METHODS MANUAL FOR LABORATORY QUALITY CONTROL TESTING OF MALARIA RAPID DIAGNOSTIC TESTS

Manual of standard operating procedures for:
Laboratory-based quality control testing of malaria rapid diagnostic tests using stored dilutions of malaria parasites and
Preparation of quality control samples from malaria parasite field collections

Version Ten
MARCH 2023

WHO Global Malaria Programme (GMP), Geneva, Switzerland

For internal use only

Methods Manual for Laboratory Quality Control Testing of Malaria RDTs

Important introductory note

This manual is intended primarly for internal use by laboratories implementing WHO-recognized malaria RDT lot testing procedures.

Careful reference should be made to the notes under 'Objectives and Scope of the Methods Manual' when using this manual.

Acknowledgements

The previous versions of this procedure manual were developed by WHO Regional Office for the Western Pacific, WHO Global Malaria Programme in collaboration with the Foundation for Innovative New Diagnostics (FIND), and the UNICEF / UNDP / World Bank / WHO Special Programme for Research and Training in Tropical Disease (TDR), in support of the WHO-FIND malaria RDT evaluation programme. This version was developed by WHO Global Malaria Programme and the Research Institute for Tropical Medicine (RITM). The manual includes inputs from numerous sources, including research laboratories, manufacturers and developers of malaria rapid diagnostic tests and quality assurance specialists.

Particular acknowledgement is due to the Parasitology Department of the Research Institute for Tropical Medicine (RITM),- Department of Health, Philippines, ; College of Public Health, University of Philippines;; Institut Pasteur, Phnom Penh, Cambodia; the National Center for Parasitology, Entomology and Malaria Control, Cambodia, the Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, USA; the Clinical Parasitology Department, Hospital for Tropical Disease, London, UK; the participants for the 2003, 2004, 2006, 2007, 2008 and 2009 WHO informal consultations on quality assurance of malaria rapid diagnostic tests; Rebecca Guy, Burnet Insititute, Australia; Ken Lilley, Army Malaria Institute, Australia; Katherine Grady, US CDC; Pernille Jorgensen, Berlin, Germany; Jennifer Luchavez, RITM, Philippines; and Wellington Oyibo, University of Lagos, Nigeria.

Version 10 includes updated procedures based on regular correspondence between the WHO programme coordinator and RITM in the Philippines, from 2017 to present.

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Key to Procedure History revisions:

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CL – Christian Luna

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RRC – Roxanne Rees-Channer, Hospital for Tropical Diseases, London, UK

QC – Qin Cheng. Army Malaria Institute, Australia

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Chapter 1: INTRODUCTION

1.01 List of Abbreviations

Term	Explanation
Ab	Antibody
Ag	Antigen
AAMI	Army Malaria Institute (Queensland, Australia)
CDC	Centers for Disease Control and Prevention (Atlanta, United States of America)
CIDEIM	Centro Internacional de Entrenamiento y Investigaciones Médicas (Cali, Colombia)
CNM	National Center for Parasitology, Entomology and Malaria Control (Phnom Penh, Cambodia)
DMR	Experimental Medicine Research Division (Department of Medical Research, Yangon, Myanmar)
DNA	Desoxyribonucleic Acid
EDTA	Ethylenediamine Tetra-acetic Acid
EHTH	Ethiopian Health & Nutrition Research Institute (Addis Abeba, Ethiopia)
ELISA	Enzyme-linked Immunosorbent Assay
EQA	External Quality Assurance
EQA LAT	External Quality Assurance Laboratory Assessment Tool
EQAP	External Quality Assurance Panel
EQC	External Quality Control
FIND	Foundation for Innovative New Diagnostics
GE	General External Qualtiy Assurance Indicator
HIV	Human Immunodeficiency Virus
HRP2	Histidine-rich Protein 2
HTD	Hospital for Tropical Diseases (London, United Kingdom of Great Britain and Ireland)
ID	Identification number
IHRDC	Ifakara Health Research and Development Center (Bagamoyo, Tanzania)
IMT	Instituto de Medicina Tropical (Universidad Peruana Cayetano Heredia, Lima, Peru)
IATA	International Air Transport Association
IPB	Institut Pasteur de Bangui (Bangui, Central African Republic)
IPC	Institut Pasteur du Cambodge (Phnom Penh, Cambodia)
IPM	Institut Pasteur de Madagascar (Antananarivo, Madagascar)
IQC	Internal Quality Control
KEMRI	Kenya Medical Research Institute (Kisumu, Kenya)

MVP	Malaria, vector-borne and other parasitic diseases
Non-Pf	Non *Plasmodium falciparum* species
	(Plasmodium *vivax*, Plasmodium *malariae*, Plasmodium *ovale*)
Pan	Plasmodium
PCR	Polymerase Chain Reaction
Pf	Plasmodium *falciparum*
pLDH	Plasmodium Lactate Dehydrogenase
Pm	Plasmodium *malariae*
Po	Plasmodium *ovale*
Pv	Plasmodium *vivax*
p/µL	Parasites per microlitre
QA	Quality Assurance
QC	Quality Control
RDT	Rapid Diagnostic Test. For the purposes of this manual, this refers to immunochromatographic lateral flow devices for the detection of malaria parasite antigens
RITM	Research Institute for Tropical Medicine (Manila, Philippines)
SD	Standard Diagnostics (Seoul, South Korea)
SOP	Standard Operating Procedure
TDR	UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases
UL	University of Lagos (Lagos, Nigeria)
WHO	World Health Organization
WPRO	Western Pacific Regional Office
'Lot-testing Coordinator'	Officer designated by WHO to coordinate overall lot-testing programme, or officer authorized to act on behalf of that person
Project Manager	Officer designated by WHO to manage the lot-testing programme, or officer authorized to act on behalf of that person

1.02 Objectives and Scope of this Methods Manual

This manual details standard operating procedures (SOPs) for the production and use of quality control (QC) samples for QC testing of malaria rapid diagnostic tests (RDTs).

The manual describes a system for QC intended to:

- qualitatively assess the suitability for field use of malaria RDTs designed to detect malaria parasite antigens in order to determine their suitability for use in diagnosis
- provide QC samples that are stable when transported and stored
- provide transparency and consistency in the production and use of the QC samples; and
- use technology and methods suitable for application in most national-level or regional-level laboratory facilities in malaria-endemic regions.

This manual includes SOPs for related areas (e.g. microscopy, laboratory practices), that are necessary to develop an adequate laboratory environment for malaria RDT QC.

It is intended that the preparation of QC samples will be performed by a more restricted number of facilities, while use of the samples for QC may be performed more widely.

The methods have been developed through a process of extensive trial and consultation. Adherence to these methods is necessary to ensure consistency of practice and accuracy of interpretation of results over a range of malaria RDT formats. Modification in the protocol and procedures should only be made with agreement of the responsible WHO coordinator of the WHO. Adaption and modification may be appropriate in certain circumstances in use outside WHO activities, but potential loss of accuracy and consistency of testing must be recognized. Comments and recommendations are welcomed by WHO and should be sent to Malaria_rdt@who.int or the address for correspondence above.

What this manual does?

This manual provides methods that will allow production of samples of relatively consistent antigen content and quality for testing malaria RDTs. These samples are intended to detect inadequacies in the lower limit of detection of parasites that are likely to result in misdiagnoses with a clinical impact in the field. Extrapolation of laboratory results to field use assumes that storage in the field is sufficiently similar to storage of RDTs in the laboratory prior to testing, and that parasites of a similar species and strain (similar antigen) are present.

These methods are therefore suitable for testing product lots after purchase, before and during deployment in the field, to ensure that the product lot fulfils basic criteria for operational use.

What this manual does not do?

This manual does not detail testing methods sufficient to distinguish minor differences in sensitivity between products, or the lower limit of detection of a product. It does not detail methods for assessing RDT specificity.

It does not include methods for assessment of RDT products for regulatory purposes and for comparative studies. However, the methods detailed here for sample collection can be modified and extended to the needs of such assessments. They should be more extensive and guided by the particular requirements of the intended field of use.

Outline of this manual

Chapter 1: Background information about the global malaria RDT evaluation programme and this manual

Chapter 2: Procedures for malaria RDT Lot-testing that serve as instructions for the staff of the Lot-testing laboratory

Chapter 3: Procedures for preparation of the quality control (QC) samples for malaria RDT QC that serve as instructions for the field and laboratory staff in the collection sites.

Part 1: Summary of the activity and requirements

Part 2: Field work procedures for patient recruitment and blood collectionPart 3: QC sample preparation in the laboratory

Part 4: Procedures for storage, internal movements, and transport of QC samples to other laboratories

Chapter 4: Procedures for malaria microscopy (minimum standard), which can be adapted from pre-existing microscopy procedures in the laboratory. These serve as instructions for the malaria microscopists involved in the QC sample preparation.

Chapter 5: Procedures for sample characterization, including malaria antigen detection and quantification by ELISA and DNA extraction and *Plasmodium* species determination by PCR, which serve as instructions for staff performing these methods in the sample characterization laboratory.

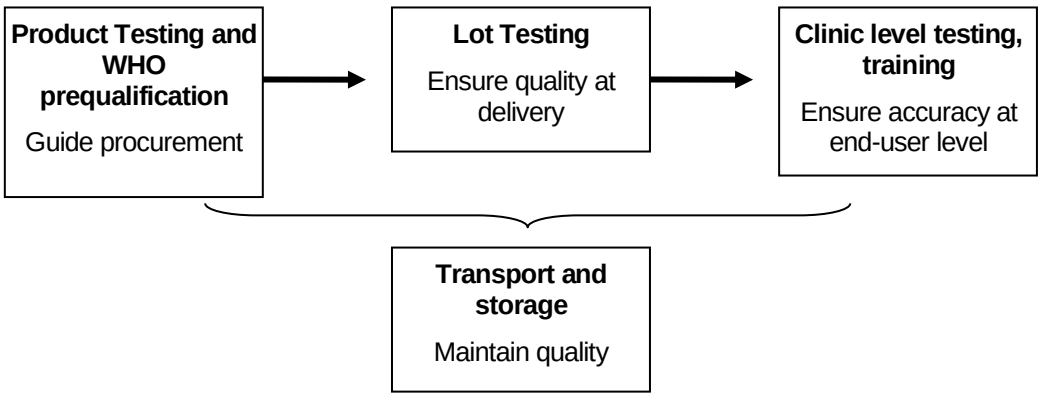
Chapter 6: Procedures for general laboratory quality assurance (minimum standard), which can be adapted from pre-existing QA procedures or manuals in the laboratory, which serve asinstructionsfor all laboratory staff.

