- monitor reagents, stains, equipment the performance of test procedures and personnel
- review test results
- 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 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.

4.1.2 Structural set up for Quality Assurance

4.1.2.1 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 respect, 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.

4.1.2.2 Confidentiality

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

4.1.2.3 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 meet 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 checking and comparing results obtained with designated Reference Laboratories (medical colleges, etc.).

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.

4.1.3 Main Components

A QA programme have two important parts: IQC and EQA. Differences between these two are shown in table 1:



Table 1. Main components of IQC and EQA

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	Release of reliable result on day to day basis	Ensure inter-laboratory comparability and assesses proficiency of participating lab.

4.1.4 Expected outcome

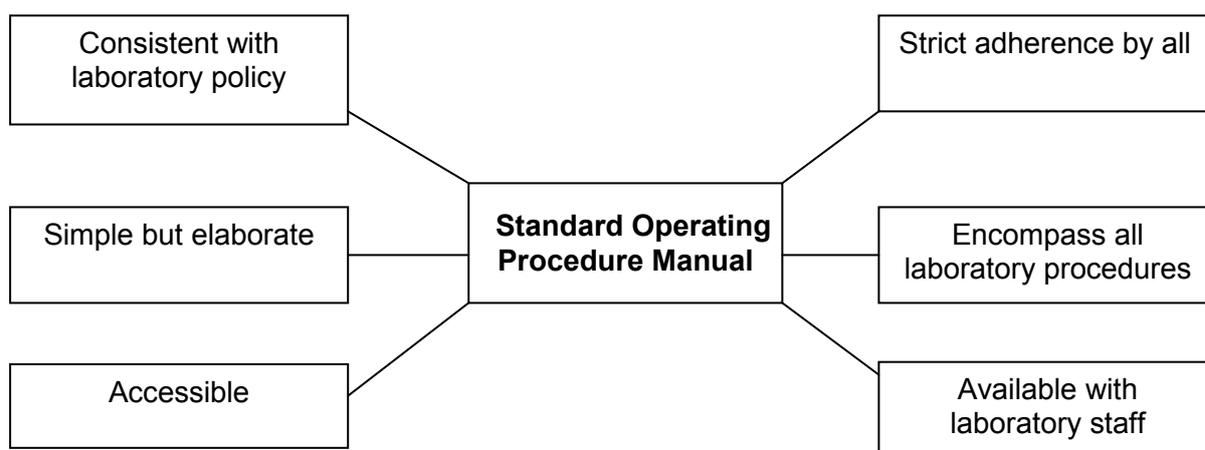
The expected outcome of a QA programme are as follows:

- 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

4.2 Standard Operating Procedures in malaria diagnosis

The Standard Operating Procedures (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 5.

Fig 5. 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.



4.2.1 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 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 EQA programme if any is available.

4.3 Proposed QAP for RDT

As mentioned under the NVBDCP, mainstay of malaria diagnosis has been microscopy. However, in recent years, for the remote and in-accessible areas where microscopy is unavailable, RDTs are being used. These are mostly used by semi skilled persons in the peripheral areas. Sometimes, they may not be exactly following the guidelines for storage of the kits. Moreover, the climatic conditions like temperature may also play a vital role in deterioration of the RDT quality in the field.

Besides, published trials and experience in various countries have demonstrated a wide variability in the sensitivity of malaria RDTs, both within and between product trials. Sensitivity is particularly variable at lower parasite densities. WHO expert consultation on Malaria Diagnosis (1999) recommended 95% sensitivity at 100 parasites/ μ l as a reasonable target for RDT performance. However, it was revised to 200/ μ l. False negative results have also occurred at higher parasite densities.

Therefore, it is important to determine the sensitivity & specificity under field conditions and to monitor any deterioration over the time. On the other hand for procurement of new RDTs for use in the programme it is of paramount importance to assess the post dispatch (post purchase) quality. Normally pre-purchase QA is mandatory for procurement of RDT under NVBDCP.

Post dispatch QA will be carried out after receiving the RDTs at the district by the DMO. Before dispatching the RDTs to the periphery, samples will be taken out randomly, and sent to the designated SRL/RRL as per SOP. R02. Thereafter randomly 7 RDTs will be collected at the interval of 3 months till the date of expiry. For detailed procedure refer to SOP. R 02.



Chapter 5

BIO-SAFETY IN LABORATORY AND SAFE DISPOSAL OF BIOMEDICAL WASTE



5.1 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 (e.g. nurses, midwives, community health workers, hospital housekeepers and doctors) or handle samples of body fluids/tissues/morbid specimens (Lab technicians, Microbiologists etc.), and thus continuously come in contact with pathogenic organisms, 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 (B & C), Human Immunodeficiency Virus (HIV) etc. and may even result in death, whereas, others are not serious and only cause morbidity.

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

Laboratories are exposed to biological hazards, besides common hazards like fire, chemical and electrical.

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

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 object.

Before undertaking any QC programme in a 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 also patients 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 acquiring hepatitis B or C is comparatively higher.



5.3 Bio-safety procedures

5.3.1 Adequate facilities

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

5.3.1.1 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 and uninterrupted water supply should be ensured.

5.3.1.2 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 equipment 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.

5.3.2 Bio-safety practices in a health care setting

5.3.2.1 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 transmission of viruses like HIV 1 and 2 and Hepatitis B and C, by the routes mentioned above. Biological and safety hazards inherent in handling human specimens, 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 infectious.

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/or with 70% alcohol before and after work and at any time before leaving the laboratory.

Barrier protection

Laboratory gown, and disposable gloves must be worn 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 material. Discard gloves whenever contaminated or perforated. 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.
- ii. 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.
- iii. 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.
- iv. ensure an effective insect and rodent control programme.

Safety procedure for QA of malaria RDT

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
- tightly close the lid of the container during transportation
- 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, 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 for details)**

iii) Pipetting

Use a rubber teat or automatic suction device properly, as outlined in SOP: G 03 for *Pipetting Techniques*. Mouth pipetting is strictly forbidden.

Biological safety cabinets, should be used whenever infectious materials are handled.



(iv) Safe handling of sharps

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

- limit use of hypodermic needles and syringes. They must not be used as substitutes for pipetting.
- never recap, bend, break or remove disposable needles from disposable syringes.
- 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. Alongwith lancets or other sharps with prior autoclaving.
- do not dispose off sharp containers in landfills.

(v) Management of accidental spill of blood

- any spilled biological material on floor/work surface must be covered with paper towel/ blotting paper/news paper/ absorbent cotton
- 10% hypochlorite solution is poured on and around the spill and left for 30 minutes before cleaning.
- all the waste is removed with gloved hands and sent for incineration in yellow bags.

(vi) Management of accidental injury

- **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 (in the laboratory first aid kit) or with sterile saline.
 - immediately seek medical attention and report to the designated nodal officer or laboratory supervisor for further management.
 - document the incident / accident in respective register for further management.

(vii) 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: _____



(viii) Bio-safety management

It is the responsibility of the laboratory supervisor to ensure the development and adoption of a bio-safety management plan. He should also ensure regular training of the laboratory staff in laboratory safety practices.

5.3.2.2 Effective sterilization and disinfection

Definitions

- (i) **Sterilization:** Complete destruction of all living microorganisms including spores.
- (ii) **Disinfection:** Destruction of vegetative forms of organisms which 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, glutaraldehyde, etc.

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. Keep the material in boiling water for 15 minutes, cool and wash the articles in running water.
- iii) **Material containing clinical specimen:** Direct on site incineration or autoclaving followed by incineration at a distant site.

5.3.2.3 Safe disposal of biomedical waste

Definitions

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

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



Management

Management of biomedical waste and disposal in a laboratory 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, segregate, package, store and transport (category 1-9) treat and dispose 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 of bio-medical waste

- i. place all bio-hazardous waste (apart from sharps) in specially designated colour coded waste containers (Table 2), separately from non-infectious waste.
- ii. all bio-medical waste containers / bags should bear biohazard symbol.
- iii. autoclave all infectious solid and non infectious waste separately in leak-proof containers e.g. autoclavable, colour- coded plastic bags, before disposal in yellow bags and incinerate. Do not dispose infectious material in landfills and do not store bio hazardous waste for more than 2 days.
- 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 disposal, regularly.
- vi. decontaminate potentially contaminated liquid waste e.g. blood before discharging in to the community sanitary sewer system.
- vii. frequently decontaminate the working area with disinfectant.

Table 2: 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 TRANSLUSCENT	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 at 121° C for 20 minutes and disposal in general waste system.
- 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 disposed by deep burial in about 2 mts. Deep pit. It should be half filled with waste then fill rest of the pit with soil. Covers of galvanized iron wire meshes should be used to cover the waste burial pit.

Records of all pits for deep burial should be maintained.

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



Chapter 6

STANDARD OPERATING PROCEDURES FOR QUALITY ASSURANCE OF EQUIPMENT

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	GENERAL QUALITY ASSURANCE - MICROSCOPE				
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

6.1.1 Purpose

It is one of the most important components of QAP 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.

For details please refer manual on Quality Assurance for laboratory diagnosis of Malaria by Microscopy.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	GENERAL QUALITY ASSURANCE – PIPETTE CALIBRATION				
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 – PIPETTE CALIBRATION

6.2.1 Purpose

This SOP describes the process for calibration of pipettes using the Gravimetric method.

6.2.2 Procedure

- if an adjustable pipette is to be calibrated, test both the maximum and minimum settings.
- place a small beaker in the balance.
- reset the balance by using the tray key and record the reading.
- while waiting for the balance to stabilize, aspirate the sample using the forward mode.
- open the balance door; add the sample to the beaker (preweighed), then close the balance door.
- record the value after the balance reading stabilizes.
- repeat steps 3 through 6 for both the maximum and minimum settings 20 times.
- calculations
 - i. calculate the mean, standard deviation, and coefficient of variation for each pipette.
 - ii the acceptable accuracy or precision in this laboratory should be within +/- 2%. If it falls outside this range, the source of error should be determined, first at the laboratory level, then, if required, at the service level.



2.3 Records

WORKSHEET

BRAND NAME: SERIAL NO: Max/Min:		BRAND NAME: SERIAL NO: Max/Min:		BRAND NAME: SERIAL NO: Max/Min:	
NO.	WEIGHT	NO.	WEIGHT	NO.	WEIGHT
1		1		1	
2		2		2	
3		3		3	
4		4		4	
5		5		5	
6		6		6	
7		7		7	
8		8		8	
9		9		9	
10		10		10	
MEAN		MEAN		MEAN	
SD		SD		SD	
CV%		CV%		CV%	
Performed by		Performed by		Performed by	
Date		Date		Date	
Remarks		Remarks		Remarks	
Action taken		Action taken		Action taken	



RECORD OF PIPETTE CALIBRATION BY GRAVIMETRIC METHOD

Laboratory Name :	Date of Calibration:
-------------------	----------------------

PIPETTE DETAILS	
a.) Type of Pipette :.....	Fix or Variable
b.) Nominal Capacity (Volume μ l) :	
c.) Pipette Number :.....	
d.) Coefficient variation (%) of Pipette given in certificate (CV% of nominal value):.....	

RESULTS OF GRAVIMETRIC TEST	
Electronic balance used :	
Accuracy of balance :	
<hr/>	
FOUND VALUES OF PIPETTE:	
Status of Pipette	
a.) Volume of Pipette for calibration	
.....	
b.) Difference in min. and max. values:	
.....	
c.) Coefficient variation % (CV%):	
.....	

TEST PERFORMED BY :
1.
2.
CERTIFIED BY
Name:
Signature:



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDPC) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	GENERAL QUALITY ASSURANCE – PIPETTING TECHNIQUE				
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 – PIPETTING TECHNIQUE

6.3.1 Purpose

This SOP describes the reverse pipetting technique.

6.3.2. Specifications

- endendorf pipettes with adjustable /variable volume
- suitable for all brands of tips
- tip ejector mechanism
- fully autoclaveable at 120° C

6.3.3 Procedure

6.3.3.1 Reverse Pipetting

Reverse pipetting, which requires an extended stroke to draw in an additional volume of liquid, is preferred because it gives better results on viscous liquids, such as blood specimens, frequently used in the laboratory.

- fit the appropriate tip.
- set the volume on the pipette counter.
- depress the pipetting button up to the second stop.
- immerse the tip about 2 to 3 mm into the sample, making sure that the pipette is held vertically.
- allow the button to retract slowly, observing the filling operation. The optimum speed for drawing depends on the sample. Also note that a larger volume enters the tip than the set value.
- when dispensing, the pipetting button should only be pressed up to the first stop.
- quickly wipe off the tip against the side of the container.
- discard tip with remaining sample.

6.3.4 Maintenance

- clean the pipette after use with 70% ethanol or 1% hypochlorite solution.
- calibrate the pipette by a standard method at regular intervals.
- maintain the data record of pipette calibration in the laboratory.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	<i>GENERAL QUALITY ASSURANCE – OPERATION OF PH METER</i>				
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 – OPERATION OF PH METER

6.4.1 Purpose

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

6.4.2 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.

6.4.3. Specifications

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

6.4.4 Reagents/Equipment

- pH meter (4-12 pH range with combined electrodes)
- pH 4.0 or pH 10.0 buffer
- pH 7.0 buffer
- distilled H₂O
- beaker

6.4.5 Procedure

This procedure is specific for various makes of pH meter.



6.4.5.1 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 in pH 7.0 buffer, then press MODE key. Calibration will be displayed on screen.
- press YES. normal 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 in sample.
- once stable, record pH reading from meter display.

Note : Subject to change with the make of pH meter

6.4.5.2 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:

<p style="text-align: center;">pH METER DETAILS</p> <p>a.) Type of pH meter:.....</p> <p>b.) Name of manufacturer:</p> <p>5. pH Meter Number:.....</p>
--

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 :

<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 – OPERATION OF THE ELECTRONIC BALANCE				
SOP No.	SOP G 05	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from “effective date”				

SOP G 05 – OPERATION OF THE ELECTRONIC BALANCE

6.5.1 Purpose

This SOP describes the process for weighing liquid for calibration of pipette using the electronic balance.

6.5.2 Specifications

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

6.5.3 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.

6.5.4 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.



RECORD OF BALANCE CALIBRATION

Laboratory Name :

Date of Calibration

BALANCE DETAILS

a.) Type of Balance :.....

b.) Nominal Capacity (Weight in grams) :

c.) Balance Number :.....

RESULTS OF THE TEST

a.) Reference weight used :

b.) Acceptable Range :

REFERENCE WEIGHT (a)	ACTUAL WEIGHT (b)		ACCEPTABLE RANGE	REMARKS
	(n)	mean		

STATUS OF BALANCE

a.) Difference in actual and reference weight :

b.) Difference in reference and test sample weight :

c.) Balance status :

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 - TEMPERATURE MONITORING			
SOP No.	SOP G 06	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP G 06 – TEMPERATURE MONITORING

6.6.1 Purpose

This SOP describes the procedure for temperature checks, as well as maintenance of all appropriate equipment.

6.6.2 Objectives

Regular temperature monitoring of incubators, refrigerators and freezers is necessary to ensure accuracy of temperature settings. Routine general maintenance of all equipment, meanwhile, is essential to keep them in good condition.

6.6.3 Procedure

- use thermometers with appropriate temperature ranges for each incubator, refrigerator and freezer in the laboratory.
- record daily temperature readings in daily temperature monitoring sheets posted in front of the equipment.
- check temperature at a set time every day. (by designated lab personnel)
- hang temperature monitoring sheet in front of the instrument.

Note : **a)** Personnel should make arrangements with other staff to perform the temperature monitoring if they are away on annual or sick leave.

b) At the end of each month, place daily temperature monitoring sheets in a folder and arrange in convenient order.

c) Relevant personnel must be notified in case of temperature deviations outside acceptable ranges.

- maintain a **Log Book** to record the temperature of each instrument with remarks as mentioned page follows:



TEMPERATURE MONITORING LOG

NAME OF THE LABORATORY MONTH / YEAR

DATE	T° C	SIGN	REMARK IF ANY	DATE	T° C	SIGN	REMARK IF ANY
1.				17.			
2.				18.			
3.				19.			
4.				20.			
5.				21.			
6.				22.			
7.				23.			
8.				24.			
9.				25.			
10.				26.			
11.				27.			
12.				28.			
13.				29.			
14.				30.			
15.				31.			
16.							



Chapter 7

STANDARD OPERATING PROCEDURES FOR RAPID DIAGNOSTIC TEST FOR MALARIA

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	RAPID DIAGNOSTIC TEST – PERFORMING A RAPID DIAGNOSTIC TEST (RDT)				
SOP No.	SOP: R 01	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from “effective date”				

SOP: R 01 – PERFORMING A RAPID DIAGNOSTIC TEST (RDT)

7.1.1 Purpose

This SOP describes the procedure for performing a Rapid Diagnostic Test supplied by the NVBDCP or for validation of a new test kit.

7.1.2 Procedure

- remove the RDT packaging.
- check desiccant for any color changes (**i.e. blue to white**). If present, discard the RDT and use another kit for testing.
- label the RDT with patient ID, date performed and other relevant information.
- Perform the test as per manufacturer’s instructions.

Note : if any RDT is discarded please inform the respective officer. (MO PHC/DMO/SPO)

7.1.3 Materials provided with the RDT

As mentioned in chapter 3, different types of RDT kits are available commercially – dipstick type, cassette type and card type. Except the test device the material supplied with a kit are almost same in all the types as mentioned below:

7.1.3.1 Dipstick type

Materials available with the kits are as follows:

- Test strip
- Reaction buffer
- Accessories:



- test tubes
- lancets

- sterile wipes
- heparinised glass capillaries / plastic loops for sample collection and application
- test tube stand
- plastic bags for storage of dipstick

7.1.3.2 Card type

Materials available with the kit

- i. Card test device
- ii. Reaction buffer/ assay diluent
- iii. Accessories
 - dropper
 - rest are same as in dipstick except test tubes

7.1.4 Procedure for testing

It is done according to manufacturers instructions (for details please see chapter 3). In general it is as follows:

- label the test strips /card with the patient identification number/name.
- allow the reagents to attain room temperature if kept in cool chain.
- select the finger for puncture, clean with antiseptic or sterile wipes (provided in the kit) allow to air dry. (same as in the case of microscopy)
- puncture the finger with a sterile lancet (provided in the kit).

7.1.4.1 Dipstick

- aspirate blood into the heparinised glass capillary upto the mark by holding the tube in the centre. If loop is provided in the kit, touch the blood drop by the loop.
- transfer the blood immediately to the test strip, just below the arrow on the absorbent pad.
- place the test strip into the reaction tube (provided in the kit) containing 200µL or 4 drops of reaction buffer.
- at the end of 15 minutes remove the dipstick from the sample tube to interpret the result. Results should **not be read beyond 20 minutes** because ghost lines may appear in negative samples due to drying of the membrane and may give false positive result. True positive results, however remain unaffected.
- the procedure performing the RDT (dipstick method) is given in the **figure : 6**
- interpret test result at 5-20 minutes, **Figure 7**.
- **do not interpret after 20 minutes.**



Fig 6. Performing a RDT by using dip stick

		
Open the box containing the test device and other accessories	Take out the above material	Take out one test device and write the patient name
		
Select the ring finger	Clean the finger with spirit swab	Prick the finger with the lancet provided with the kit
		
Allow a drop of blood to ooze out	Collect the blood by capillary tube provided in the kit	Put the blood in the absorbent pad
		
Put 4 drops of reaction buffer into the test tube	Dip the test strip into the reaction tube and leave for 15-20 minutes	If 2 lines (bands) appears after 15-20 minutes the person is suffering from <i>P. falciparum</i>
		
If only 1 line (band) appears the test is working and the patient is negative for malaria	If no line appear after 15-20 minutes discard the test	Keep the RDTs for record



7.1.4.2 Card type

- remove the test device and the dropper from the foil pouch and place it on flat, dry surface.
- slowly add 10µl (1 drop) of whole blood, serum or plasma to the sample well and add 70µl (2 drops) of the assay diluent.
- as the test begins to work, a purple colour is seen moving across the result window in the center of the test device.
- Interpret test result at 5-20 minutes, **Figure 8**.
- **Do not interpret after 20 minutes.**

Note: The above interpretation time is based on reading the test results at room temperature of 15-30°C. If the room temperature is lower than 15°C, then the interpreting time should be increased proportionately.

7.1.5 Interpretation of results

7.1.5.1 Dipstick type

NEGATIVE RESULT



If only 1 line (band) appears the test is working and the patient is negative for malaria

POSITIVE RESULT



If 2 lines (bands) appear after 15-20 minutes the person is suffering from *P. falciparum* malaria

INVALID RESULT



If no line appears after 15-20 minutes discard the test and repeat the test

Fig 7. Result of Dipstick type test

7.1.5.2 Card type

Fig 8. Result of Card type test

NEGATIVE RESULT



If only 1 line (band) appears the test is working and the patient is negative for malaria

POSITIVE RESULT



If 2 lines (bands) appear after 15-20 minutes the person is suffering from *P. falciparum* malaria

INVALID RESULT



If no line appears after 15-20 minutes discard the test and repeat the test

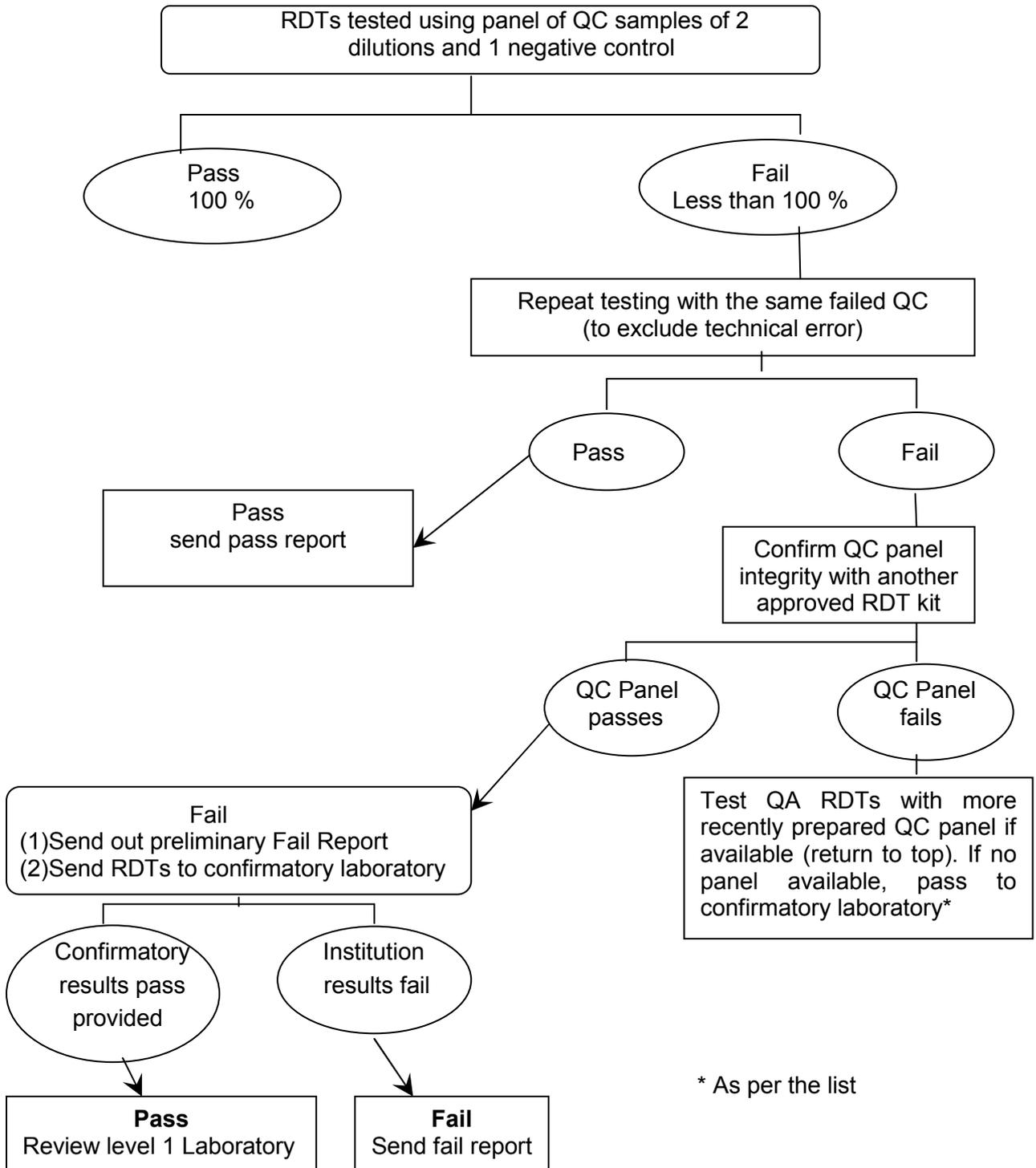


Note: Any positive test without control line (Repeat the test)

7.1.6 Procedure for Passing/Rejecting new RDT lots :

When new RDT are tested for validation of sensitivity and specificity the criteria adopted from WHO (WPRO) as indicated in the figure 9 should be followed:

Fig 9. - Flow Diagram of Proposed testing for validation of RDTs



* As per the list



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	RAPID DIAGNOSTIC TEST - QUALITY ASSURANCE OF MALARIA RDTs				
SOP No.	SOP: R 02	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: R 02 - QUALITY ASSURANCE OF MALARIA RDTs

7.2.1 Purpose

This SOP describes the process of Quality Assurance of malaria RDT kits.