1.03 Introduction

Malaria Rapid Diagnostic Tests (RDTs) have a major role in malaria management, particularly in providing blood-based diagnosis in remote locations where microscopy-based diagnosis is unavailable. Like other diagnostic pathology tests, various conditions of manufacture, transport, storage and use may impair their accuracy. Malaria RDTs have frequently performed well in diagnostic trials, but unexplained poor sensitivity has also been recorded in field and laboratory trials [1-10] and in operational use (unpublished reports).

Most manufacturers recommend that RDTs be stored between 2 and 40°C. To be used in tropical/subtropical areas, an ideal RDT should be able to tolerate temperatures of at least 40°C, with peaks of 50°C, under storage for up to 2 years [11]. There are limited data on the stability of many RDTs under such conditions at present, and more extreme conditions may occur temporarily during transport. The stability and sensitivity of products may also vary between lots. It is important that users minimize exposure to high temperatures, and to monitor the performance of each lot [12-13].

In 2008, the WHO-FIND malaria RDT evaluation programme began a product testing programme for malaria RDTs, to assess the performance of product lots under ideal conditions submitted specifically by manufacturers for this purpose. The results served as a guide to RDT procurement for a decade, while an increasing number of products became WHO prequalified In view of the implications of impaired sensitivity to case management, it is vital to have a mechanism in place to ensure continued adequate performance of the tests after delivery of future production lots to countries. This includes a reliable system for laboratory-based assessment of performance on delivery and throughout the expected shelf life of the tests. These manual details quality assurance for lot-testing of malaria RDTs.



TECHNICAL ASPECTS OF TESTING MALARIA RDTS

Malaria RDTs, as referred to in this manual, are immunochromatographic lateral flow devices that detect parasite antigen. Capture of dye-labelled 'signal' antibody-antigen complex by a fixed 'capture' antibody produces a visible line on a nitrocellulose strip, signifying a positive test result. Different products target various antigens specific to plasmodia. Blood, product reagent and labelled antibody-antigen complex are drawn along the nitrocellulose-fibre strip by capillary action and flushing with a reagent / buffer solution.

Performance of malaria RDTs is therefore dependent on several factors, including the rate of flow of blood up the nitrocellulose strip, the adherence of capture antibody (Ab) to the strip, ability of the Ab to bind antigen (Ag), and the integrity of the signal Ab-dye conjugate. All these factors are subject to deterioration in adverse transport and storage conditions, and rates of deterioration and their effect on outcomes can vary between products.

The relationship between antigen concentration and parasite density can vary with the degree of sequestration of parasites, the stage of parasite growth, and the persistence of antigen after reduction or elimination of the parasite population. The antigen concentration of QC samples with a given parasite

density may therefore vary within certain limits, and the parameters used for preparing QC samples must take this into account. Wild parasites and cultured parasites are used for preparing the QC samples.

Variation in the structure of some parasite antigens affects binding to antibody [14]. This variation should be taken into account when interpreting failure of tests against samples with low parasite density, and in the choice of QC samples to verify these results.

The QC samples described in this Manual are derived from fresh blood and prepared and stored in a manner designed to minimize loss of antigen or other changes that may affect RDT performance.

DEVELOPMENT OF THIS METHODS MANUAL

The SOPs within this manual related directly to the production and use of QC samples for malaria RDTs are derived from discussions with a range of developers and manufacturers of malaria RDTs, staff of malaria diagnostic laboratories, published research, and field and laboratory development coordinated by the World Health Organization in collaboration with a number of institutions. Versions of the manual have been reviewed at WHO informal consultations on quality assurance for malaria RDTs at Manila 2003 [15], Manila 2004 [13], Geneva 2006 [16], Kisumu 2006, Atlanta 2006, Philadelphia 2007, Geneva 2008, Bangkok 2010, London 2011, Geneva 2012, Phnom Penh, 2013, Singapore 2015, Atlanta 2016, and London UK 2018. A number of further SOPs relating to ancillary laboratory procedures (e.g. microscopy, and equipment calibration) necessary for preparation of the quality control samples are included. These latter SOPs are written specifically for malaria RDT QA and are not necessarily applicable to other laboratory procedures.

The control copy is based in the World Health Organization Global Malaria Programme. Correspondence should be addressed to Malaria_rdt@who.int, or the addresses for correspondence.

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13. WHO, Informal consultation on laboratory methods for quality assurance of malaria rapid diagnostic tests; Manila, 20-22 July 2004. 2004, World Health Organization: Regional Office for the Western Pacific: Manila
14. Baker, J., et al., Genetic diversity of Plasmodium falciparum histidine-rich protein 2 (PfHRP2) and its effect on the performance of PfHRP2-based rapid diagnostic tests. J Infect Dis, 2005. 192(5): p. 870-7.
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1.04 Major changes from Version 9

Version 10	**List of major changes from Version 9**
Chapter 2	
SOP 2.01	Defined criteria for selecting/replenishing stock RDTs. Inclusion of invalid and false positive fail criteria on Figure 2-1.
SOP 2.03	Inclusion of invalid result and false positive against negative samples fail criteria on Figure 2-2
SOP 2.05	Inclusion of false positive result for pass criteria. Interpretation of false positive result against negative samples and and instructions for repeat testing, and false posiive result on the wrong Plasmodium species. Addition of repeat testing instructions for invalid and false positive results on figure 2.06. Maximum number of lots tested per lot-testing request updated.
SOP 2.06a	Addition of repeat testing instructions for invalid and false positive results on figure 2.07
Forms	
Form 2.08	Updated text in QC testing method and inclusion of non-routine testing information
Annex	
Annex 2	Formatting changes. Addition of indistinct shadowing comment.

1.05 Terms of reference for malaria specimen collection and RDT lot QA testing sites

BACKGROUND

As part of a WHO initiative to improve quality of malaria diagnosis based on Rapid Diagnostic Tests (RDT), WHO is coordinating a network of laboratories to collect and prepare parasite samples suitable for use in testing RDTs, and to test RDTs submitted by national malaria programmes and other bodies to ensure sufficient quality for use in the field. The network will include central reference laboratories/specimen bank(s) where more extensive product testing will take place.

The specimen collection/lot-testing sites will collect and prepare samples of wild-type parasites according to standard operating procedures, characterizing by microscopy and screening for blood-borne viruses. Some of these samples will be transferred to the central specimen banks, where further characterization will take place. Some collection sites may perform further characterization, depending on pre-existing capacity.

The collection sites may also test locally procured lots of RDTs using retained specimens, according to the SOPs. Some sites may perform only one of the two functions, specimen collection or testing using external specimens.

TERMS OF REFERENCE

1. Follow the SOPs detailed in this manual;
2. Maintain local specimen bank;
3. Complete database information and transmit collected information on a regular basis as required by SOP;
4. Maintain local database of available specimens;
5. Characterize specimens by parasite density, white cell count, red cell count, haemoglobin;
6. Screen for bloodborne viruses (HIV, Hepatitis B, hepatitis C);
7. Provide suitable specimens to reference banks;
8. If requested to lot-test RDTs:
    - Test and monitor RDTs from purchased lots from countries, manufacturers, NGOs, procurement agents etc.;
    - Test RDTs from field on request (non-routine testing);
    - Provide rapid feedback on results of RDTs testing (7-14 working days after receipt);
9. Research to refine protocols, in collaboration with WHO and others laboratories;
10. If requested, test new rapid tests and testing formats for malaria in collaboration with WHO;
11. Be overseen by institutional (ethics) review board, and external quality assurance (EQA) programme, including participation in a malaria microscopy EQA programme;
12. Publication of results must be done in collaboration with WHO and cleared by WHO;

Disclosure of any possible conflict of interest to WHO

TECHNICAL AND COMPETENCE REQUIREMENTS

1. Access to cases suitable to provide specimens, within reach of preparatory facility;

2. Availability of prequalified expert microscopists;
3. Ship and receive international biological specimens;
4. Receive, test, and store RDTs according to standard protocols;
5. Storage (-80°C) and archiving of specimens;
6. Consistently monitor storage conditions;
7. Prepare paperwork, summaries of testing/records and collate returned reports;
8. Maintain electronic database of panels and results;

EQUIPMENT AND SPACE REQUIREMENTS

1. Set of automatic pipettes, binocular microscope and centrifuge
2. pH meter (if Giemsa stain is prepared by the laboratory)
3. Slide dryer, staining station
4. Thermometer, vortex mixer
5. Access to blood cell count analyser
6. Computer with internet connection
7. Freezers (-80°C) with alarm and uninterrrupted power supply (UPS), generator set
8. Refrigerators and freezers
9. Incubators dedicated to project
10. Adequate bench space

PRINCIPLES GOVERNING THE USE OF SAMPLES AND THE ROLE OF LABORATORIES IN MALARIA DIAGNOSTICS EVALUATION

Samples derived from wild type parasites should only be used in laboratories within the WHO network. These samples may be used as follows:

1. Routine lot testing for malaria programmes and other organizations as required.
2. For development of methods for evaluation of malaria diagnosis falling under the WHO network.
3. Specific testing for manufacturers and developers on a non exclusive basis in consultation with WHO network.

Note: Samples should not be available for commercial purposes. In situation where there is a possible conflict of interest between its role of a Diagnostic Evaluation Centre under WHO network or with any specific manufacturing company, the relationship should be dicussed with WHO network by laboratories.

1.06 List of laboratories involved in laboratory QC testing of malaria RDTs

GLOBAL MALARIA SPECIMEN BANK AND SAMPLES CHARACTERIZATION

Centers for Disease Control and Prevention
Malaria Branch, Division of Parasitic Diseases and Malaria
1600 Clifton road
Bldg 23, Room 10-169 Mailstop D-67
NE Atlanta GA 30329
USA

Department of Clinical Parasitology
Hospital for Tropical Diseases (HTD)
Mortimer Market, Capper Street
London WC1E 6AU
UK

Australian Defence Force Malaria and Infectious Disease Institute
Weary Dunlop Drive
Gallipoli Barracks
Enoggera QLD 4051

WHO SUPPORTED LOT TESTING LABORATORIES

2009 - present
Research Institute for Tropical Medicine (RITM)
Filinvest Compound
Alabang, Muntinlupa City
PHILIPPINES

2009 – 2017**
Laboratory of Molecular Epidemiology
Institut Pasteur du Cambodge (IPC)
#5, Monivong Blvd, P.O. Box 983
Phnom Penh
CAMBODIA

OTHER LABORATORIES FOLLOWING WHO PROCEDURES FOR LOT TESTING

Department of Medical Microbiology and Parasitology
College of Medicine (RM 308) of the University of Lagos (UL)
University of Lagos
Idiaraba, Lagos
NIGERIA National Institute of Malaria Research

ICMR-National Institute of Malaria Research
Sector 8, Dwarka, New Delhi-110077 (India)

MALARIA SAMPLE COLLECTION SITES FOLLOWING THE SOPS IN THIS MANUAL

Ethiopian Public Health Institute (EPHI)
Patriot Street
P.O. Box 1242
Addis Abeba
ETHIOPIA

Experimental Medicine Research Division,
Department of Medical Research (DMR)
No. 5, Ziwaka Road, Dagon P.O., Yangon 11191
MYANMAR

Department of Medical Microbiology and Parasitology
College of Medicine (RM 308) of the University of Lagos (UL)
University of Lagos
Idiaraba, Lagos
NIGERIA

Institut Pasteur de Bangui (IPB)
BP 923 Bangui
CENTRAL AFRICAN REPUBLIC

Centre for Clinical Research
Kenya Medical Research Institute (KEMRI)
Po Box 54
Kisumu
KENYA

Ifakara Health Research and Development Centre (IHRDC)
360 Kiko Avenue
Mikocheni, Dar-es-Salaam
UNITED REPUBLIC OF TANZANIA

Institut Pasteur de Madagascar (IPM)
Unité du Paludisme - Malaria Unit
Institut Pasteur de Madagascar
BP 1274 - Antananarivo 101
MADAGASCAR

Service de Parasitologie - Mycologie
Faculté de Médecine, Pharmacie et Odonto Stomatologie
Université Cheikh Anta DIOP Dakar.
Avenue Cheikh Anta DIOP,
BP : 5005 Dakar Fann / 16 949 Dakar Fann
SENEGAL

Centro de Entrenamiento y Investigaciones Médicas (CIDEIM)
Avenida 1-N 3-03
Cali
COLOMBIA

Universidad Peruana Cayetano Heredia
Av. Honorio Delgado 430
Urb. Ingenieria, San Martin de Porres
AP 4314 Lima
PERU

1.07 Noting and Varying Procedures in this Manual

VARYING CHAPTER 2 AND CHAPTER 3

Procedures in Chapter 2 and Chapter 3 of this Methods Manual refer to procedures specific for quality control testing of malaria RDTs. When used for specimen collection and storage and QC testing within the WHO RDT QC network, these procedures should only be varied from the written procedures with the agreement of the WHOProject Manager, unless otherwise specified in the "SCOPE" section of each SOP. Agreed variations from the written procedures should be *noted in the table* at the end of each SOP, with a letter confirming the agreement with variation by Project Manager.

VARYING CHAPTER 4 TO CHAPTER 6, AND CHAPTER 7 (FORMS LIBRARY)

Procedures in Chapter 4 and Chapter 6 refer to general laboratory procedures. Many institutions will have pre-existing standard operating procedures and quality control guidelines for these procedures. The procedures in these chapters should be viewed as minimum standard for RDT QC testing and may therefore be varied by each institutions providing all essential elements of the procedures are retained, unless otherwise specified in the "SCOPE" section of each SOP. Variations /replacement forms should be *noted in the table* at the end of each chapter of the reference copy for the Methods Manual.

The Forms in Chapter 7 should be viewed as minimum standard and may be modified according to local needs of participating institutions, providing the replacement forms retain the information sought on the existing forms.

SIGNING OF PROCEDURES AND ARCHIVING

For quality assurance purposes, a reference copy of the Methods Manual should be kept and procedures signed and noted by responsible officer/technicians overseeing the procedures to confirm it is understood (in the table at the end of each SOP). A further copy should be available in the laboratory /specimen collection site.

Hard copies of all forms should be retained, in addition to electronic archiving.

See SOP 6.10 and 6.11 for Documents Control and Storage.

Methods Manual for Laboratory Quality Control Testing of Malaria RDTs

Chapter 2: RDT QUALITY CONTROL PROCEDURE

LIST OF FORMS FOR CHAPTER 2:

SOP 2.01 Organization of the Lot Testing laboratory

PURPOSE

This Standard Operating Procedure (SOP) describes a short overview of the malaria RDT lot-testing process, and the basic components required to set up a laboratory for this.

BACKGROUND

Reliance on RDTs to guide malaria case management is expected to continue to increase. Therefore, a quality assurance (QA) system for RDTs is needed to ensure there are good practices related to manufacturing, purchase, transport, storage, and technical use by health workers. A method of monitoring these practices is to implement quality control (QC) procedures at a number of different stages:

a) Prior deployment to the field (lot testing)

b) By health workers prior to use in the field.

This document specifically relates to quality control lot testing prior to deployment to the field. An integral component of the lot testing is the development and use of quality control samples to test the threshold sensitivity of RDTs to determine if deterioration has occurred. To ensure that each lot of a product has the high standards specified for the product by the manufacturer, RDTs should be tested on receipt from a manufacturer prior to use in the field (initial testing).

This initial lot testing of RDTs will provide some confidence about the quality of RDTs used as a basis for determining malaria therapy.

For the purposes of this document, RDTs detecting only *P. falciparum* are designated Pf-only RDTs, and combined RDTs detecting *P. falciparum* and pan-specific or *P. vivax* specific antigens are designated Combination RDTs. The methods may be adapted to RDTs detecting antigens specific for non-*P. falciparum* parasite species.

SCOPE

This procedure is part of the methods for the quality control of malaria RDTs described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the WHO Project Manager/RDT lot-testing coordinator.

PROCEDURE

A. Overview of the lot-testing (Figure 2-1)

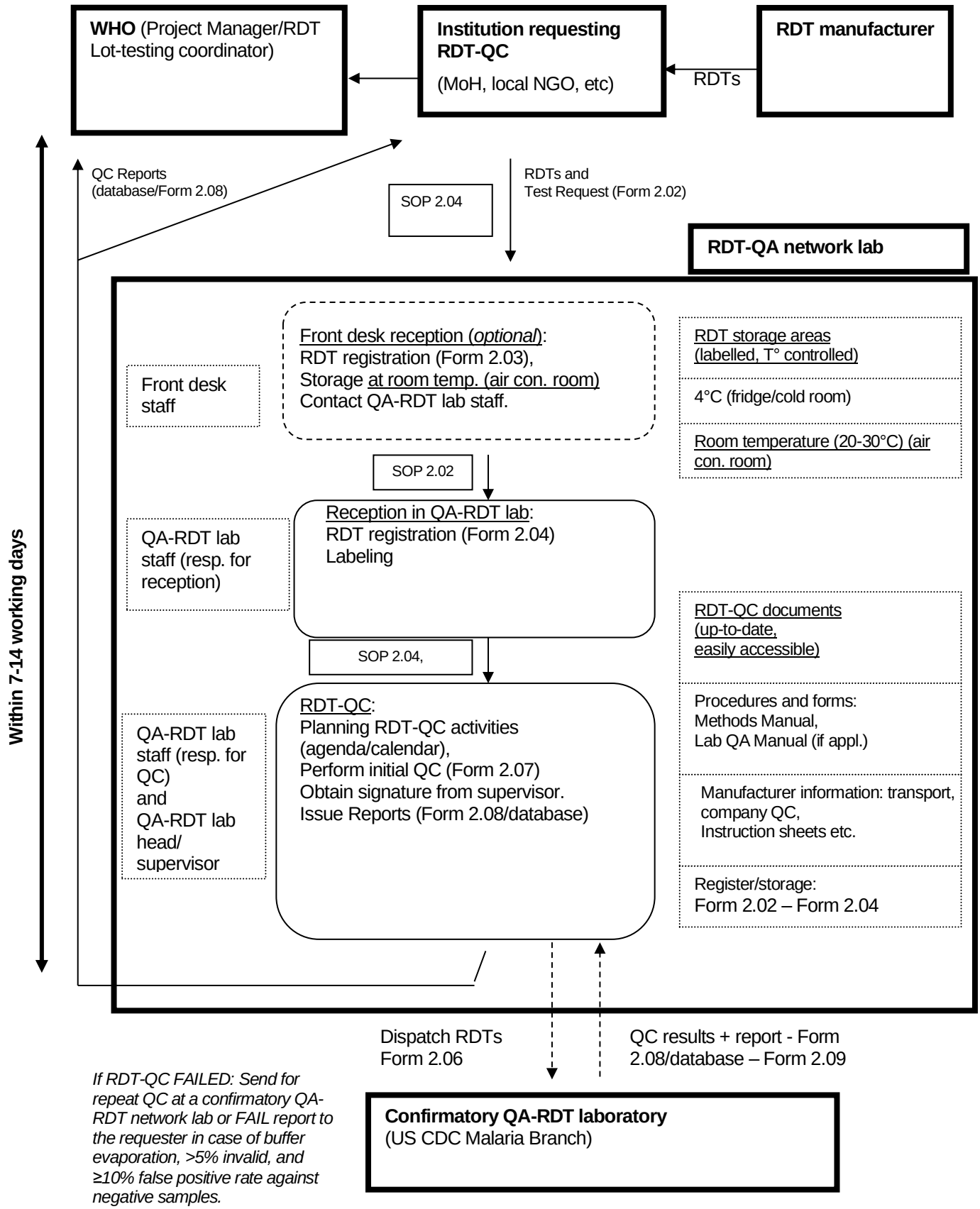
1. The lot testing activity in the WHO Lot Testing Laboratories is supervised and coordinated by the laboratory head as well as a WHO Project Manager/ RDT lot-testing coordinator.
2. The lot testing laboratories are organized appropriately by:

- designating a supervisor and responsible staff for each of the tasks related to lot testing.
- organizing areas for temperature-controlled storage of RDTs.
- organizing all required documents: Methods Manual for procedures and forms, laboratory QA Manual for the laboratory's own QA system, documents related to all received RDTs, completed forms for all QC tested RDTs, etc.
- organizing the receipt, registration, storage, lot testing, dispatch of RDTs, as well as the communication of lot testing results and reports with WHO, the Institution requesting lot testing, and other lot testing laboratories if required

3. Lot Testing of RDTs can be required by Institutions distributing and/or using malaria RDTs in the field, such as the National Malaria Control Programs (Ministry of Health), Non-Governmental Organizations, and others. This manual refers to 'Routine Lot-testing' of commercially available RDTs prior to use for clinical management. The laboratory also has to perform QC of RDTs for its own activities (RDTs for screening patients in the field, stock RDTs for validation of QC samples, see paragraph D). The laboratories in the network also test RDTs under development or required for specific research protocols, in which case other agreed 'non routine' testing protocols may be used.
4. The Institutions requesting the lot testing send a completed Lot Test Request (LTR) form and the required number of RDTs to the RDT-QA laboratory.
5. The lot testing staff undertakes reception, registration, storage and lot testing of the RDTs. After signature of the final lot testing report by the supervisor, the report is sent to the the WHO RDT lot-testing coordinator and to the requester (institution having requested the lot testing) within 7-14 working days after RDT receipt, depending on the workload of the lot-testing laboratory).
6. If RDTs fail the lot testing, RDTs are dispatched to US Centers for Disease Control and Prevention (Malaria Branch) for confirmatory testing. However, the lot testing laboratory doing the primary testing for a particular lot is responsible for final reporting of the results, including the results from the confirmatory laboratory in that report.