7.2.2 Objective

To provide guidelines for the testing of RDTs using Quality Control (QC) samples to assess whether the sensitivity and specificity of the RDT batch is acceptable for use in the field.

7.2.3 Background

Reliance on RDTs to guide malaria case management is expected to increase over the years. A QA system for RDT is therefore needed before and during deployment of this diagnostic tool in the field. An integral component of the QA system is the development and use of standardized QC panel samples to test the threshold sensitivity of RDTs and to determine if any deterioration has occurred. RDTs should be tested on receipt from a manufacturer prior to use in the field and further testing should be performed at a later date with the RDTs in the field to ensure that acceptable sensitivity is maintained on the lot that passed the initial QA testing.

The present QA programme on RDT will strengthen the NVBDCP in bringing confidence about the quality of RDTs used.

7.2.4 Quality assurance of RDTs

Malaria RDTs are affected by various conditions of manufacture, storage and use can impair their accuracy and reliability.

QA program for malaria RDTs aims to ensure high accuracy of tests in the hands of end-users. Besides quality, this programme also aims to monitor technical standards of the RDTs and processes to minimize environmental impacts.



7.2.4.1 Requirements for QC of RDT manufacturers

- approval of manufacturing facility by Drug Controller General of India's office.
- evidence of GMP / ISO certification.
- post-manufacturing QC testing by using parasite based QC samples prepared by national/regional reference laboratories. These should be from geographically diverse areas.
- pre-release QC testing of RDT lots performed by using same samples used for post-release testing by QC laboratories.
- evidence data of stability up to the expiry date.
- source and details of raw materials and format of products used in RDTs and also if there is any change in the same should be clarified.
- real-time heat stability data on each lot, prior to release. product type, lot numbers and expiry dates should be clearly labeled on all packaging – sufficient labeling containing essential information on individual RDT envelopes.

7.2.5 Equipment/reagents

- HRP 2 and / or pLDH, RDT kit
- Pipettes (One each): 1-20 µl; 20-200 µl; 200 – 2000 µl
- - 20°C freezer
- Thermometer (range: -20°C to +20°C)
- Thermometer (range: 0°C to 100°C)
- + 4°C Refrigerator
- Incubator (range: 20°C to 80°C) – only at NRL
- Pipette tips (as per pipettes)
- QC samples: 200, 2000 parasites/µl

Note: in addition to above

- P. falciparum* infected blood from four patients
- Malaria negative blood from two healthy subjects

7.2.6 Procedure of testing RDT kits using QC samples

- follow the procedure for receipt of RDTs
- use QC samples from 2 different sources to test RDTs.

Select the following samples:

- *P. falciparum* positive with atleast 20000 parasites/µl and a negative control (0 parasites/µl).
- use the QC samples (200 and 2000 parasites/ µl) from each patient to test two sets i.e., two replicates
- use the negative control to test only two RDTs.
- one hour before RDT testing, thaw 50 µl aliquot each of the required QC samples.

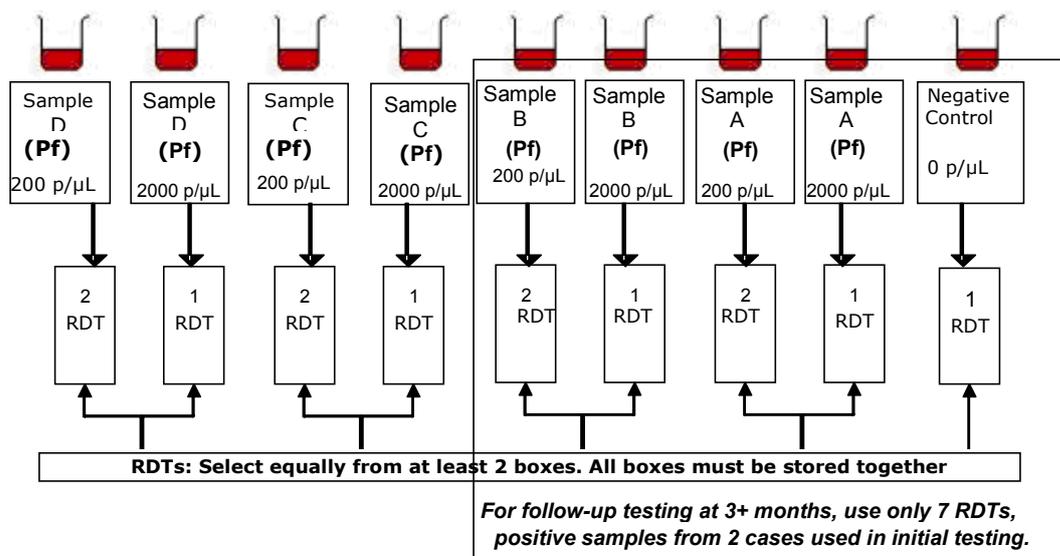


- RDTs should be brought to room temperature BEFORE OPENING the package for testing, if stored in cold chain.
- from the RDT lot, remove a total of 13 RDTs, using at least 2 different boxes at each dilution.
- check integrity of RDT packaging when opening.
- ensure no signs of moisture (e.g. silica gel desiccant, if present, should be colourless). If signs of moisture DO NOT use the RDT.
- test the RDT kits with the QC samples.
- record the results on the QA result sheet.

7.2.6.1 Immediate QA

- follow the procedure for receipt of RDTs (SOP - R 11).
- total RDT tested = 13 RDTs (Figure 10)
- select QC sample from 4 *P. falciparum* cases; testing 2 RDTs against 200 parasite/ μ l and 1 against high (2000 parasites/ μ l) from each case. Test 2 RDTs against a negative sample (Figure 10)
- one hour before the RD testing, thaw the selected QC aliquots. Refer to SOP: R 04 for thawing procedure.
- from the respective RDT lot, remove a total of 13 kits each from 4 different boxes if available. (Figure 10).
- check the RDT packaging when opening, ensure no signs of moisture. If moistened, DO NOT use the RDT.
- perform RDT testing as per manufacturer's instructions. Transfer the blood (QC samples) to the RDT strip by pipette (See SOP:G 03).
- record the results on the QA result sheet RF 01.

Fig 10. Flow diagram of immediate RDT QA procedure



Notes

- According to WHO recommendations 95% sensitivity and a minimum parasite count of 200 parasites/ μ l to be taken as lowest parasitaemia to determine the quality assurance of RDTs. The same criteria i.e. 95% sensitivity and a



Quality Assurance of Malaria Diagnostic tests

minimum of parasite count of 200 parasites/ μ l is proposed as lowest detectable parasitaemia for QA of RDTs under NVBDCP.

- dilutions will be prepared based on an initial parasite count (refer Manual on Quality Assurance of Laboratory Diagnosis of Malaria Microscopy SOP 03), and therefore some variability in malaria microscopy is unavoidable and exact parasite densities will vary around the designated value.
- there may also be variation between the relationship between parasite density and antigen concentration due to sequestration and antigen persistence.
- ideally, four samples from different cases should be used for QA testing. If four cases are not available, two cases may be used.

Do not re-use frozen aliquots of QC samples – ONE USE ONLY.

7.2.6.2 Long term QA

- sufficient stock should be available for testing of the RDTs every 3 months.
- note the expiry date i.e. 12 months then calculate the number of intervals at which RDTs should be tested i.e. 3, 6, 9, 12 = 4 intervals.
- store the RDTs reserved for QC testing below 28°C or according to manufacturer’s instructions/protocols.
- after initial test of 13 RDTs, 7 RDTs should be tested at each interval with 6 positive and 1 negative sample (**Figure 10**).
- retain extra RDTs for repeat testing or if extra RDTs are required to be sent to a confirmatory laboratory.
- a total quantity of 69 RDTs (41 + spare 28) from each lot of supply is required for the complete QA testing including spare RDT-kits (**Figure 11**)
- for validation of a new RDT, if a +28°C incubator is not available, store RDTs in an air-conditioned room and record minimum and maximum temperature daily.

Note – These tests will be carried out designated laboratories (SRL/ RRL/ NRL), not in the PHC or district malaria laboratories

Fig 11. Requirement of RDTs for immediate and long term QA

Immediate QA	13 RDTs	
3 months	7 RDTs	
6 months	7 RDTs	
9 months	7 RDTs	
12 months	7 RDTs	
Total	41 RDTs	Plus spare kits (minimum 28)



7.2.7 Results

For a lot of RDTs to pass the QA assessment, all QC dilutions must be positive (100%) and the negative control must be negative total no. of dilutions required are indicated in **Table 3**:

Table 3. Dilutions for QC

QC Dilutions <i>Pf</i> (parasites / μ l)	Desired number positive / number tested	
	Initial	subsequent
200	8/8	4/4
2000	4/4	2/2
0	1/1	1/1

If the above criteria are not met on initial testing, then further procedures should be undertaken, see algorithm (Figure 12)

7.2.8 Reporting

- for immediate QA, prepare a report using a *Report* form RF 01, within five working days of receipt of the RDTs. The report should be submitted to the Dte. of NVBDCP with a copy endorsed to NRL.
- issue long term QA report every 3 months, within the expiry date. Use RF 03 for record and report. The report should be submitted to the Dte. of NVBDCP with a copy endorsed to NRL as above.
- all the reports should be submitted within 48 hours of the testing.
- if an error is detected in a report and the report has already been sent to the NVBDCP and NRL, the original report should **NOT** be amended but a new report generated (with a new date) with the original results enclosed and a supplementary section detailing the correct information and explanation regarding the error that occurred should be sent.



Notes

- the integrity of the QC samples can be checked with stock RDT i.e. RDTs stored in the laboratory that are considered to have good sensitivity and are of high quality.
- presently QC samples will be used for QA of RDTs only against *P. falciparum* parasites.
- RDTs should have a sensitivity of 95% for *P. falciparum* at 200 parasites/ μ l. However, there are no such criteria as to how RDTs should perform for *P. vivax*. Therefore, if QA of RDTs for *P. vivax* is carried out, then QC samples at 500 p/ μ l and 5000 p/ μ l are recommended by WHO.
- QC dilutions are prepared based on an initial parasite count (refer Manual on Quality Assurance of Laboratory Diagnosis of Malaria by Microscopy SOP 03). The relationship between parasite density and antigen concentration may vary. Consequently, RDT lots that do not pass initial testing should be checked at a second facility, before rejection, in consultation with the Dte. of NVBDCP. While referring to the second laboratory, complete the RF 04 and send along with the RDT sample.
- for long term QA, the RDTs are stored at +28°C (room temperature) rather than +4°C, as RDTs are supposed to be stored below 30°C. In the field, RDTs are likely to be kept closer to 28°C than 4°C.
- **Results must be interpreted with caution and reported without a comment or 'pass' and 'fail' to the end user.**
- **for any complication in result the testing laboratory should consult the QA coordinator at Dte. of NVBDCP.**

7.2.9 Quality Assurance of Malaria RDTs used at peripheral levels

The process of QA at peripheral levels (upto village) has to be monitored by DMO of the respective district to determine any deterioration in the kit. Both immediate and long term QA will be performed with the RDT kits supplied to the periphery. It will be the responsibility of the DMO to pick up 2 samples of different packages to check the sensitivity and specificity of RDTs on quarterly basis by selecting the villages randomly. The procedure of testing of RDT has to be strictly followed as laid down in the SOP.