The following paragraphs and other SOPs of Chapter 2 provide more detail for each of these steps.

• Figure 2-1: Overview of the lot testing



B. Organization of the lot testing staff

1. The responsibility for each of the tasks is assigned to the staff as listed in Form 2.01, and a copy provided to each of them.
2. Explain and distribute copies of all relevant procedures and forms to each of the staff.

C. Organization of lot testing documents

1. Keep one folder with all procedures and forms needed for lot testing in the laboratory or in a place easily accessible by laboratory staff (at least Chapter 2 of the Methods Manual and the relevant forms).
2. Prepare folders for each of the following documents (compiled by date):
    a) Documents accompanying the RDTs shipped to the laboratory (transport documents, manufacturer'sQC, etc.),
    b) product insert from the RDT manufacturer
    c) lot testing request form (Form 2.02) provided by the requester
    d) completed forms for RDT registration and description (Form 2.03, Form 2.04)
    e) completed forms for RDT storage, internal movements, and dispatch (Form 2.05, Form 2.06)
    f) completed forms for record of the QC results (Form 2.07)
    g) completed forms for lot testing reports (via database or Form 2.08)
    h) complete forms for accessory assessment (Form 2.09)
3. Refer to SOP 6.10 and SOP 6.11 for control and storage of all documents.

D. Definitions guiding the storage conditions and QC of received RDTs

Lot-RDTs are RDTs received for routine lot testing are stored in room temperature (20-30°C) and lot-tested as described in the procedures of this chapter 2 (initial testing).

RDTs used for patient screening for the laboratory's sample collections are stored at 4°C and similarly lot-tested as per these chapter 2 procedures (initial testing only, unless there is doubt about stability).

Stock-RDTs are used for validation of the QC samples which have produced negative results with lot-RDTs. They are stored at 4°C and similarly lot-tested as per these chapter 2 procedures (initial testing only, unless there is doubt about stability). It must be a WHO prequalified product and not have any active notice of concern, not expiring within 18 months, passed the routine lot-testing with no erroneous results (i.e. no false positive and false negative, and invalid) and has a clear background within reading time (i.e. no red background).

The RDTs for patient screening and the stock-RDTs are selected among the products meeting WHO performance requirements according to results obtained in the WHO malaria RDT Product Testing.

E. Overview of RDT register, storage and QC planning

1. RDTs received at the QC Laboratory should first be registered (SOP 2.02).
2. RDTs should immediately be transferred to the appropriate storage temperature as defined above (D.).
3. Initial testing of RDTs is performed and results are reported within 7-14 working days (SOP 2.05).

PROCEDURE HISTORY

Date	Version	Comments	Initials
11 MAY 2008	5	SOP introduced	DB/JL/PJ/SI/WO/CS
MARCH-JUNE 2010	6	Testing interval changes to 6 months	SI, DB, AA
MAY 2014	7	Room temp. instead of <25°C	DB, SI, NC
APRIL 2016	1	Inclusion of the Form 2.09 (accessory assessment)	SI, NC
JUNE 2019	9	Revised Figure 2-1, Updated reporting time of testing results, Removal of Long-term testing Information and SOP references	JC, JL, CAL
MARCH 2023	10	Defined criteria for selecting/replenishing stock RDTs. Updated Figure 2-1, addition of false positive against negative samples and invalid results fail criteria	JC, JL

SOP 2.02 Receipt, storage, and dispatch of Malaria RDTs

PURPOSE

This Standard Operating Procedure (SOP) describes describes how to organize and ensure proper receipt, storage, and dispatch of Rapid Diagnostic Tests (RDTs).

SCOPE

This procedure is part of the methods for the quality control of malaria RDTs described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the WHO Project Manager/RDT Lot testing coordinator.

PROCEDURE

A. Front Desk Receipt (optional)

This step is only relevant if RDTs are received at a front desk or reception area and cannot be immediately delivered to the lot testing laboratory.

1. The person receiving the RDTs (front desk guard or receptionist) completes the RDT Front Desk Register (Form 2.03), and contacts one of the lot testing staff listed in Form 2.01 **as soon as possible** (Responsibilities of RDT QC staff).
2. If none of the responsible laboratory staff are available, or if the RDTs are received outside office hours, immediately place the RDTs in an air-conditioned room. If air-con room is not available, store RDTs as per manufacturer's specifications. Do not leave the RDTs exposed to direct sunlight, heat, or rain. Do not put the RDTs in a freezer.
3. Contact the responsible laboratory staff as soon as possible (*must* be within 5 days of receipt).

B. Receipt in the Lot Testing Laboratory

1. Inspect the RDTs (damage of boxes, traces of humidity, etc.) and complete Form 2.04.
2. File any accompanying transport documentation in the designated folder. Record on the documentation the name, lot number, and reception date of the RDTs to which it belongs.
3. File the RDT product instructions from the manufacturer in the designated folder. Record on the instruction sheet the name, lot number and reception date of the RDTs to which it belongs.
4. File the RDT manufacturer QC results (if available) in the designated folder, and record the name, lot number and reception date of the RDTs to which it belongs.
5. Label all RDT boxes with the reception date. If RDTs are transferred in plastic bags, then label them additionally with the RDT product name, the catalog number, lot number and expiry date.

C. Storage of RDTs

1. RDTs for lot testing can be kept at room temperature (20-30°C) until the initial testing.
2. RDTs for patient screening and stock-RDTs must be stored at 4°C, and the 4°C storage temperature indicated on the boxes or bags. Temporary storage at room temperature (20-30°C) in an air-conditioned room is acceptable, as long as it doesn't exceed 2 weeks.
3. The storage of all RDT lots is recorded in Form 2.05.
4. RDT storage spaces should be well organized with labeling (stickers on shelves, doors of incubators, etc.) or organization charts.
5. Temperatures should be recorded daily with calibrated thermometers of appropriate temperature range, with clearly identified staff responsible for these records (use SOP 6.08 and Form 6.07 or equivalent).

C. Movement and dispatch of RDTs

1. Movement of RDTs to another storage area must be recorded in Form 2.05.
2. Dispatch of RDTs to another laboratory must be recorded in Form 2.06.

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	3	Routine Revision, minor changes only	RG
28 MARCH 2006	4	Modification of storage temperature	DB
11 MAY 2008	5	Re-numbered from SOP 2.1 (version 4) to 2.02 (version 5). Front desk receipt: specified delay, changed storage temperature, Lab receipt: more detail, referred to new SOP and form for RDT storage, and added information on temperature monitor; Dispatch: mentioned shipment arrangements.	DB/JL/PJ/SI/WO
MAY 2014	7	Form 2.06 no longer completed at reception RDT boxes to be labelled only with date of receipt Added procedures for RDT storage, movement and dispatch	DB, SI, NC
JUNE 2019	9	Removed long-term storage instructions	JC, JL, CAL

SOP 2.03 RDT QC Communication pathway

PURPOSE

This document describes the procedure for the communication of results obtained for lot testing of malaria RDTs to requesters and WHO.

SCOPE

This procedure is part of the methods for quality control testing of malaria RDTs described in Methods Manual for Laboratory Quality Control Testing of Malaria Rapid Diagnostic Tests. The SOP is only to be modified with agreement of the WHO Project Manager/RDT Lot testing coordinator.

PROCEDURE

Definition of terms:

Lot testing coordinator: is the person based at WHO and is tasked to coordinate all activities related to lot testing.

Lot testing requester: can be any institution asking for lot-testing of RDTs (*e.g. National Malaria Programme that orders RDTs from manufacturer through a local supplier or a manufacturer responding to a pre-shipment testing request*)

Sending institution: is the institution that ships RDTs for lot testing to the lot testing laboratories. It can be different from the requester, e.g.in the case of lot testing requested by a National Malaria Program but RDTs being sent directly by the manufacturer.

Primary Lot Testing Laboratory: is the laboratory first receiving RDTs for lot testing, and conducting the primary lot testing.

Confirmatory Lot Testing Laboratory: is the laboratory receiving RDTs from the primary lot testing laboratory (in case of a DEFERRED result) and conducting the confirmatory testing.

The QC Assessment Communication Pathway is summarized below and in Figure 2-2.

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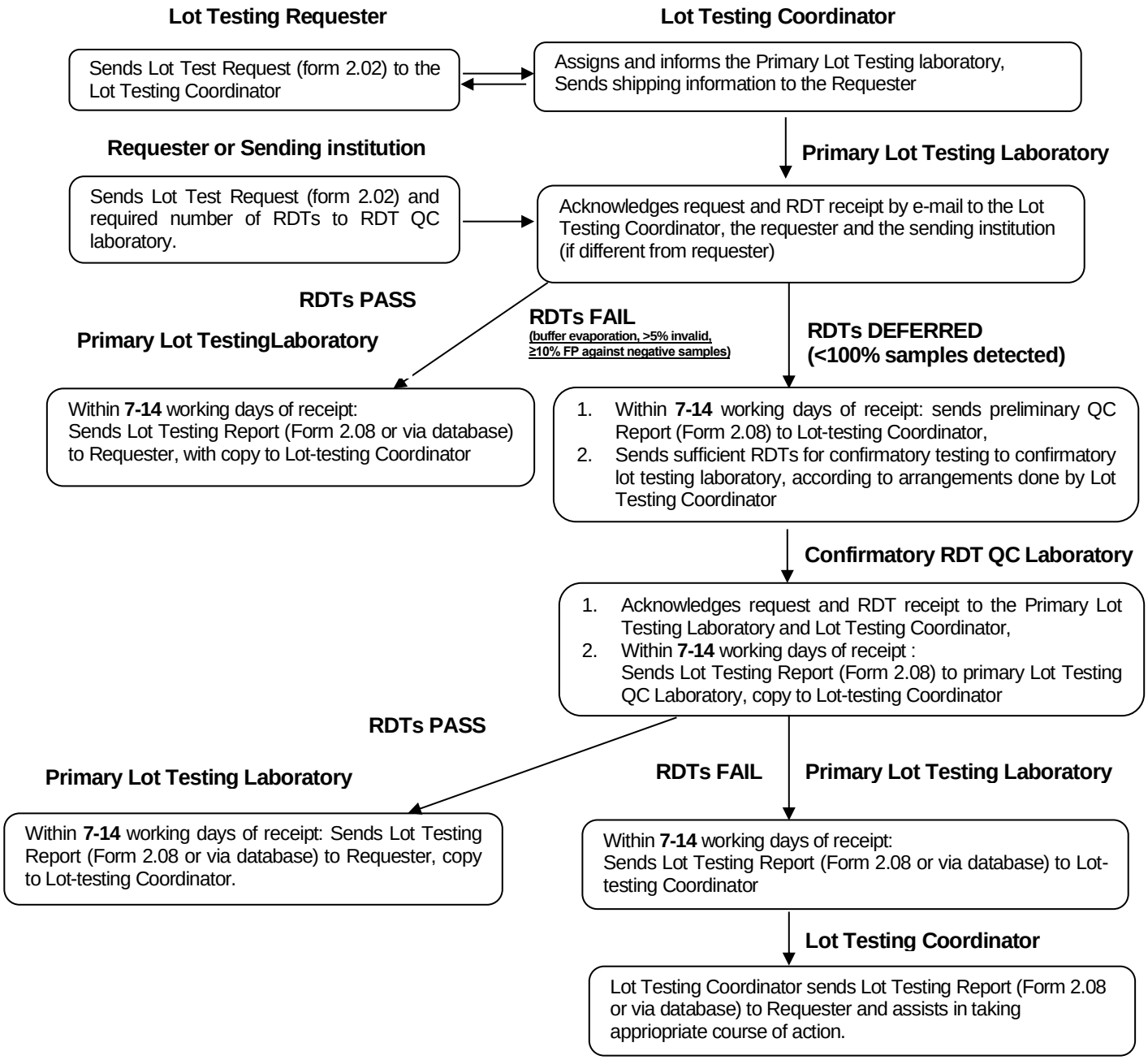
1. The requester completes the Lot Testing Request Form (Form 2.02), obtainable from the website, and sends it to the WHO RDT lot testing coordinator. The lot testing request form cannot contain more than 15 RDT lots.

   Shipping instructions are sent to the requester and/or sending institution (e.g., manufacturer shipping the RDTs) within maximum10 working days.

2. Sending Institution ships RDTs with a copy of the Lot Testing Request Form (Form 2.02) to the laboratory.

3. As soon as the RDTs are received in the lot-testing laboratory, the requester, sending institution and lot-testing coordinator are informed through email.
4. If the RDTs are not received at the QC Laboratory, as expected, and as determined by the requester, the requester should contact the lot-testing coordinator by phone or e-mail (not the lot testing laboratory).
5. After the lot-testing is finished (within 7-14 working days depending on the workload after receiving the RDTs), "PASS" QC lot-testing reports are sent directly to the person(s) named in the lot-testing request form as recipient(s) of the report and to the lot-testing coordinator.
6. "DEFERRED" QC lot-testing reports are sent to the lot-testing coordinator, who will subsequently organize for confirmatory testing and shipping of RDTs to the other lot-testing laboratory. Once the RDTs are received in the confirmatory laboratory, the lot-testing coordinator is informed, who may give additional instructions on the manner of testing.
7. After the confirmatory testing (within 7-14 working days depending on the workload after receiving the RDTs), lot-testing reports, whether "PASS" or "DEFERRED", are sent to the primary laboratory (first lab that performed the lot-testing) and to the lot-testing coordinator.
8. The primary laboratory immediately prepares the final lot-testing report (PASS or FAIL).
9. For "PASS" reports, they are sent directly to the requester(s) and lot-testing coordinator.
10. For "FAIL" reports, they are sent first to the lot-testing coordinator for review, and if determined to be accurate, are sent to the requesters.

- Figure 2-2: Organization and Communication Pathway for RDT lot testing



PROCEDURE HISTORY

Date	Version	Comments	Initials
1 JULY 2004	D	Draft developed after site visit to Cambodia	RG
14 OCTOBER 2005	2	Routine Revision, minor changes only	RG
17 DECEMBER 2005	2	Revised	DB/RG
11 MAY 2008	5	Re-numbered from SOP 2.5 (version 4) to SOP 2.04 (version 5). Added reference to Test Request form and to SOPs for registration and storage of RDTs, changed numbers of RDTs to test, (re-)defined contact persons for notification and reporting at WHO and FIND. Communicaton path and flow charts.	DB/JL/PJ/SI/WO/CS
MAY 2010	6	Clarification of number of RDTs required for confirmatiory testing and of required confrmatory testing during long-term follow-up	DB,SI, AA, NC
MAY 2014	7	Rewording and formatting changes	DB, SI, NC
APRIL 2016	1	Figure 2-2 updated with failure in case of buffer evaporation	SI, NC
JUNE 2019	9	Updated contact person of lot-testing coordinator, turn around time for sending of reports revised, removed long-term testing and reporting time changed in Figure 2-2	JC, JL, CAL
MARCH 2023	10	Updated figure 2-2, inclusion of false positive against negative samples and invalid result fail criteria	

SOP 2.04 Performing an RDT with QC sample aliquots

PURPOSE

This SOP describes the procedure for performing a malaria Rapid Diagnostic Test, by appropriately using QC sample aliquots as a blood sample.

SCOPE

This procedure is part of the methods for quality control testing of malaria RDTs described in Methods Manual for Laboratory Quality Control Testing of Malaria Rapid Diagnostic Tests. The SOP is only to be modified with agreement of the Project Manager.

PROCEDURE

A. Appropriate use of QC sample aliquots for RDT QC testing

1. Take out the required QC sample aliquots from the freezer and place on a rack.
2. Leave on the bench and let stand at room temperature (20-30°C) for a minimum of 20 and a maximum of 60 min.
3. Store inside the refrigerator at 4°C if not to be used immediately and use it within a maximum of 12 hours of thawing.
4. QC sample aliquots should be used only once (do not re-freeze unused and/or left-over samples).
5. Discard left-over samples as per safety SOP 6.01.

B. Performing an RDT using a QC sample aliquot

1. Before performing the RDT QC testing, study the RDT manufacturer instruction sheet.
2. Prepare the required number of QC sample aliquots as described above.
3. Approximately 30 minutes before testing, bring RDTs to room temperature (20-30°C) BEFORE OPENING the package. This applies only to RDTs stored under different conditions than room temperature (20-30°C) (e.g. incubator, fridge).
4. Remove the RDT packaging.
5. Check integrity of RDT packaging when opening. If signs of moisture are present, DO NOT USE the RDT.
6. Check desiccant for any colour changes if visible (e.g. blue to white). If the color change indicates moisture, discard RDT and use another RDT for testing.
7. Label the RDT with at least the QC sample ID and dilution, using a marker pen.
8. Mix the QC sample aliquot vigorously (flick or use vortex) prior to opening and pipetting the blood.

9. Test the RDTs with the QC sample as per manufacturer instructions BUT use a micropipette to transfer the specified blood volume to the RDT.
10. Use a timer to record all steps exactly as per manufacturer instructions.
11. Read RDT results within the manufacturer recommended time.
12. Record the results on RDT QC Result Form (Form 2.07).
13. Refer to the WHO standard color chart **(ANNEX 1)** for rating the band intensity from 0 (negative) to 4+.
14. Refer to the 'Guide for observations noted during malaria RDT Lot Testing' **(ANNEX 2)** for noting comments about any abnormalities observed on the lot-RDTs.
15. Take digital photographs of all tested RDTs and file at least an electronical copy with the Lot Testing report (via database or Form 2.08).
16. Discard left-over samples as per safety SOP 6.01.

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
11 MAY 2008	5	Re-numbered from SOP 2.3 (version 4) to 2.04 (version 5). Combined with former SOP2.2 (RDT QC procedure) and SOP 3.4 (Use of QC samples). Specified SOP for performing RDT with QC sample aliquots. Changes include: use of timer, specified of the time until RDTs have reached room temperature (20-30°C), thawing of samples	DB/JL/PJ/SI/WO
MARCH 2010	6	Addition of digital photographs of non-critical abnormalities	
MAY 2014	7	Re-specified minimum and maximum time for thawing QC samples. Use color charts for rating band intensities Note comments according to the guide for observations (abnormalities) Save electronical copies for all photos together with the reports	DB, SI, NC
JUNE 2019	9	Minor changes only	JC, JL, CAL

SOP 2.05 RDT Lot Testing Procedure

AIM

To provide guidelines for the testing of RDTs using quality control samples in order to assess if the threshold of detection (sensitivity) of the RDT lot (batch) is acceptable for use in the field.

BACKGROUND

Published trials and experience in various countries has demonstrated a wide variability in the sensitivity of malaria RDTs, both within and between products trials [1-9]. Sensitivity is particularly variable at lower parasite densities. The WHO expert consultations of 1999 and 2003 recommended 95% sensitivity at 100 parasites/μl as a reasonable target for RDT performance [10-11]. However, with the limitations of microscopy accuracy, dilution accuracy, loss of Ag during preparation and storage, and the natural variation in the ratio of parasite density to antigen concentration, a higher level (200 p/μl) was chosen to prevent incorrect rejection of good quality tests. False negative results have also occurred at higher parasite densities [1, 6-8],

PURPOSE

This Standard Operating Procedure (SOP) describes the process of initial testing of malaria RDTs.

SCOPE

This procedure is part of the methods for quality control testing of malaria RDTs described in Methods Manual for Laboratory Quality Control Testing of Malaria Rapid Diagnostic Tests. The SOP is only to be modified with agreement of the WHO Project Manager/RD lot testing coordinator.

MINIMUM REAGENTS AND EQUIPMENT

Items	Quantity Required**
1-20 μL Pipette	2
Pipette tips (1-20 μL capacity)	
Timer	4
Vortex mixer	1
-70°C freezer	2
+4°C refrigerator	1
Refrigerator thermometer (range: -20°C to +50°C)	1
RDTs for lot testing, including spares*	See paragraph A.
QC aliquots of Pf, Pv and malaria parasite negative cases	See paragraph A.
Rack for QC aliquots	3
Stock (quality assured) RDTs for each type of antigen‡	100 (per antigen type)
pLDH and HRP2 ELISA (optional)	-
RDT QC Result Form 2.07	1
RDT QC Report Form 2.08 or via Database	1
Marker pen	2
Camera	1
Waste bin for biological samples	2

* Spare RDTs are from the same lot under assessment. They are required for re-testing failed/deferred RDTs within the QC laboratory and for confirmatory testing in another laboratory within the WHO Malaria RDT QA network.

‡ Stock RDTs are quality assured RDTs from a different lot that may be of the same or different RDT brand as the RDTs under assessment. They must detect the same malaria parasite antigen as the RDTs to be assessed (i.e., if the RDT to be assessed is HRP2-based, a similar RDT type whether of the same brand or not, should be used as stock. They are used only when it is necessary to check the integrity of malaria positive QC aliquots that gave negative results with RDTs under assessment.