- at the time of distribution of RDTs, DMO should collect the lot number and batch number of the consignment. He should retain randomly 20 kits out of the entire lot.
- out of these 13 kits should be send to the designated laboratory for performing Quality Assurance by immediate testing as given at 2.6:
- rest 7 kits should be retained by the DMO and store properly to refer in future if required.
- after 3 months randomly he should select some PHCs, out of which from one sub center he will pick up 1 RDT. The process should be repeated to collect total 7 RDTs from different sub centers after every 3 months.
- the next batch of 7 RDTs should be collected from different centers at an interval of 3 months.
- the process of QA should be continued till the date of expiry (DOE) as mentioned on the kits by the manufacturer. (e.g. if DOE is 12 months from the date of manufacturing and consignment is received after 3 months, than on receipt the 1st round of QA, thereafter 2nd, 3rd and 4th round should be carried out.



e.g., if the kits are manufactured in January 2008 and the shelf life is 12 months from DOE. Then the DOE will be January 2009.

The DMO receives the consignment of RDT in the month of April 2008, then the QA rounds will be :

April 08	-	1 st round immediate testing (13 + 7 kits)
July 08	-	2 nd round (7 kits)
Oct. 08	-	3 rd round (7 kits)
Jan. 09	-	4 th round (7 kits)

- the DMO should send these RDTs collected from field to the designated state referral laboratory for long term testing. Form No. RF 95 should be used for this purpose. One copy of the RF 05 should be retained by him and the other should be dispatched to the laboratory alongwith the kits to provide the results.
- after getting the results from the laboratory a copy will be forwarded to the State Programme Officer and one copy to the Dte. of NVBDCP.

Note : the overall activity of QA for the RDTs used/supplied under the programme will be monitored by ROH&FW.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	RAPID DIAGNOSTIC TEST - PREPARATION OF QC TEST PANELS FROM WILD PARASITES: FIELD COLLECTION PROCEDURE				
SOP No.	SOP: R 03	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: R 03 - PREPARATION OF QC TEST PANELS FROM WILD PARASITES: FIELD COLLECTION PROCEDURE

7.3.1 Purpose

This SOP describes the procedure for collection of samples of malaria parasites from the field (from malaria positive patients) for preparation of Quality Control (QC) test panels.

7.3.2 Objective

To collect field samples of malaria parasites with parasite density sufficiently high for use in the preparation of (QC) panel for testing malaria RDTs.

Malaria RDTs are designed for use with fresh human blood. QC samples should therefore mimic fresh blood infected with wild parasites as closely as possible.

7.3.3 Background

The preparation of QC panels for testing malaria RDTs require sufficiently high parasite densities, to allow preparation of dilutions of 2000 parasites (p) per μL . The condition of blood should be as close as possible to fresh blood when dilutions are prepared to minimise loss of antigen or other changes, which may affect RDT performance.

For proper storage, the sample after collection should be stored immediately in a cool box.

7.3.4 Equipment and reagents

See *Field Supplies Checklist RF 06*

7.3.5 Procedure

- select patients without any history of anti-malaria treatment in the last month (record on *RF 07*).
- perform RDT using a finger-prick blood sample (SOP R 01)



- if RDT is positive, prepare a blood smear and stain the slides (refer Manual on Quality Assurance of Laboratory Diagnosis of Malaria Microscopy, SOP 01 and SOP 03).
- examine the slides for parasite density and make a count (refer Manual on Quality Assurance of Laboratory Diagnosis of Malaria Microscopy, SOP 01 and SOP 03) and record the findings on RF 08.
- if the parasite level is sufficiently high, i.e. 20000 or more parasites/ μ l of blood, select the patient as donor.
- select the patient for venepuncture complete RF 09.
- give an area specific ID code (it is **important** to record the ID on *RF 07 as well as 08*).
- apply a tourniquet, clean skin with alcohol swab, collect venous blood in 2x 5 ml EDTA tubes and 1x5 ml plain tube of venous blood and gently agitate EDTA tubes. Plain tubes are to be sent for serological screening (Hepatitis B, Hepatitis C, and HIV 1 and 2).
- when Polymerase Chain Reaction (PCR) is to be done, collect 2/3 drops of blood on a piece of filter paper.
- label tubes and blood slides with ID, time, place and date.
- allow the blood smear to dry, free from dust, flies and direct sunlight.
- complete *RF 10 – venepuncture*.
- store blood samples immediately at +4°C and maintain at +4°C during shipment.
- transport blood samples to laboratory at +4°C, ideally within 6 hours of venepuncture.

Processing of plain tube

- use the plain tube to test for HBs Ag, HCV and HIV 1 and 2 antibodies.
- centrifuge the plain tube, aliquot serum into a plastic sterile screw-capped tube, and store at + 4°C.

For processing of the parasitized blood, refer SOP R 03.

- Note:**
1. Avoid infants, small children, seriously ill/anaemic/pregnant subjects as donors
 2. Blood smears should be made on fresh blood, as films made from EDTA dry poorly.
 3. The areas may be selected within reach of adequate laboratory facilities.
 4. For species identification, PCR will be used in the NRLs. Gradually the technology would be transferred to RRLs and SRLs.

7.3.6 Organization of blood collection from field

The blood collection from field should be according to the steps described in the **figure 13** if PCR is available, otherwise as in **figure 14** when PCR is not available.



Fig 13. Selection and collection of blood from malaria patients for preparation of panel for RDT when PCR is available

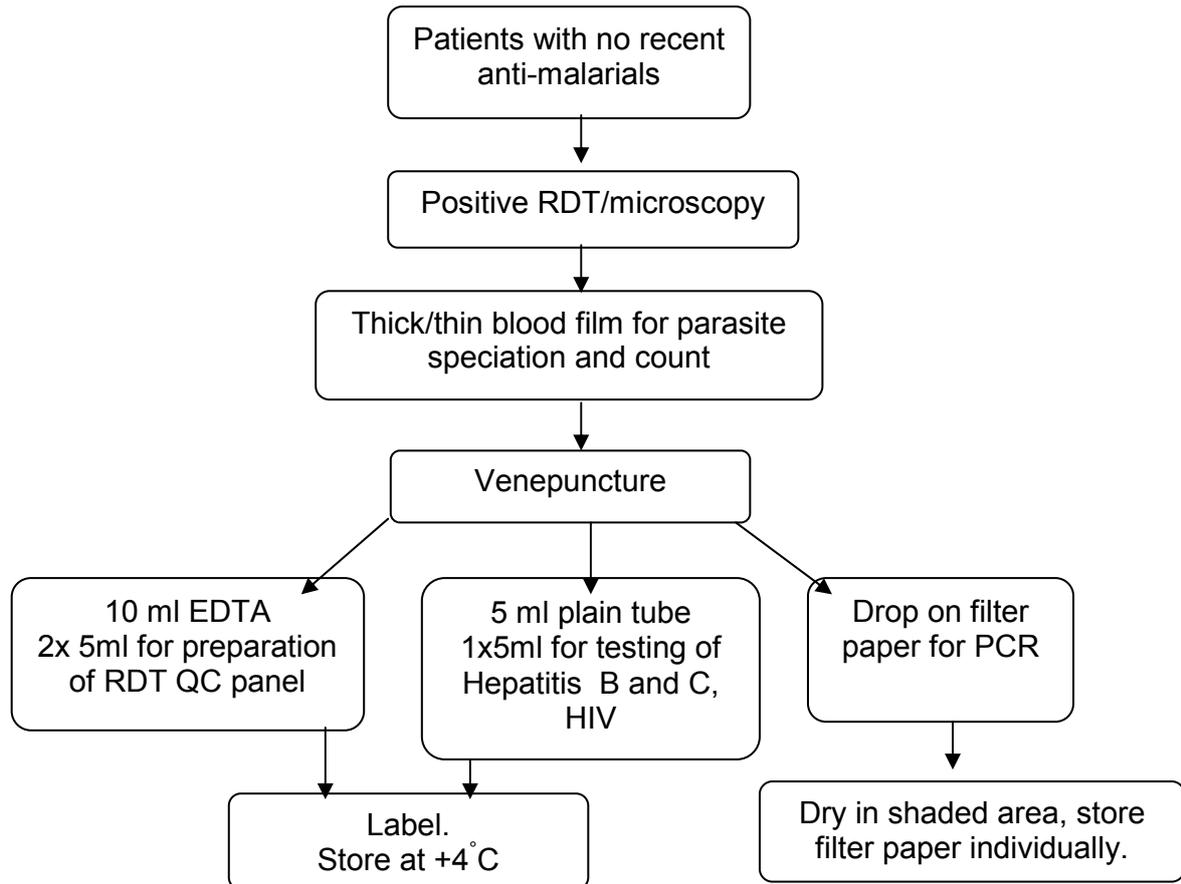
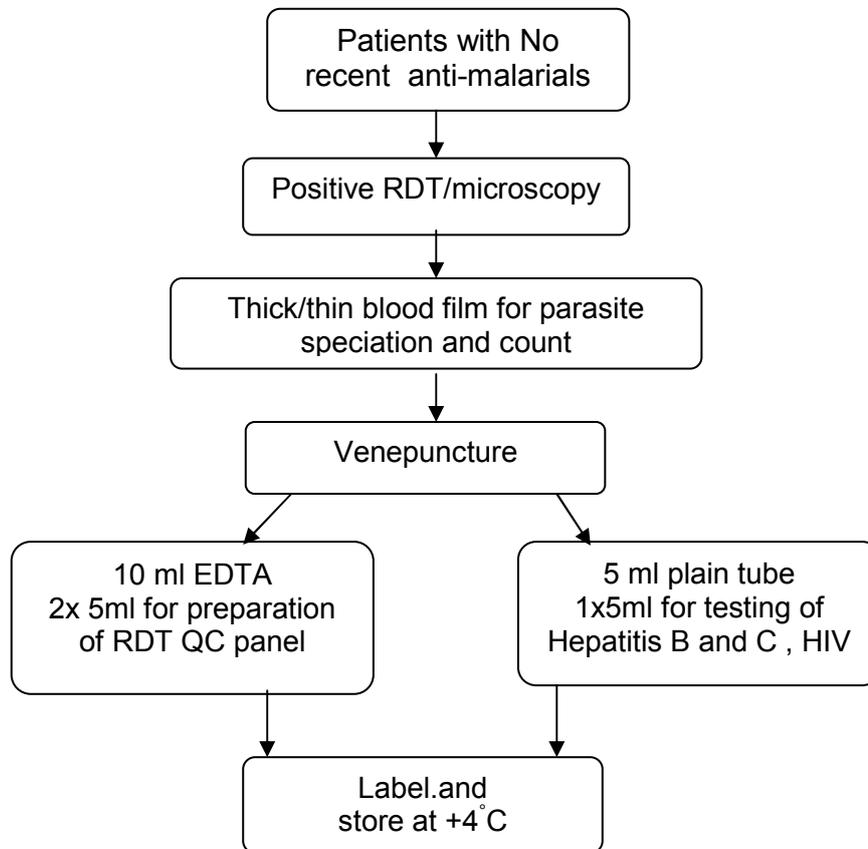




Fig 14. Selection and collection of blood from malaria patients for preparation of panel for RDT when PCR is NOT available





NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR AQ					
SOP Title	RAPID DIAGNOSTIC TEST - PREPARATION OF QUALITY CONTROL SAMPLES AND DILUTION PROCEDURE				
SOP No.	SOP: R 04	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: R 04 - PREPARATION OF QUALITY CONTROL SAMPLES AND DILUTION PROCEDURE

7.4.1 Purpose

This SOP describes the process for preparing dilutions of QC samples from wild parasites for QA of malaria RDTs.

7.4.2 Principle and objective

To prepare QC samples from wild parasites to be used in QA of malaria rapid diagnostic tests (RDTs), the sample should simulate fresh clinical specimens of parasites in blood. Therefore, it should have a parasite density close to the lower limit of detection of RDTs i.e. 200 parasite/ μ l of blood. It must be stored at +4°C to avoid deterioration, allowing qualitative detection of parasites.

7.4.3 Background

An important component of the EQA scheme is the development and use of QC samples to test the threshold sensitivity of RDTs to determine if deterioration of RDTs has occurred. The method followed to develop QC samples is preparation of antigen-based or parasite-based samples. Wild parasites may have greater variation in antigen production between samples but are likely to be more representative of field conditions. For these reasons, wild parasites will be used for the production of QC samples.

7.4.4 Equipment /reagents

See *field supplies checklist* – RF 06

7.4.5 Procedure



7.4.5.1 Parasite-free donor blood for dilutions –Complete donor preparation from (RF 11).

Blood collection from donor: either fresh blood from donor or fresh frozen plasma (FFP) from blood bank may be used for dilution.

- i. donors must be either blood group O positive or O negative.
- ii. one month prior to the field trip, arrange for screening of donors for Hepatitis B surface Antigen (HBsAg), Hepatitis C (HCV) and HIV 1 and 2, (5ml of blood in a plain tube is required for these tests).
- iii. if any of these tests are positive, exclude the donor.
- iv. commence donor preparation just 2-3 hrs before dilution preparation. Collect a finger-prick blood sample for RDT to exclude malaria positives (HRP2 and if possible pLDH to rule out *P. vivax/P. malaria* as well).
- v. also prepare thick/thin film for malaria microscopy. Exclude positive samples.
- vi. if negative by RDT and microscopy, collect a total of approximately 600 ml of blood in EDTA tubes (**40 ml from each of 15 donors**).
- vii. the total volume of parasite free donor blood required is based on the following assumptions:
 - parasite positive sample selected for panel has parasite density of 25,000p/μl
 - from each of the above sample, two QC dilutions at 200 p/μl, and 2000 p/μl has to be prepared
 - 400 aliquots of the above panels are needed (50μL in each)
 - each dilutions will be of total 20 ml volume (21 ml preferably to account for any loss when pipetting)

7.4.5.2 Procedure for dilutions

Follow the procedures for *Malaria Parasite Dilution Calculation* – RF 12 on *Dilution Preparation* – RF 13 (*For details see table 4*)

- i. mix gently on rocking tray before preparing dilutions
- ii. prepare dilutions of “parasitized blood” in the “parasite-free” donor blood
- iii. prepare approximately 21 ml of each dilution in sterile containers
- iv. prepare dilutions at 200 and 2000 parasites/μl
- v. prepare a 1ml dilution at 200 parasite /μl **PRIOR** to commencing dilutions. This dilution must be positive by RDTs to be able to proceed. If the RDT is negative, the blood cannot be used for preparation of quality control samples, (in such case do not process further for dilution, discard the sample), if positive, proceed for dilution.
- vi. use RF 12 (Dilution Calculation) to calculate quantities of “parasitised blood” and “parasite-free” donor blood required.
- vii. use SLOW REVERSE pipetting (see SOP : G 03) and use a different tip for every sample of blood dispensed.



- viii. after dilutions are made, mix gently on a rotator (which inverts tubes) for 1 hour at 4° C. donor blood with high parasite counts (i.e. >20,000 parasite / μ l) will require at least 3 hours mixing.
- ix. after 1 or 3 hours of mixing, test samples against known good RDT sample of HRP2. Proceed if all dilutions are positive. Record results on RF 14.
- x. check for red blood cell (RBC) clumping (*SOP : R 05*).
- xi. if clumping occurs, record the result and do not process any further.
- xii. if no clumping occurs, continue processing.
- xiii. using a multi-dispense pipette, prepare 50 μ L aliquots of each dilution in low-absorption cryotubes.
- xiv. prepare 2 x 100 μ l aliquots to be used for ELISA/RDT.
- xv. label the cryotubes with patient ID and dilution i.e. a 200 (a = patient ID, 200 = 200 p/ μ l).
- xvi. freeze at -20° C as soon as possible.
- xvii. discard contaminated waste as mentioned in chapter 5 on bio-safety.
- xviii. when dilutions are not to be used, they should be kept at 4° C and gently rotated.

7.4.5.3 Preparation of negative controls

- these samples should be prepared prior to the field trip.
- collect 40ml of donor blood in (4 x 10 ml) EDTA tubes.
- make 50 μ l aliquots and freeze at -20° C.
- label freezer box with “Negative control” and date.

7.4.5.4 Notes on methodology

7.4.5.4.1 Plasma replacement

Replacement of plasma of type "O" donor blood with AB plasma is necessary to avoid clumping of red cells due to ABO incompatibility with parasitized blood.

Clumping of blood should be avoided for the following reasons:

- it may prevent accurate confirmation of parasite density
- QC samples should be as close to the quality of fresh blood (for which the products were designed)
- clumping may possibly influence the ability of the lysed blood products to pass through the pores of the nitrocellulose strip in RDTs.

7.4.5.4.2 Antibiotics

As QC samples are prepared using aseptic technique, adding antibiotics to the dilutions is not required.



7.4.5.4.3 Parasite concentrations for QC testing

WHO expert consultations have recommended 95% sensitivity at 100 p/μl as a reasonable target for RDT performance. However, they suggested QC samples of 200 p/μl to test the lower limit of detection for QA of RDTs. 100 p/μl were not chosen as sufficient antigen concentration could not be guaranteed in some trials for a fair evaluation of RDTs due to the following reasons:

- some variability in malaria microscopy during preparation of dilutions is unavoidable and exact parasite densities will vary around the designated value.
- there may also be variation in expression and structure of antigens and wide variation between the relationship between parasite density and antigen concentration due to sequestration and antigen persistence.

Dte. of NVBDCP also envisages 95% sensitivity at 200 parasites/μl as a reasonable target for RDT performance for the same reason.

7.4.5.4.4 Preparation time (venepuncture to freezing) should be minimized to ideally less than 24 hours. Samples should be kept at +4°C at all times while not being processed.

7.4.5.4.5 It should be aimed to prepare dilutions on several patients with malaria counts above 5000 parasites/μl and 50μL aliquots should be prepared. As part of the QA testing of RDTs, each dilution will be used to test 2 RDTs; one RDT requires approximately 5 to 10 μl of blood.

7.4.5.4.6 Reverse pipetting

Reverse pipetting is recommended for pipetting of viscous fluids (*SOP: G 03*)

7.4.5.4.7 Preparation of 1mL dilution at 200 parasite/μL

HRP2 and pLDH production are likely to vary between patients. Therefore, despite the most accurate technique to prepare a 200 p/μl, the level of HRP2 or pLDH production may be less than the detection limit of the assay. To avoid unnecessary wastage of donor blood, a 1mL sample of the 200 p/μL dilution should be made up before commencing the actual dilutions. This 1mL 200 p/μl dilution must be positive by RDT before proceeding to make rest of the diluted stock.

7.4.6 Preparation of quality control panels

7.4.6.1 Principles for developing methods to produce panels

To develop quality control testing panels which could reliably predict the accuracy of RDTs in the field, it will be necessary to clearly characterize the range of antigen variation in wild parasites, particularly with HRP2 in falciparum malaria. To determine the relationship of antigen concentration to parasite density, this should characterize the contents of a panel in terms of the following:

- antigen structure
- antigen concentration
- characteristics of blood



Note: It is necessary to determine which characteristics of preserved blood affect RDT sensitivity and then characterize the panel accordingly.

7.4.6.2 Principles for contents of a quality panels

- QC panels used for testing malaria RDTs must contain antigen that is representative of the range of structural variation (epitope expression) encountered in the field.
- in at least a part of the panel, the antigen must be at a concentration, representative of the lowest level of malaria infections that NVBDCP would expect to detect and treat.
- the panel should also represent the range of antigen concentrations that are likely to be encountered or at least predict the sensitivity of the RDT to this range.
- the substrate in which the antigen is held must mimic the action of fresh blood on the RDT as closely as possible.
- the panel must be stable and reproducible.
- test panel samples can be stored upto a period of six months.
- cryopreserved samples can be stored up to period of six months.

With these prerequisites, it is necessary to determine the effects of antigen structure on RDT sensitivity, the relative antigen concentrations in different epidemiological situations, stage of infection and stability of the panel.

All samples in a panel will need to be characterized by:

- geographical origin
- microscopy (stage, species, parasite density)
- species, confirmed by PCR (where ever possible)
- ELISA (antigen concentration)

These samples will be used for the QA of RDTs. (SOP: R 02) "Quality Assurance of RDTs")

Note: Do not RE-USE frozen aliquots of QC samples – ONE USE ONLY.

7.4.7 Transport of quality control samples

The frozen samples are required to be sent to another institution. Therefore, the dilutions should be tested by HRP2 and pLDH types RDTs, before transport to ensure that they are working adequately.

7.4.8 Records

Field supplies checklist	RF 06
For venepuncture	RF 10
Donor preparation	RF 11
For dilution calculation	RF 12
For dilution preparation	RF 13
RDT testing results sheet	RF 14



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title		<i>RAPID DIAGNOSTIC TEST - LIGHT MICROSCOPY FOR RED BLOOD CELL CLUMPING</i>			
SOP No.	SOP: R 05	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: R 05 - LIGHT MICROSCOPY FOR RED BLOOD CELL CLUMPING

7.5.1 Purpose

This SOP describes the procedure for determining the presence of red blood cell clumping using light microscopy.

7.5.2 Background

Red blood cell clumping must be avoided in dilutions of wild parasites for QC because it may prevent the accurate confirmation of parasite density. QC samples must likewise be prepared as close to fresh whole blood as possible, as these are the samples for which the products were specifically designed.

7.5.3 Procedure

- use a frosted-end glass slide.
- label with patient ID number, dilution, date performed and other relevant information.
- add a drop of saline to the slide.
- touch the blood with a pipette tip, add to the saline and mix gently using the same pipette tip.
- add a cover slip.
- look under a light microscope and check for presence of red cell clumping at 10x and 40x magnification.
- record the result as positive or negative.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAM (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	RAPID DIAGNOSTIC TEST - THAWING OF FRESH FROZEN PLASMA				
SOP No.	SOP: R 06	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: R 06 - THAWING OF FRESH FROZEN PLASMA

7.6.1 Purpose

This SOP describes the procedure for thawing Fresh Frozen Plasma (FFP) for use in QC sample dilutions.

7.6.2 Equipment required

- water bath
- thermometer (0 °C to 100 °C)
- plastic bags

7.6.3 Procedure

- set the water bath at 37° C.
- remove the FFP from the freezer.
- put the FFP inside a plastic bag and place on the water bath, with the container in an upright position.
- make sure that the entry ports are above water level to avoid water contamination.
- keep FFP at 37° C water bath for 20-30 minutes. Invert occasionally.
- store thawed FFP at 4° C in the refrigerator prior to use.
- at a temperature range of 1- 6° C, thawed FFP can be used up to 24 hours.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	RAPID DIAGNOSTIC TEST - USE OF QC SAMPLES				
SOP No.	SOP: R 07	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: R 07 - USE OF QC SAMPLES

7.7.1 Purpose

This Standard Operating Procedure (SOP) describes the method for the use of QC samples.

7.7.2 Procedure

- take out required number of QC samples from the freezer and place on a foam rack.
- leave on the bench and let them stand at room temperature for an hour. Mix occasionally.
- once thawed, QC samples should always be utilized.
- store inside the refrigerator at 4⁰ C if not yet ready for use.

NOTE: Do not reuse the qc sample



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	RAPID DIAGNOSTIC TEST - PACKAGING OF RDT SAMPLES FOR TRANSPORT				
SOP No.	SOP: R 08	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: R 08 - PACKAGING OF RDT SAMPLES FOR TRANSPORT

7.8.1 Purpose

This SOP describes methods for proper packaging prior to transport of QC samples for testing malaria RDTs.

7.8.2 Background

Proper packaging and labeling of the material being shipped is vital in maintaining the integrity of the specimens, preventing accident and ensuring that there are no delays due to violations of regulations.

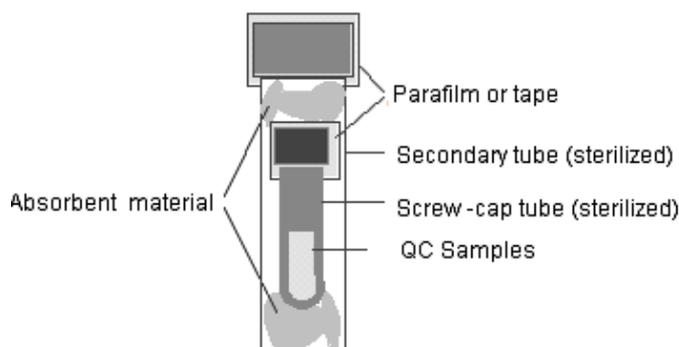
7.8.3 Procedure

For the purpose of transport of malaria RDT QC specimens, the samples are treated as risk 3 i.e. high individual risk and low community risk.

- pack all quality control samples in sterilized screw-cap tubes with an external thread, such as 1.8 mL cryovials (currently this is the method of storage). Seal the tube caps with parafilm or waterproof plastic tape.
- do not seal samples from different QC dilutions in the same bag.
- place the sealed tubes in a suitably sized plastic bag together with a small amount of absorbent material, for example cotton wool. Seal the bag, either using a bag heat-sealer or waterproof adhesive tape.
- place the sealed bags containing the isolates inside iata approved plastic secondary containers with screw-cap lids (**Figure 15**). place the additional absorbent material inside the container to absorb any leakage that may occur. the total number of samples that can be packed inside a single container will depend on the number of samples to be transported. the maximum volume that can be legally packed in a single package is 50µl (i.e. 100 quality control samples of 50 µl volume).