** Quantities are for 2 staff per laboratory, working on approx. 3 RDT lots at the same time.

PROCEDURE

The Lot Testing Laboratory should perform an Initial QC Testing of a specific number of RDTs using malaria QC samples. Initial QC testing is performed for each RDT lot received for assessment at the QC laboratory In case of insufficient buffer (e.g. low volume of buffer) to perform a testing, a 'fail' report will be sent to the requester. Photos of the testing of each RDT lot and accessory assessment performed on each RDT lot are sent to the requester with the lot testing report.

A. Number of RDTs required for QC per lot

i). For Pf-only RDTs:

34 RDTs are required for initial testing Spare RDTs should be retained in case of repeat testing or if extra RDTs are required to be sent to a confirmatory laboratory. A total quantity of **100 RDTs** will be required for completing the entire QC testing:

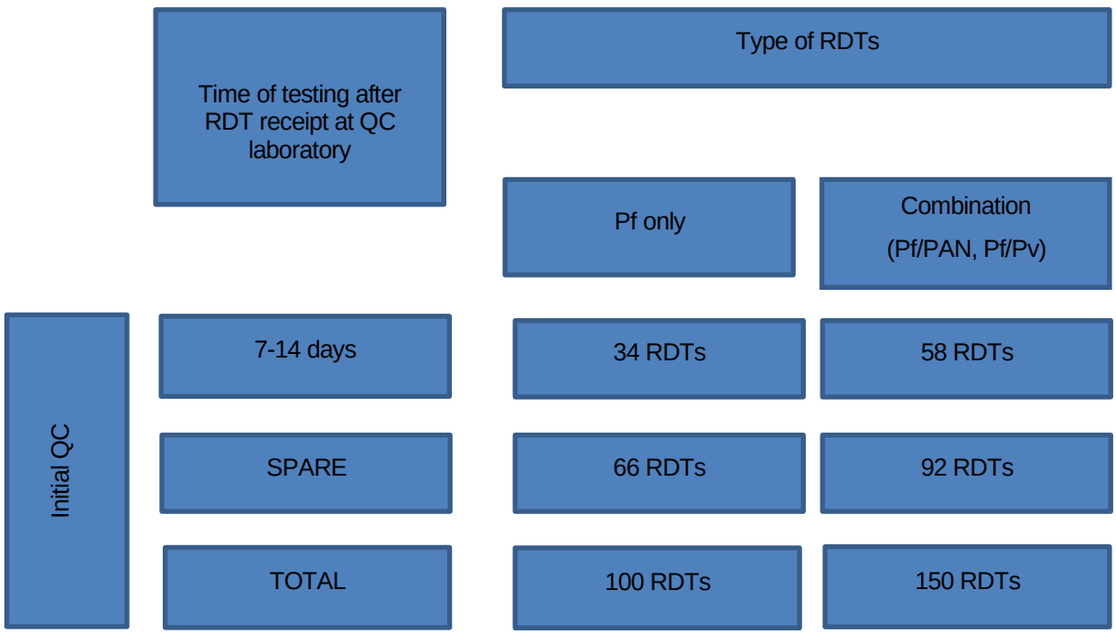
Initial QC: RDTs	34
Back-up for repeat testing and confirmatory testing	66
Total	100

ii). <u>For Pf and Pv combination RDTs:</u>

58 RDTs are required for initial testing. Spare RDTs should be retained in case of repeat testing or if extra RDTs are required to be sent to a confirmatory laboratory. A total quantity of **150 RDTs** will be required for completing the entire QC testing.

Initial QC: RDTs	58
Back-up for repeat testing and confirmatory testing	92
Total	150

- Figure 2-3: Summary of RDTs required for initial QC for RDTs



B. Selection and management of the QC samples

The samples used for the lot testing are prepared according to the procedures in chapter 3 of this Manual, and subsequently characterized according to procedures in chapter 5. For inclusion of samples for lot testing, the following criteria must be fulfilled:

- Single species infections only, as confirmed by PCR
- Consistent ELISA quantification results obtained in the ≥3 runs of ELISA experiments performed for each of the three antigens and each dilution, with the results obtained at the 200 p/µL and the 2,000 p/µL being consistent with each other as well (factor of roughly 10 between results)
- Antigen concentrations of dilutions at 200 p/µL should be within the same range as the wild-type panels selected for the Malaria RDT Product Testing (Rounds 1 to 5):

	HRP2	Pf LDH	Pf aldolase	Pv LDH	Pv aldolase
Minimum	0.6 ng/mL	0.2 ng/mL	0 ng/mL	1.6 ng/mL	1.7 ng/mL
Maximum	74 ng/mL	53.5 ng/mL	9.9 ng/mL	47.9 ng/mL	15 ng/mL

- If the pool of samples available for lot testing is sufficiently large in numbers, then the antigen concentration range at the 200 p/µL dilution should be restricted to the following range:

	HRP2	Pf LDH	Pf aldolase	Pv LDH	Pv aldolase
Minimum	5.0 ng/mL	10.8 ng/mL	0 ng/mL	15 ng/mL	1.7 ng/mL
Maximum	9.5 ng/mL	53.5 ng/mL	9.9 ng/mL	47.9 ng/mL	15 ng/mL

- The samples should be selected according to their antigen concentrations for the antigens that are targeted by the malaria RDT being tested. However they can be out of the recommended ranges for any other antigen that is NOT targeted by the RDT.

  e.g. for testing of a Pf-only RDT targeting HRP2 only, the selected *P. falciparum* samples must be in the recommended ranges for HRP2, but they can be out of the recommended ranges for Pf LDH and/or Pf aldolase

  e.g. for testing of a Combination RDT targeting HRP2 and pLDH, the selected *P. falciparum* samples must be in the recommended HRP2 and Pf LDH ranges, and the selected *P. vivax* samples must be in the recommended Pv LDH ranges, however they can be out of range for Pf aldolase and/or Pv aldolase.

- The selection of the samples is to be agreed with the Project Manager, with agreed samples listed in the dedicated masterfiles.
- The samples use is monitored in the dedicated masterfiles, with stocks of samples aliquots to be updated based on aliquots use calculations every month, and based on a physical inventory at least once a year.

C. Summary of the QC Testing

Take out the required number of RDTs from at least 2 boxes. Bring RDTs to room temperature (20-30°C) and thaw the required number of QC aliquots minimum 20 minutes, maximum 60 min before testing, then perform RDTs, all according to SOP 2.04. Note that more than one aliquot may be needed for the testing of each sample. Record results on RDT QC Result Form 2.07. The detailed steps and algorithm of the QC testing are described below in paragraph E.

In addition to the testing with QC samples, an assessment of the RDT kit accessories is also conducted. This is described in paragraph D.

D. Assessment of accessories

The assessment of the kit accessories is conducted during the initial testing, using form 2.09. For one lot testing request, only one lot (per product) will undergo this assessment (to reduce workload), i.e. if

one lot testing request contains more than one lot (for the same product), only one of these will be randomly selected for the accessories assessment.

- At initial testing, the product insert sheet is verified for a number of essential items, to answer all questions listed on form 2.09. Then, the alcohol swabs, desiccant and buffer vials are checked to answer the questions about the accessories. The form 2.09 is then completed and photos are taken of all the accessories. Both the photos and the completed form 2.09 are provided along with the lot testing report form 2.08, as described under the 'reporting' paragraph below.

Notes:

For the question related to the desiccant, it needs to be known what aspect (color) the desiccant included in this particular RDT product has in case of exposure to humidity. If this is unknown, a small quantity of desiccant can be taken out of the kit and purposely exposed to some water, to observe any color change.

For the question related to the buffer vials, the answer 'yes' is checked if there is sufficient buffer to conduct the entire QC testing procedure. If however there is insufficient buffer, or if it is noted that the kit contains buffer vials that are empty or have nearly no buffer inside, then the answer 'no' is checked. A failure on this particular question signifies a failure of the RDT lot, so this observation must be noted in the 'comments' field of the accessories form 2.09, and a FAIL result must be indicated in the lot testing report form 2.08.

E. Initial Testing (0 months)

The initial testing (0 months) is done within 5 working days of reception of the lot RDTs at the lot testing laboratory.

Step 1a: First testing

The first testing is the testing of lot RDTs with a first series of QC samples (step 1a of the flowchart shown in Figures 2-6 and 2-7)

i) Pf-only RDTs:

1. Select 4 different Pf QC samples (*A*-*D*) (Pf panel 1), and 10 different negative QC samples (*I*-*R*). (*Different* QC samples refer samples prepared from different patient blood samples, i.e. with different ID number) (Figure 2-4).
2. For each of the 4 Pf QC samples, perform 6 RDTs with the 200 p/μL QC aliquot(s) (total 24 RDTs).
3. For each of the 10 negative QC aliquots, perform 1 RDT (total 10 RDTs).
4. Use a total of 34 RDTs taken from at least 2 boxes.
5. All RDTs should be performed, and QC samples used according to SOP 2.04.
6. Record results on the RDT QC Result Form 2.07, according to SOP 2.04.

ii) Pf and Pv combination RDTs:

1. Select 4 different Pf QC samples (*A*-D) (Pf panel 1), 4 Pv QC samples (*E*-*H*) (Pv panel 1), and 10 different negative QC samples (*I*-*R*) (*Different* QC samples refer samples prepared from different patient blood samples, i.e. with different ID number) (Figure 2-5).
2. For each of the 4 Pf QC samples perform 6 RDTs with the 200 p/μL QC aliquot(s) (total 24 RDTs)..
3. For each of the 4 Pv QC samples perform 6 RDTs with the 200 p/μL QC aliquot(s) (total 24 RDTs).
4. For each of the 10 negative QC aliquots, perform 1 RDT (total 10 RDTs).
5. Use a total of 58 RDTs taken from at least 3 boxes.

6. All RDTs should be performed, and QC samples used according to SOP 2.04.
7. Record results on the RDT QC Result Form 2.07, according to SOP 2.04.

At this stage, lot-RDTs ***pass*** the lot testing only if:

- *Pf*-only lot-RDTs test positive on all 6 RDTs for each of the 4 different *Pf* QC samples (total 24 RDTs positive on 24 RDTs tested on Pf panel 1, i.e. 100%).
- Combination lot-RDTs test positive on all 6 RDTs for each of the 4 different *Pf* QC samples and each of the 4 different *Pv* QC samples (total 48 RDTs positive on 48 RDTs tested on Pf panel 1 and Pv panel 1, i.e. 100%).
- Invalid testing result is less than 5% of total RDTs tested including the test with negative QC samples.
- False positive is less than 10% on RDTs tested against negative QC samples.

Send a PASS Lot Testing Report, using the Lot Testing database or report in word format.