Fig 15. Packaging of primary and secondary receptacle for transportation of QC samples



- pack QC samples of the same ID number but different dilutions of parasite concentration in the same secondary container and samples of different ID numbers in separate secondary containers.
- the insulated container and outer packaging must conform to *IATA Dangerous Goods Regulations Packaging Instructions* and must be part of a matching set. This ensures that strict performance tests on the package which include a nine-meter drop test and a puncture test, have been met. The box must have the appropriate markings on the outside.
- for best results, precondition the insulated packaging by storing in a freezer or filling with dry ice for at least 6 hours before putting the tube-set in place. Alternatively, for shipments of short duration (overnight), frozen ice packs or additional plastic containers containing ice may be used.
- fill the spaces around the secondary container with dry ice and the lid of the insulated container placed on top. To allow venting of the dry ice, **the top must be sealed in a way to permit release of carbon dioxide gas** (this can be achieved by puncturing the top of the carton). Use of dry ice requires a declaration of Dangerous Goods class 9, UN1845 and must comply with the prescribed packaging instructions. **The instructions given here comply with these rules.**
- to ensure that samples remain frozen during transport, the packaging will require an outer container to place cooling material inside. Place the completed set in an over pack. Fill the outer packing with dry ice. An extra label is required on the outside of the over pack stating:

INNER PACKAGES COMPLY WITH PACKING INSTRUCTIONS

- include a list of QC samples contained in the package in an envelope, tape to the top of the insulated lid, placed under the external fiberboard packaging.
- label the outer packaging with the following information
 - the sender's name, address and contact telephone/fax numbers
 - the classification numbers and proper shipping names
 - the weight of dry ice included in the package at commencement of shipment
 - the receiver's name, address and contact telephone/fax numbers.



- **Infectious substances** label showing class 6
- **Miscellaneous label class 9** (if dry ice is being used)
- include an additional label requesting: “Keep package at -70° C”. Seal the box using wide sealing tape, taking care not to obscure the labels with the tape and leaving a gap for venting of the dry ice.
- all infectious substances must be accompanied by a **Sender’s Declaration for Dangerous Goods**, indicating shipment of infectious substances and if appropriate, the use of dry ice in the shipment.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	RAPID DIAGNOSTIC TEST - DOCUMENTATION OF TRANSPORTED QUALITY CONTROL SAMPLES FOR TESTING MALARIA RDTS				
SOP No.	SOP: R 09	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
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SOP: R 09 - DOCUMENTATION OF TRANSPORTED QUALITY CONTROL SAMPLES FOR TESTING MALARIA RDTS

7.9.1 Purpose

This SOP describes the essential documents required when transporting QC samples, in addition to documentation required by consignee and consignor for transport of human blood products.

7.9.2 Procedure

For the transport of QC samples, the following documents need to be prepared:

7.9.2.1 Documents completed within Institution

- specimen Referral RF 15 and Receipt Logs RF 16. The institutions (NRL:NIB and RRLs and SRLs) should keep a standard record detailing dispatch and receipt of QC samples. The record should contain a description of specimen including volume, destination and source, mode of transport, date sent and received and designated laboratory personnel (consignee and consignor). Problems encountered during transport or receipt should be documented. The record should be kept in the laboratory at all times.

7.9.2.2.1 Documents to attach to package for transport

- sender's declaration of Dangerous Goods. It is recommended that 2 copies for domestic packages should be attached.
- a packing list: which includes shipping company's name, the receivers address, the number of packages, detail of contents, source, weight, value (required for international shipping only)
- customs declaration: Only in case of international shipping.
- instruction sheet: This document describes the nature of the specimens, prescribed manner of handling and the purpose for which the material will be used. Appropriate background on the material, such as screening tests done, potential hazards and storage conditions are also included.
- airway bill. The airway bill should be marked with the following information:



- name, address, telephone/fax of receiver
- number of specimens
- "Highly perishable" label
- "telephone receiver upon arrival" (include telephone number)

"URGENT: DO NOT DELAY"

Biological specimens -- highly perishable -- store at -20° C



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	RAPID DIAGNOSTIC TEST - COORDINATION OF TRANSPORT FOR QC SAMPLES AND MALARIA RDTS				
SOP No.	SOP: R 10	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: R 10 - COORDINATION OF TRANSPORT FOR QC SAMPLES AND MALARIA RDT KITS

7.10.1 Purpose

This SOP describes guidelines for proper shipment of QC samples for testing malaria RDTs. The transport of QC samples requires careful planning and coordination between the consignor, the carrier and the consignee.

7.10.2 Procedure

7.10.2.1 Transport planning

It is the responsibility of the sender (NRL) to ensure the correct designation, packaging, labeling and documentation of all materials sent from the laboratory.

The efficient transport of infectious materials requires good co-ordination between the sender (the shipper), the carrier and the receiver (the consignee or receiving RRL), to ensure that the material is transported safely and arrives on time and in good condition. Such co-ordination depends upon well-established communication and a partner relationship among the three parties.

Advance arrangements with the consignee

Once it has been decided that materials need to be transported from the laboratory, the receiver at RRL should be contacted and informed.

- notify the consignee (receiving party) beforehand of specimens being transported and send acknowledgement of preparedness for receipt to the consignor.
- if permits are needed, the RRL will need to obtain the CURRENT permit and send it (usually a faxed copy) to the shipping laboratory so that the permit can be given to the carrier.



- the sender (NIB) and the receiver (RRL) should then make advance arrangements for a mutually convenient time for shipment to ensure that the appropriate staff is available to receive the shipment. It is recommended to avoid weekend arrivals, while planning.
- Information should pass on to the respective ROH&FW, for coordination.

7.10.2.2 Advance arrangements with the carrier

- once a shipment is necessary, contact a carrier familiar with handling infectious substances and diagnostic specimens and make arrangements to ensure that:
 - i. the shipment is undertaken by the most direct routing, avoiding weekend arrival.
 - ii. the conditions of the shipment while in transit will be monitored.
 - iii. the sender and consignee will be notified of any delay.
- send any necessary shipping documents that the carrier may require or any specific instructions, necessary to ensure safe arrival of the shipment. Any advice on packaging by the carrier should be considered.
- in cases of delay, the consignor must arrange for both the consignee and consignor to be notified immediately by the carrier and advised on expected arrival arrangements.

7.10.2.3 Notification of the consignee of departure

Once the package has been sent, notify the RRLs (consignee) of the following:

- number of specimens (nature and quantity)
- flight details (airline, flight number, arrival date and time)
- airway bill number
- instruction "please inform if not received"

7.10.2.4 Notification of the consignor

Once the package has been received, the RRLs should immediately notify the NRL of the receipt and condition of the shipment (including temperature) problems, if any, encountered. This can be facilitated by including a 'fax back' form in the shipment that the RRLs can fill and return.

7.10.3 Cold chain guidelines on transport and storage of Rapid Diagnostic Test Kits

Malaria RDTs are biological tests that can be rapidly degraded by exposure to high temperatures, moisture and freezing. Most manufacturers recommend controlled-temperature storage between +2°C and +30°C. Expiry dates are generally set according to these conditions. RDTs stored at temperatures exceeding the recommended limits are likely to have a reduced shelf-life. Obtain real time temperature stability data and accelerated data on the current lot from manufacturers, prior to purchase.



Transport and storage at temperatures above +30°C is sometimes unavoidable in many remote locations where RDTs are intended for use. In such cases, storage conditions should be as cool as possible and exposure to direct sunlight or storage under hot roofing should be avoided. Major storage facilities should have temperature monitoring and recording. All RDT lots should have regular monitoring of sensitivity while in use. Procedures should be in place for monitoring and replacing lots when the specified storage conditions are breached.

Note : *Entire process would be monitored by the QA section of the Dte. of NVBDCP. A copy of the detailed consignment sent by the nodal laboratory would be endorsed to the QA section of the Dte. of NVBDCP. Similarly, the consignee on receipt of the consignment would inform the details including nos. of sample received, status of package, maintenance of cold chain, etc. to the QA section of the Dte. of NVBDCP. Under NVBDCP supply system, consignee are districts, which are mostly not connected by air. Therefore, during the purchase procedure, the purchase agent would ensure supply of the consignment up to the district level maintaining the cold chain. **Necessary legal action would be taken against the manufacturer if these conditions are breached.***

In light of the above, a “cold chain” should be developed for shipment and storage of RDTs. This should commence at the site of manufacture and extend as far as possible to the end-user. Where ever recommended storage conditions are breached, procedures should be in place to monitor sensitivity or replace the suspected lot of test kits. Referral laboratories involved in QA of RDTs should identify one nodal officer with responsibility for overseeing all aspects of transport, storage and QA of RDTs at PHC level.

The following procedures should be in place:

- before shipping from manufacturer, the manufacturer contacts consignees with details of numbers of containers and expected arrival time.
- the manufacturer initiates shipment only when the consignee confirms receipt of the shipping notification. Shipping time should be arranged to avoid delay due to weekends/public holidays.
- ground transportation during any stage of delivery is carried out without delay and with attention to temperature within the vehicle at all times, including when parked.
- storage at central and final field facilities at +30°C or less, wherever possible.
- RDTs with moisture-proof packaging damaged in transit should be discarded.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	RAPID DIAGNOSTIC TEST - RAPID DIAGNOSTIC TEST REGISTER				
SOP No.	SOP: R 11	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: R 11 - RAPID DIAGNOSTIC TEST REGISTER

7.11.1 Purpose

This SOP describes the process for receipt and dispatch of RDTs. By SRLs/RRLs For QA of RDT as described in SOP R 02. RDTs supplied to the periphery under NVBDCP would be collected by the respective DMO and send these to the identified SRLs/RRLs for testing.

7.11.2 Procedure

7.11.2.1 Receiving outside the laboratory

- complete the *RDT receiving Register* (RF 16).
- contact one of the listed responsible officers.
- if the samples arrive outside office hours, make arrangements locally to receive the samples.

7.11.2.2 Receiving in the laboratory

- complete the *RDT Register* (RF 17).
- complete the *RDT description Log* (RF 18).
- attach and complete the *RDT Movement Log* (RF 15) - for SRL and RRLs.
- file any accompanying transport documentation in the RDT register folder.
- file the company method sheet received with the RDT in the designated folder.
- file the QA results of RDT received from the company in the designated folder.
- store the RDTs at 4° C immediately.

7.11.2.3 Dispatch

- complete the RDT register.
- complete the RDT movement registers.

At district level please ensure:

- complete RF 05 (in duplicate), send one copy to SRL/RRL and retain one copy for record.
- that RF 15 and RF 18 is filled and sent with RDT sample to the SRL/RRLs.
- copies of RF 05, RF 15 and RF 18 are endorsed to the Dte. of NVBDCP.

At SRL/RRL



- complete RF 16/17, check RF 05 and RF 18 acknowledge the receipt of RDT.

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	RAPID DIAGNOSTIC TEST - DOCUMENT CONTROL				
SOP No.	SOP: R 12	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: R 12 DOCUMENT CONTROL

7.12.1 Purpose

To detail the control of Quality Documents for QA of Rapid Diagnostic Tests under NVBDCP.

7.12.2 Background

Dte. of NVBDCP will control the issue, approval and updating of all quality-related documents and data. The role of the laboratory in-charge on QA is to maintain a Document Master List and to ensure that laboratory personnel perform their own internal checks of their documents and data and that the quality audits adequately address the issue of document control in their checklists.

The SOP acts as a register for quality documentation such as proformae, methodology to be followed and work instructions which are maintained in the operational guide.

7.12.3 Procedure

- no amendments of the SOPs are allowed. Suggestions if any, for amendments may be communicated to the Dte. of the NVBDCP. **Only the Director, NVBDCP can amend the SOPs in consultation with the experts.**
- all the laboratories involved in QA programme would be connected through the electronic networking. The data and documents are to be maintained in the electronic format. The Dte. of the NVBDCP would have access to all the data.
- obsolete documents are to be removed from the active electronic documentation system from time to time and placed in archive folders. Hard copies are to be maintained. Dte. of the NVBDCP envisages retention of back up of all important folders. No data should be removed from the archives; besides none other then the Director NVBDCP can decide for any change in the documentation.

Note : Dte. of NVBDCP envisages that all the documents of QA testing would be completed within the same day and the report would be sent to the NVBDCP and NRL within 48 hours of the test.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	RAPID DIAGNOSTIC TEST - DOCUMENT STORAGE				
SOP No.	SOP: R 13	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: R 13 DOCUMENT STORAGE

7.13.1 Purpose

This SOP describes the process for storage of documents produced as part of SOP : R 13 for quality assurance of RDTs.

7.13.2 Procedure

- all documents generated as part of the QA are to be **archived** for 5 years.
- records must be legible.
- if paper-based records are kept they are to be filed in an organized manner.
- records stored electronically are to be well organized.
- data stored on the computer would be backed-up regularly and the back-up ideally stored in a separate place.
- an electronic copy would be sent to QA section of Dte. of the NVBDCP for analysis, reviewing and archiving.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	RAPID DIAGNOSTIC TEST- SUPERVISORY VISITS FOR ON-SITE ASSESSMENT				
SOP No.	SOP: R 14	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: R 14 SUPERVISORY VISITS FOR ON-SITE ASSESSMENT

7.14.1 Purpose

This SOP describes the process and guidelines for assessment of the performance of laboratories conducting QA for RDTs.

7.14.2 Need for supervision

A major component of any QA scheme is EQAS, which is a process to assess performance of a laboratory. EQAS may be achieved by on-site assessments (supervisory visits) and/or comparison of results of panel testing with another laboratory and confirmatory testing of routine work is performed at another laboratory.

Advantage of "on-site" evaluations is that assessment of the laboratory occurs under actual working conditions and necessary corrective actions are implemented immediately. It provides an environment where there is direct contact between staff and the assessor. Therefore, the assessor/supervisor must be appropriately trained and have considerable expertise. (SOP : M 8, Manual on Quality Assurance of Laboratory Diagnosis of Malaria by Microscopy).

7.14.3 Procedure

- "On-site" assessments should ideally be performed annually. However, initially the NVBDCP envisages for quarterly assessment.
- it is preferable for an external organization/institute to perform the evaluation. Dte. of NVBDCP would identify organization/institute and provide logistic support for the pupose.
- the organization/institute selected would coordinate with the laboratories (NRL, RRL and SRL) regarding proposed visit under intimation to the Dte. of NVBDCP. This ensures that the relevant staff will be present at the time of the evaluation.
- the evaluation will be carried out by means of the assessor making general observations and using checklists (RF 19), which covers both the



administrative issues and technical issues like safety, QC panel preparation and testing of RDTs for QA as well as corrective measures as and where needed.

- the same checklists would be used each year to assess improvement in performance over time.
- at the end of the evaluation, the assessor/supervisor would discuss the observations made according to the checklists with the laboratory staff and suggest the corrective measures as and where needed.
- the supervisor would see the report of the previous assessment, look for recommendations given and actions taken for improvement.
- if there is no improvement after the previous visit, the supervisor should discuss the issue with the in-charge of the laboratory and highlight the issue in his/her report.
- after the visit, a report on the visit with recommendations would be forwarded to the Dte. of NVBDCP.
- these reports would be reviewed by QA section of the Dte. of NVBDCP and the NRLs and feedback would be to the laboratories supervised, with suggestions for improvement.
- if required, NVBDCP would organize refresher training for the laboratory staff on QA through NRLs.
- ROH&FW will co-ordinate the supervisory visits.



NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	RAPID DIAGNOSTIC TEST - QUALITY AUDITS OF RDTs				
SOP No.	SOP: R 15	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: R 15 QUALITY AUDITS OF RDTs

7.15.1 Purpose

This SOP describes the process and guidelines for a structured internal assessment of performance of Internal Quality Control (IQC) of the laboratories conducting QA of RDT.

7.15.2 Objective

IQC would be an on-going process in all laboratories, including structured review by staff of routine work and formal auditing at set intervals. The in-charge of the laboratory networked for conducting QA of RDTs would be assigned the responsibilities of IQC in the concerned laboratory.

7.15.3 Procedure

- quality audits would be performed at least 6 monthly and also after new staff involved in the laboratory for the activities related to QA is recruited/joins.
- the evaluation would be carried out by means of a formal check list, based on the external evaluation through supervisory visits and the same checklists used for supervisory visits should be referred for this purpose.
- the suggestions/recommendations will be given by the external supervisor on the issues covering preparation and use of QC panel for testing of RDT and issues related to administration and bio-safety.
- the feedback of the Dte. of NVBDCP would be discussed with staff of the laboratory and improvement will be assessed.
- if required, request would be sent to the Dte. of NVBDCP to organize refresher training for the laboratory staff on QA, as and when required, to improve the performance .
- the same checklists would be used each year to assess improvement in performance over time.
- during and after the evaluation, the checklists will be completed.
- completed checklists would be retained for EQAS purposes and a copy of the assessment report would be communicated to the QA section of the Dte. of NVBDCP.



CHAPTER 8

REPORTING FORMATS (RF) FOR QA OF RDT

RF- 01: RDT KIT QA RESULT SHEET FOR RECORD

Name of the Laboratory: _____

Date of Testing

RDT KIT

RDT Kit	Manufacturer	Lot Number	Expiry date

RESULTS

Sample	Dilution (Parasites/ μ L)	RESULTS			
		Pf	non-Pf	Control	Result
Negative Control	0				
Parasite dilution per mm³ Panel member I	200				
	200				
	2000				
	2000				
Panel member II	200				
	200				
	2000				
	2000				
Panel member III	200				
	200				
	2000				
	2000				
Panel member IV	200				
	200				
	2000				
	2000				

Signature
Name of the Laboratory staff

Countersigned by Lab Incharge
Name :
Designation:



REPORTING FORM (RF) FOR EQA OF RDT

RF- 02: EXTERNAL QUALITY ASSESSMENT OF MALARIA RAPID DIAGNOSTIC TEST FOR REPORT

Date report prepared: dd /mm/ yyyy

RDT receipt details

Sent from:

Transport method:

Date sent: /...../.....

Date received: /...../.....

Place/Institute received: ROH&FW Medical College.....

RDTs received:

	RDT
Name:	
Manufacturer:	
Lot no:	
Expiry:	
Quantity:	
Previous storage conditions:	
Description of RDTs on receipt:	
Condition of RDTs on receipt:	

Total Nos of samples used for External Quality Assessment (QA) testing

Quality control samples of dilutions from wild-parasites, stored at -70°C/-20 °C

Samples containing

- 0 parasites/ μ L of blood (negative control)
- 200 parasites/ μ L of *P. falciparum*
- 500 parasites/ μ L of *P. falciparum*
- 2000 parasites/ μ L of *P. falciparum*

Method

RDTs were tested as per manufacturer instructions

QA RDT RESULTS:

Quality control dilutions (parasites/ μ L)	RDTs tested	RDTs positive	% positive
200	4		
500	4		
2000	4		
	RDTs tested	RDTs negative	% negative
0 (negative control)	1		

Conclusion:

**RDT 1:
PASS/FAIL
RDT 2:
PASS/FAIL
RDT 3:
PASS/FAIL**

Comment:



For a lot of RDTs to pass the QA assessment, all quality control dilutions must be positive (100%) and the negative control must be negative.

Interpretation of results:

- PASS: This RDT lot passed the QA assessment and has sufficient sensitivity **FOR USE** in the field.
- PRELIMINARY RESULTS: This RDT lot failed the initial QA assessment, and has been sent to another institution for confirmation. A final report will be issued on receipt of the confirmatory results. It is recommended that the lot is **RETAINED** until a final report is received.
- FAIL: This RDT lot failed the initial QA assessment and also failed at another institution. It is recommended that lot should **NOT BE USED** in the field as it lacks expected sensitivity. It is recommended that the manufacturer be contacted and advised of the results.

Note: This RDT lot will be retained for long term Quality Assurance. A further report will be only be issued if the RDTs lot fails the QA assessment.

Name :
Designation:
Sign:

REPORTING FORM (RF) FOR EQA OF RDT

RF- 03: LONG TERM QUALITY ASSESSMENT LOG

Name of the Laboratory: _____

RDT	TIME INTERVAL							
	3 MONTH	6 MONTH	9 MONTH	12 MONTH	15 MONTH	18 MONTH	21 MONTHS	24 MONTHS
Name: _____ Manufacturer: _____ Lot No. _____ Expiry Date: _____ Date of receipt: _____	Date of testing:							
	Result							
Name: _____ Manufacturer: _____ Lot No. _____ Expiry Date: _____ Date of receipt: _____	Date of testing:							
	Result							
Name: _____ Manufacturer: _____ Lot No. _____ Expiry Date: _____ Date of receipt: _____	Date of testing:							
	Result							

REPORTING FORM (RF) FOR EQA OF RDT

RF- 04: FORMAT FOR SPECIMEN REFERRAL

SPECIMEN e.g Malaria RDT QC samples, ID	DESCRIPTION (50 uL blood in cryotubes) 100 vials, total vol = 5 mL	REASON FOR REFERRAL	DESTINATION	MODE OF TRANSPORT	DATE SENT	DATE RECEIVED	REMARKS	Dated SIGNATURE of Lab I/c

REPORTING FORM (RF) FOR EQA OF RDT

RF - 05: Testing for Sensitivity and Stability of RDTs at the Periphery

Name of the State District.....

Product name Manufacturer Batch/lot no

Date of manufacturing Date of expiry Received on (date).....

Total quantity received..... Date of distribution to PHC.....

Date of 1st sample drawn (13 RDTs randomly) at the time of dispatch to PHC.....

Date of subsequent samples drawn for lot testing: Interval: 3/6/9/12/15/18/21/24 months

Name of the PHC	Name of the Sub-center	Name of the Village	Date of sending	Sent to (Name of the SRL)	Date of receiving results	Results	Remarks

Sign of DMO

Note: A copy of the above form should be sent to SPO and Dte. of NVBDCP after each quarter



RF- 06: CHECKLIST FOR COLLECTION OF WILD PARASITES FOR QC PANEL

Supply	Stock required	Stock available	Amount ordered	Date ordered	Company ordered from	Date received	Total Stock available
I. Materials required for collection							
1. Lancets							
2. Vacutainer (10 ml. EDTA)							
3. Vacutainer (10 ml. Plain, no additives)							
4. Vacutainer needles							
5. 5 ml EDTA tubes							
6. 5 ml plain tubes (no additives)							
if Vacutainer not available 7-10							
7. 10 ml syringes,							
8. 5 ml syringes							
9. 21g Needles							
10. 23g Needles							
11. Tourniquet							
12. Alcohol swabs							
13. Gloves—small, medium, large							
14. Glass slides							
15. Sterile plastic disposable screw capped tubes (15 ml.)							
16. Filter paper (Whatmann 3M) for PCR							
17. Small individual plastic bags for filter paper.							
18. Saline							
19. Freezer boxes (10x10)							
20. 1% Hypochlorite solution							
21. Cotton balls							
22. Sharp containers							
II. Storage vials							
23. Cryotubes 1 ml (low absorption) for aliquots							
III. Stains and kits							
24. JSB Stain I & II							

Contd....



25. Staining container							
26. Methanol – 250 ml.							
27. RDTs (eg make A)							
28. RDTs (eg make B)							
IV. Equipment							
29. Pipetting equipment							
a) Multi dispensing							
b) pipette (50 –100 µl)							
b) 20 ul micropipette							
c) 200 ul micropipette							
d) 1000 ul micropipette							
e) Pipette tips- yellow							
f) Wide-bore pipette tips							
g) Sterile plastic pipettes							
30. Microscope with 100x oil immersion objective							
31. Counter							
32. Water bath							
33. Refrigerator 4 ⁰ C							
34. Freezer –20 ⁰ C							
35. Centrifuge							
V. Other laboratory/ stationary items							
36. Group AB fresh frozen plasma							
37. Pens –pencils, marker pens							
38. Paper towels							
39. Coolers with ice for storage							
40. Dry Ice (15 kg)							
VI. Forms							
RF 07 Patient record							
RF 08 Malaria microscopy							
RF 09 Donor’s consent form							
RF 10 Blood sampling							
RF 11 Preparation of donor							
RF 12 Malaria parasite density							
RF 13 Dilution of parasitized							
RF 14 RDT testing results							
RF 15 RDT kit movement							
RF 16 Register for receiving							
RF 17 RDT kit register							
RF 18 Description of RDT kits							
RF 19 Checklist for supervision							



REPORTING FORM (RF) FOR QA OF RDT

RF- 07: PATIENT RECORD

GENERAL INSTRUCTIONS: Encircle number or item; fill in blanks and boxes as appropriate.

ID NO. _____ DATE: ___/___/___ TIME:

PATIENT DETAILS

M F

NAME _____ AGE _____ (in yrs) Gender 1 2

CLINICAL HISTORY

Symptoms

(In the past two weeks, have you suffered from.....?)