Step 1b: Repeat testing

If at least 1 lot-RDT is negative (<100% positive) with any of the QC samples in *Pf*-Panel 1 and/or *Pv*-Panel 1, proceed with repeat testing:

1. Select as many **new** QC samples as the number of QC samples that produced a negative result in the first testing step,

Example 1: If lot-RDTs are negative with QC sample 'A' from *Pf*-Panel 1 →

select 1 new *Pf* QC sample 'X',

Example 2: If lot-RDTs are negative with QC sample 'A' from *Pf*-Panel 1 and with QC sample 'E' from *Pv*-Panel 1 → select 1 **new** *Pf* QC sample 'X' and 1 new *Pv* QC sample 'Y'

(Note: it may be necessary to replace more than one QC sample for each parasite species, depending on how many of the QC samples test negative in the first testing step).

2. For each new QC sample, perform only a **first set of 3 RDTs** and record the result on a new copy of form 2.07, according to SOP 2.04.

2a. If at least 1 RDT of the **first set of 3 RDTs** is negative on at least one of the new QC samples, then testing can be stopped, results can be recorded, and it must be proceeded to verification of all the failing QC samples with stock RDTs (see below)

2b. If all 3 RDTs of the **first set of RDTs** are positive, then perform **the second set of 3 RDTs**, on the same QC sample, and similarly record the results.

→ If at least 1 RDT of this **second set of 3 RDTs** is negative on at least one of the QC samples, then results can be recorded, and it must be proceeded to verification of all the failing QC samples with stock RDTs (see below)

→ If all lot-RDTs are positive (100%) with all 6 RDTs performed with all the new QC samples, then the RDT lot has ***passed*** QC testing.

→ Send a PASS Lot Testing Report (SOP 2.04**,** Form 2.08 or using the Lot Testing database) and proceed with:

- validation of the QC samples from *Pf*-Panel 1 and/or *Pv*-Panel 1 which tested negative on the lot-RDTs in the first testing step, using stock-RDTs that target the same antigen as the lot RDTs.

Step 2a: Testing with stock RDTs:

For validation of QC samples that have failed in the first testing step (lot-RDTs have *passed* repeat testing on new QC samples)

Using stock-RDTs, re-test all QC samples from *Pf*-Panel 1 and/or *Pv*-Panel 1 that were negative on lot-RDTs in **step 1a**, by performing 6 RDTs on each sample. The stock RDTs used must target the same antigen as the lot-RDT.

1. All stock-RDTs are positive (100%).

   This suggests that the QC sample(s) that was negative on lot-RDTs in the first testing step: i) may have had lower parasite concentration than the other QC samples in *Pf* and/or *Pv* Panel 1, or ii) that the antigen concentration of the QC sample decreased since initial preparation. The QC sample should be considered for future use, but results monitored, and future use reviewed with the WHO lot-testing coordinator if repeated RDT failures are noted on that sample.

2. At least 1 stock RDT is negative (<100% positive).

   Concerning the QC sample: This suggests that the QC sample(s) which is now both negative on lot-RDTs and stock-RDTs has reduced antigen concentration and should not be used in future QC testing until the QC sample(s) has been tested further. Decision to use that particular sample must be discussed with the WHO lot-testing coordinator.

Step 2b: Testing with stock RDTs:

For validation of QC samples that have failed in the first testing AND in the repeat testing steps (lot-RDTs have *failed* on repeat testing with new QC samples)

Using stock-RDTs, re-test all QC samples from *Pf*-Panel 1, *Pv*-Panel 1, and all **new** QC samples that were negative on lot-RDTs in the first testing (**step 1a**) and the repeat testing (**step 1b**), by performing 6 RDTs on each sample. The stock-RDTs must target the same antigen as the lot-RDT.

1. All re-tested QC samples are positive on stock-RDTs (100% positive). This suggests that the threshold of detection of the lot-RDT may not be acceptable. The RDT lot is temporarily deferred, and the following action should be taken;
   a. Send preliminary DEFERRED Lot Testing report Form 2.08, using a word document), and inform the WHO Lot Testing Coordinator,
   b. The lot testing coordinator will confirm the number of RDTs to be dispatched to the confirmatory laboratory depending on the remaining number of RDTs and testing interval. For RDT dispatch, use Form 2.06 (SOP 2.02)
   c. The Confirmatory Lot Testing Laboratory proceeds to confirmatory testing.

2. If at least 1 stock RDT is negative (<100% positive), this suggest that the QC sample(s) which is now both negative on lot-RDTs and stock-RDTs has a reduced antigen concentration, and should not be used in future QC testing until the QC sample(s) has been tested further, e.g. with ELISA, or with a different set of stock RDTs targeting the same antigen, if available (according to arrangements made by the WHO lot-testing coordinator). Decision to use that particular sample must be discussed with the WHO lot-testing coordinator).

3. The following action should be taken: Test lot-RDTs with as many new *Pf* QC sample(s) and/or *Pv* QC sample(s) as have been negative on both the lot- and the stock-RDTs, e.g. if *Pf* QC sample 'X' is negative on both lot-RDTs and stock-RDTs, test the lot-RDTs with a new Pf QC sample 'Z' , by performing 6 RDTs for each QC sample, then follow the procedure from there. If the QC Laboratory does not have additional QC samples, discuss with the WHO lot-testing coordinator. If instructed, dispatch the appropriate number of RDTs for confirmatory testing according to arrangements made by the WHO lot-testing coordinator (SOP 2.05). For RDT dispatch, use Form 2.06.

Step 3: Confirmatory testing

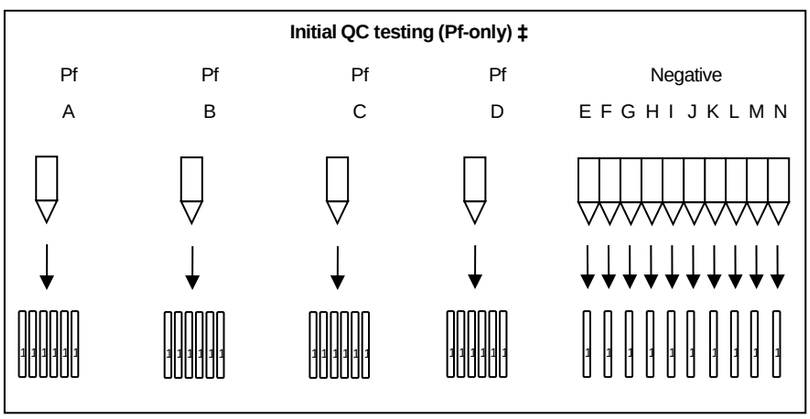
The confirmatory testing takes place at a different Lot Testing Laboratory designated by the WHO lot-testing coordinator. The confirmatory testing is to be done within 7-14 working days of receipt of the lot-RDTs at the Confirmatory Laboratory. It follows the same testing procedure and algorithm as described above except that **there is no testing against negative QC samples.**

Once the confirmatory testing is complete with the entire algorithm followed, the Confirmatory Lot Testing Laboratory will notify the Primary Lot Testing Laboratory about the results of QC testing (use Form 2.07 and Form 2.08, in word format), i.e. whether lot-RDTs fails or passes.

Step 4: Issue of final lot testing report, after confirmatory testing

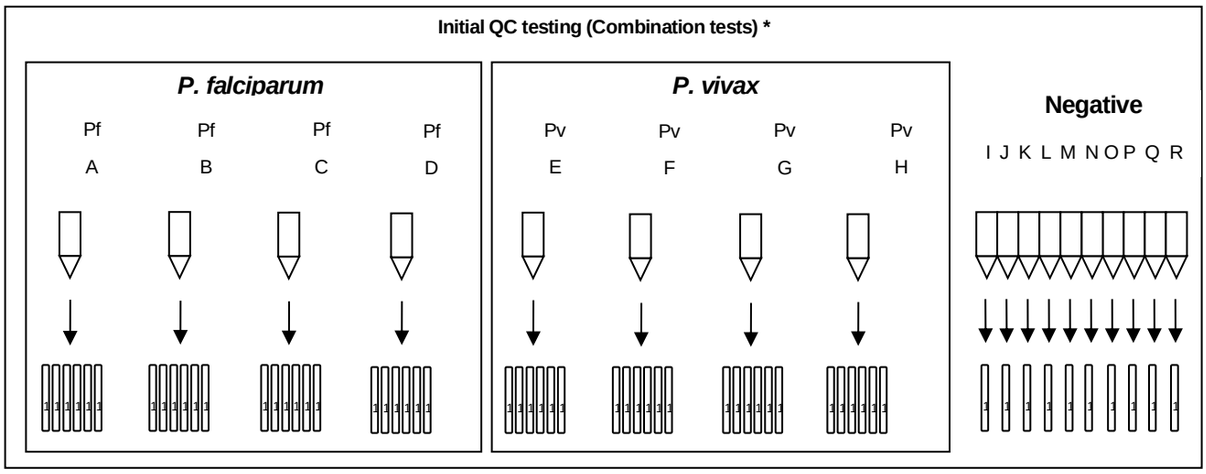
- If Lot-RDTs passed the confirmatory testing, the Primary Lot Testing Laboratory sends a PASS Lot Testing Report (Form 2.08 or using the Lot Testing database) and sends lot-RDTs to the Confirmatory Lot Testing Laboratory, according to arrangements made by the WHO lot-testing coordinator who will confirm the number of RDTs to be sent. For RDT dispatch, use Form 2.06 (SOP 2.02).
- If Lot-RDTs failed the confirmatory testing, the Primary Lot Testing laboratory sends a FAIL Lot Testing Report Form 2.08 or using the Lot Testing database or word report).

• Figure 2-4: Flow Diagram of initial QC Testing of Pf-only RDTs



***Initial QC testing:** Use 24 RDTs and use QC samples from 4 different Pf cases (A, B, C, D) and 10 different malaria CASES (E-N).

- Figure 2-5: Flow Diagram of initial QC Testing of combination RDTs (Pf -pan and Pf -Pv)



*** Initial QC testing :** Use 48 RDTs, and use QC samples from 4 different Pf cases (A, B, C, D) , 4 different Pv cases (E, F, G, H) and 10 different malaria parasite negative cases (I-R).

- If parasite-negative samples give false positive results this needs to be clearly reported in the QC Report Form 2.08 or creating a report via the database and its significance needs to be considered by the institute using RDTs. Further, false positive results are to be repeated using the same QC sample/s they tested positive - this result will replace the findings in initial testing and will be used to determine the final result.If the false positive result is ≥10% of the RDTs tested (determined by repeat testing), then it is considered as FAIL and a final report is issued by the primary testing laboratory. For example: (a) Initial testing: 1FP/10, Repeat testing: no FP, Final: PASS; (b) Initial testing: 1FP/10, Repeat testing: 1FPs/1, Final: 1/10 = 10% FAIL ; (c) Initial testing: 2FP/10, Repeat testing: 2FPs/1, Final: 2/10 = 20% FAIL.