			No	Yes	if yes specify
Chills	Fever		0	1	
	Sweating	0	1		
	Headache		0	1	
	Others		0	1	

Treatment

(In the past month, have you taken any of the following medicines?)

	No	Yes
Chloroquine	0	1
Sulfadoxine/Pyrimethamine	0	1
Primaquine	0	1
Others	0	1

(Do you keep any malaria medicines at home?)

Access to medication	0	1
----------------------	---	---

RDT RESULTS (finger-prick) – with pLDH to rule out non Pf

Microscopy results

No of BS	Date of BSE	Result		Parasite count
		Pf	Mix	

RDT	Manufacturer	Lot No. /Expiry	Pf	Non-Pf	Control	Result

If the patient meets the following criteria, then perform a venepuncture

- 1) Patient is Pf positive
- 2) RDT – negative for non Pf
- 3) Age 7 years and above
- 4) No malaria medicine in the past month



REPORTING FORM (RF) FOR EQA OF RDT
RF- 08: MALARIA MICROSCOPY RECORD

Name of the laboratory: _____

Microscopy date: / /
DD /MM/ YYYY

White cell count

Microscopy

Species	Pf		Pv.	
	Ring	Gametocyte	Trophozoite/ schizont	Gametocyte
Parasite count				

500 white blood cells must be counted

However if the parasite count is very high 200 WBC may be counted.

Comments.

Signed (Lab Tech.):

Parasite count: I II

Average of I and II

Asexual parasites counted Av count

$$\frac{\text{Av count}}{\text{White cells counted}} \times \text{WBC } \mu\text{l}^{-1} \quad 8000 = \text{para } \mu\text{l}^{-1}$$

White cells counted



REPORTING FORM (RF) FOR EQA OF RDT

RF- 09: Donor's Consent Form

- To be modified to fulfill local requirements.
- Further consent or counseling may be required for HIV testing.

I, _____, give consent for blood to be taken from myself/my child
Name/Guardian Name

_____, under the supervision of Dr The
Name of child

following have been explained to me and I understood before I signed this consent form.

1. I will have 10 to 20 ml of venous blood drawn for this study. The blood will be assessed to see if it is suitable to be used to check the performance of rapid diagnostic tests, which are used to test for malaria.
2. Case management and treatment will be provided to me according to the result of the blood tests already performed.
3. If my blood is found to be suitable for use it will be tested for HIV-1 & 2, Hepatitis B & C for the safety of people who will be using it. I will get proper care, if any of these tests are found to be positive.
4. All my identifiable records and information will be kept strictly confidential.
5. My participation is voluntary and I can withdraw from the study any time for any reason.
6. For further enquiries, I can contact the following persons:

IDNO.....

Signature: _____

Name: _____ Relationship: _____
 (Name of the guardian in case of Children)

Witness: _____

Date: _____

Informed consent obtained by: _____



REPORTING FORM (RF) FOR QA OF RDT

RF- 10 : BLOOD SAMPLING

If the patient meets the following criteria, then perform a venepuncture

- 1) Patient is Pf positive
- 2) RDT – negative for non Pf
- 3) Age 7 years and above
- 4) No malaria medicine in the past month

Date: ___/___/___ ___ ___ Time: Patient Consent Read

dd /mm/yyyy

Required:

<i>Tube</i>	Quantity	Total volume
EDTA		
Plain		

Please ensure samples are labeled with the allocated ID

Filter paper sample prepared

Microscopy results are handed over for redical treatment

Time blood refrigerated/ Temperature of refrigerator

Name and signature

Supervisor

Lab Tech.



REPORTING FORM (RF) FOR EQA OF RDT

RF- 11: PREPARATION OF DONOR

Parasite free blood *

Preparation of "parasite-free" blood

Donor blood (EDTA) - source:

Blood group

Date/time collected:

Volume

AB plasma – date/time thawed.....

Plain tube – serum refrigerated

Temperature.....

Time

Parasite free blood - refrigerated

Temperature.....

Time

RDT RESULTS

Finger-prick

Date: ___/___/___
dd/mm/yyyy

Time:

RDT	Lot No. /Expiry	Reading	Pf	Non-Pf	Control	Result
pLDH						
HRP2						

0 (no band), 1+ (weak band), 2+(medium band), 3+ (strong band)

Hepatitis B,C & HIV testing

Lab performing test

Date sent for testing: ___/___/___
dd/mm/yyyy

Hepatitis B Negative Positive

Hepatitis C Negative Positive

HIV 1,2 Negative Positive

*** If donor blood is not available in the blood bank**

use separate form for each donor



REPORTING FORM (RF) FOR EQA OF RDT

RF- 12: MALARIA PARASITE DENSITY CALCULATION

Name of the Laboratory: _____

Parasite density

Parasite density microscopy (form 03)	1)	2)
Calculate mean: (Count 1 + Count 2)/2		
Calculate discrepancy (%): (Diff/mean) x 100		

If discrepancy is ≤ 20%- Use mean parasite count

If discrepancy is >20% - Repeat counting

Parasite density microscopy (Repeat counting)	3)	4)
---	----	----

Of the 4 readings, choose (circle) the two closest (one each from the different

Calculate mean: (Count 1 + Count 2)/2	
Calculate discrepancy (%): (Diff/mean) x 100	

If discrepancy is ≤ 20%- Use mean parasite count

If discrepancy is >20% - DO NOT use this sample for dilutions

Mean parasite density: _____ p/μl

Dilution calculations

Dilutions should always be made up to 5ml, unless otherwise specified

Vol. required (V)	Parasite density required (d)	Mean parasite density (p/μl) (n)	$v_p = \frac{d}{n} \times V$	Vol. parasitised blood (v _p)	Vol. stock blood (V-v _p).
e.g 5 ml	50 p/μl	10,000	50/10,000 x 5	0.025 ml	4.975 ml
Dilutions					
	50 p/μl				
	100 p/μl				
	150 p/μl				
	200 p/μl				
	500 p/μl				
	5000 p/μl ¹				



REPORTING FORM (RF) FOR EQA OF RDT
RF- 14: RDT TESTING RESULTS SHEET

Screening of newly-prepared dilutions

Date.....

Name of RDT Kit	Manufacturer	Lot Number	Expiry date

RESULTS

PANEL MEMBER SAMPLE (ID)	DATE AND TIME PREPARED	DATE AND TIME TESTED	DILUTIONS OF PARASITES /μL	NO OF TESTS PERFORMED BY	RESULTS		REMARKS IF ANY
					HRP II	pLDH	
1			200				
			2000				
2			200				
			2000				
3			200				
			2000				
4			200				
			2000				
5			200				
			2000				
6			200				
			2000				

Name and Signature of the Lab Tech

Countersigned by Lab-In-Charge

REPORTING FORM (RF) FOR EQA OF RDT

RF- 15: RDT KIT MOVEMENT REGISTER

Attach this sheet (in a plastic sleeve) to the outside of the RDT box

RDT DETAILS

Product Name:

Manufacturer:

Batch/Lot No:

Expiry date: (dd/mm/yy)

RECEIPT						DISPATCH				
Received By : Name & Sign	Place received (Name of Lab)	Date received dd/mm/yy	Transported by:	Quantity received Boxes/ tests per box	Place stored	Removed by: Name & Sign	Date removed dd/mm/yy	Destination	Quantity removed Boxes/ test per box	Transport storage i.e. cold box

NOTE - RDTs SHOULD BE STORED At 4°C AT ALL TIMES



REPORTING FORM (RF) FOR EQA OF RDT

RF- 16 : REGISTER FOR RECEIVING RDT KITS OUT SIDE LABORATORY: RECEIPT LOG

Name of the Laboratory: _____

Received by (sign)	Date received (dd/mm/yy)	Label on box	Person contacted	If someone can't be contacted, where and what refrigerator was the box stored in?	Signature of the Lab supervisor I/C



REPORTING FORM (RF) FOR QA OF RDT

RF- 17 : RDT KIT REGISTER

Name of the Laboratory: _____

RECEIPT									DISPATCH				
Received by:	Date received	Shipped by: Transported by:	Name of the kit	Source/ Manufacturer/Field	Lot/ Batch	Expiry	Quantity received	Place stored	Removed by:	Date removed	Destinati on	Quantity removed	Transport method
Name	dd/mm/ yy					dd/m m/yy			Name	dd//mm/ yy		Boxes/ tests per box	i.e. cold box

RDTs SHOULD BE STORED AT + 4°C AT ALL TIMES



REPORTING FORM (RF) FOR QA OF RDT

RF- 18: DESCRIPTION OF RDT KITS

Name of the Laboratory: _____

Sr. No	RDT	RDT condition on receipt
1	Date:	Type of packaging (i.e. box, bag):
	Name:	
	Manufacturer:	Condition of packaging (i.e. damaged, hot, cold, frozen) frozen) :
	Lot no:	
	Expiry date:	Other comments:
	Received by	Sign: Name:
2	Date:	Type of packaging:
	Name:	
	Manufacturer:	Condition of packaging:
	Lot no:	
	Expiry date:	Other comments:
	Received by	Sign: Name:
3	Date:	Type of packaging:
	Name:	
	Manufacturer:	Condition of packaging:
	Lot no:	
	Expiry date:	Other comments:
	Received by	Sign: Name:

Signature of the supervisor



REPORTING FORM (RF) FOR EQA OF RDT

RF-19 CHECKLIST FOR SUPERVISION (ON SITE EVALUATION)

Name of the Institute: _____ Date..../...../

Name of the District _____ Name of the State _____

Name of the Laboratory Technician	
Name of the Laboratory in Charge	
Name & Designation of the supervisor	

A. General

Section	Checks	Supervisor's comment
Workplace	<ul style="list-style-type: none"> • Dedicated work area • Efficient workflow • Cleanliness, tidiness • Water, electricity services 	
Human resource	<ul style="list-style-type: none"> • Total No of laboratory staff, No. involved in QA • No of trained staff, Nos trained on QA • Last training attended (place) 	
Standard Operating Procedures	<ul style="list-style-type: none"> • Aware about the SOP • SOP available in the laboratory • SOP being followed strictly • SOPs & methods followed are reviewed by the laboratory in charge • Date of last review (a copy of the observation) 	
Maintenance of Laboratory results/ records	<ul style="list-style-type: none"> • Results register available in the laboratory • Register updated • Total no of registers being maintained • Certified by supervisor 	
Equipment	<ul style="list-style-type: none"> • Maintenance records of all equipment available • Laboratory inventory indicating date of receiving, sl no etc • Temperature checking regularly • -70°/-20° freezer working condition (alarm operating) • Calibration of pipettes, PH meter, analytical balance • Pipetting technique 	
Computer	<ul style="list-style-type: none"> • All documentation computerized • Maintenance • Security of the documents • Using internet 	
Logistics	<ul style="list-style-type: none"> • Adequate supplies of laboratory logistics • Any problems or discontinuity in supply chain 	
Laboratory Safety <i>Medical colleges & Research Institutes only</i>	<ul style="list-style-type: none"> • Protective clothing worn in laboratory • Bio-safety hood • Appropriate biohazard waste disposal • Sufficient sharps bins 	



B. Section: QA procedure RDT

Sub Section	Checks	Assessor's (Supervisor's) comment
RDT register storage	<ul style="list-style-type: none"> • Details of RDTs received recorded in the laboratory register. • RDTs inspected on arrival • RDTs kept at 4°C • Check RDT register 	
RDT transport	<ul style="list-style-type: none"> • Check RDT Movement Register • No of RDTs received from the periphery • Time interval between two batches received from field 	
Panel transport	<ul style="list-style-type: none"> • Check QC panel transport registers • Time taken during the transport • Whether SOP was adhered 	
QC Panel use	<ul style="list-style-type: none"> • Adequately thawed prior to use • Used only once • Appropriately labeled • The ID number recorded on worksheet • Date of receipt • No. received 	
RDT QA	<ul style="list-style-type: none"> • RDT testing technique correct • QA method performed as per SOP • Any negative QA results are repeated and confirmed • Check previous QA worksheets • Check RDT package inserts are accessible 	
Reporting	<ul style="list-style-type: none"> • All reports are in standard format • Results reported within 5 days 	
Feedback	<ul style="list-style-type: none"> • Any feedback received from <ul style="list-style-type: none"> - NVBDCP - NRL - RRL (in case of SRL only) • Action taken report 	

Signature of the Supervisor
(With remarks if any)



Chapter 8

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