- If the lot RDT tested positive for the wrong Plasmodium spp will be reported in the 'Observations' section of the QC Report Form 2.08.

- If invalid results are noted during testing, then it must be clearly reported in the QC report Form 2.08 or using the database and RDT(s) are to be repeated. If the test line is not visible for any reason such as strong red background or ghost lines, then the RDTs are to be repeated (refer to the guide compiling all anomalies encountered during testing). Invalid rate of >5% of overall RDTs tested is considered failure and a final report is issued by the primary testing laboratory.

- **Table 2-1: Number of lot-RDTs that must be positive in Initial QC testing for the RDT Lot to pass.**

Parasite positive QC samples : species / dilutions (parasites/μL)	Number positive tests / number of tests performed for the entire QC lot testing
P. falciparum / 200 parasites/μL	24/24*
P. vivax / 200 parasites/μL	24/24*

** Discounting QC samples that have been replaced due to initial test failure (Figure 2-6, 2-7).*

F. Reporting of results

The below steps summarise how to report results from Initial testing (for more details on the communication pathway, see SOP 2.03)

1. Results of the initial testing (Form 2.08) or using the database should be sent by email within 7-14 working days of receipt of RDTs. The lot testing report is sent with the photos of the testing of RDTs, photos of the accessories, and the completed form 2.09 of the accessories assessment.
2. Up to 10 RDT lots may be reported on a single report form, when all lots;
   - are from the same RDT product (same catalog number), AND
   - were tested for the same test requestor, AND
   - were listed in the same lot testing request.

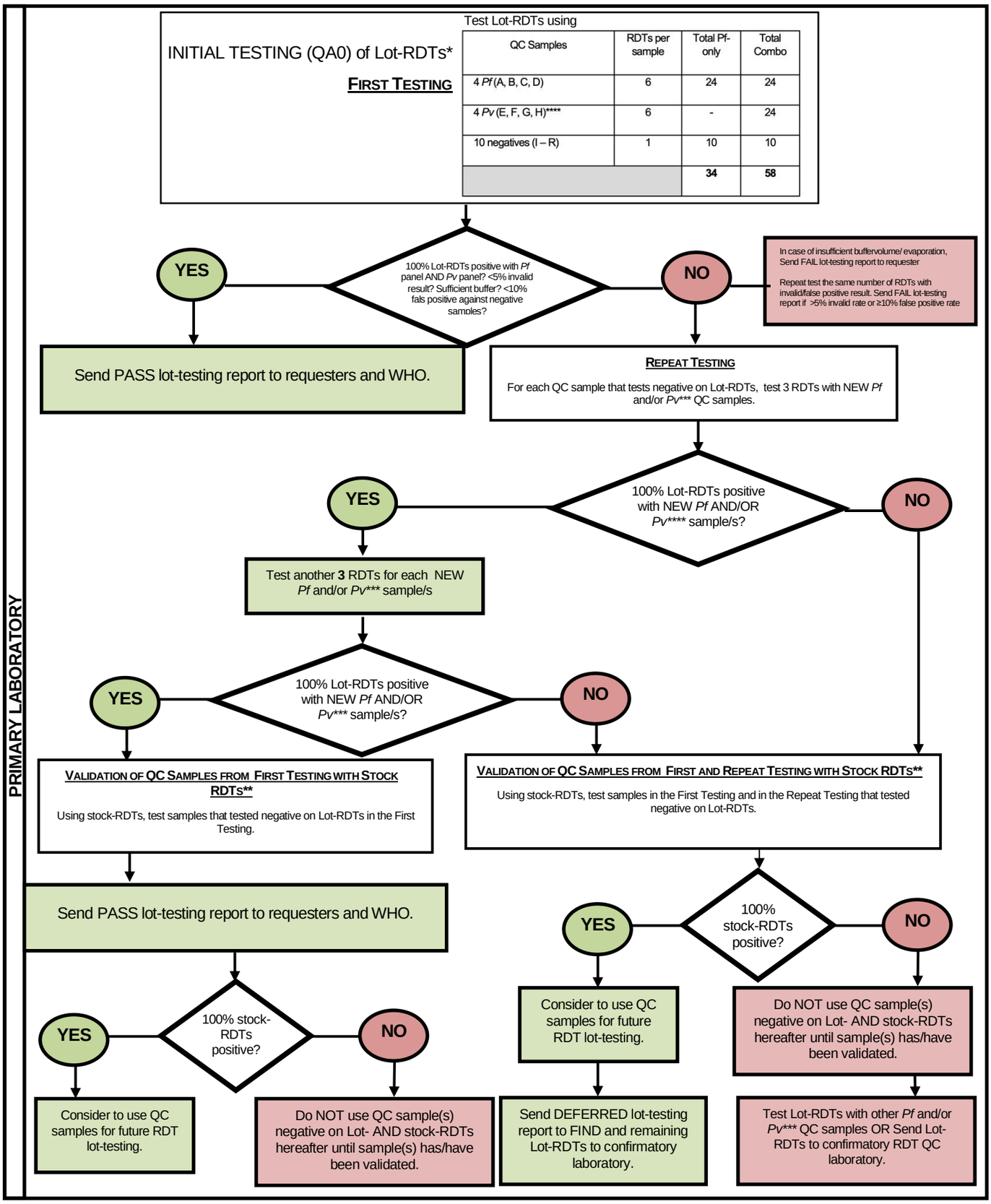
   Separate reports should be completed for different products, or for data intended for different recipients, or for lots listed in different lot testing requests.
3. All reports including photos of the testing, of the accessories and the accessory assessment form must be checked by the supervisor of the Lot Testing Laboratory or by one of the Lot Testing laboratory technician, by paying particular attention to items such as: the product catalog number(s), the lot number(s), the expiry date(s), the e-mail addresses of the requester(s), as well as the consistency between the comments noted in the report (including the number of RDTs noted for each comment) and the photos provided with the report (if photos have been taken) making sure report is sent according to recipient list mentioned in the lot testing request form.
4. After checking, the supervisor or laboratory technician signs a hard copy of the report which is then filed in a dedicated folder.
5. The report (including photos and accessory assessment form) and email to be sent to the requester cannot be released before being double checked by the supervisor or another technician following a detailed checklist provided by the lot-testing coordinator.
6. Electronic and hard copies of all reports, as well as the electronic version of the RDTs and accessories photos, must be retained in the Lot Testing Laboratory.
7. If an error is detected in a report after having been sent to the Project Manager requester and/or the WHO Lot Testing Coordinator, the original report should NOT be amended but a

new report with a new version number should be generated, with adding a short comment that explains what correction has been applied (e.g. corrected catalog number).

8. Follow the reporting guidelines in SOP 2.04.

- Figure 2-6: Algorithm for QC Testing of Pf only and Combination RDTs

This flowchart is the process to follow when performing initial testing at the primary laboratory. The same process is to be followed during confirmatory testing except that the RDTs do not need to be tested against negative QC samples.



- * Lot-RDTs refer to the RDT lot under evaluation;
- ** Stock-RDTs refer to a RDT lot considered to have high quality and good sensitivity, based on previous QC testing results. They are stored at 4°C.If available, ELISA can be used instead of stock RDTs (see Chapter 5).
- *** Pv samples are used for testing of combination (combo) RDTs only

NOTES

1. *The 1999 WHO expert consultation Malaria Diagnosis: new perspectives recommended 95% sensitivity at 100 parasites/μL as a reasonable target for RDT performance [11]. However, for quality assurance of RDTs, quality control samples of 200 p/μL were chosen to test the lower limit of detection. At 100 p/μL, sufficient antigen concentration could not be guaranteed for a fair evaluation of RDTs as:*

   QC dilutions were prepared based on an initial parasite count (see Chapter 3), and therefore some variability in malaria microscopy 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.

   Consequently, RDT lots that do not pass immediate testing should be checked at a second facility before rejection. This should be arranged through the Project Manager.

2. *The integrity of the QC samples can be checked with stock RDT i.e. RDTs stored in the laboratory that is considered to have good sensitivity and are of high quality. It can also be checked with ELISA (HRP2 and/or pLDH and/or aldolase) if available.*

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11. WHO, Informal consultation on laboratory methods for quality assurance of malaria rapid diagnostic tests; Manila, 20-22 July 2004. 2004, World Health Organization: Regional Office for the Western Pacific: Manila

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	3	Routine review, type and quantity of QC panels modified	RG
AUGUST 2006	4	Corrections to RDT numbers, parasite densities	DB
11 MAY 2008	5	Re-numbered from SOP 2.2 (version 4) to SOP 2.06 (version 5) Changed numbers of RDTs to test. Changed parasite densities for testing increased number of negative control samples, new figures revised flowchart, adapted reporting from SOP 2.04,	DB/JL/PJ/SI/WO/CS
MARCH-JUNE 2010	6	Increase in number of RDTs initial testing, and reduction in frequeny of interval testing, in line with WHO Malarai Specimen Bank Steering Committee recommendation, Bangkok, 2010. Clarification of number of RDTs to be transferred for confirmatory testing	DB, SI, AA, NC
MAY 2014	7	No more testing against 2,000p/µl samples and repeat testing when at least one RDT fails. No testing against negative samples during confirmatory testing. Interval of testing: 6 months before expiry instead of testing after 18 months of receipt. Updated number f RDTs for testing. New version number if reports are corrected. Repeat testing in case of obscured test lines.	DB, SI, NC
APRIL 2016	1	Failure in case of buffer evaporation (figure 2.6 updated accordingly)	SI, NC
JUNE 2019	9	Removed incubator in list of minimum required equipment. Modified figure 2.03 with removal of long-term testing information, Inclusion of invalid testing result in determining a pass assessment, Deleted figures 2.04b and 2.05b, Updated Table 2-1 with the number of lot-RDTs that must be positive in initial testing, Figure 2.06 Modified with removal of long-term	JC, JL

		testing and addition of invalid testing result criteria,	
MARCH 2023	10	Inclusion of false positive result for pass/fail criteria and instructions for its repeat testing. Addition of repeat testing instructions for invalid and false positive results on figure 2.06.	JC, JL

SOP 2.06 RDT Laboratory External Quality Assurance Programme

AIM

To outline the processes required for RDT lot-testing and QC sample collection laboratories to be assessed with the External Quality Assurance Laboratory Assessment Tool (EQA LAT) and to participate in the External Quality Assurance Programme.

BACKGROUND

To ensure there are high standards in specimen collection/preparation and testing of RDTs it is essential to have a robust and well-documented QA programme. Two important components of this EQA programme are the EQA LAT and proficiency testing using a set of 'good quality' and 'degraded' (e.g. heat stressed) RDTs, called an External Quality Assurance Panel (EQAP). A third component, parallel testing of RDT lots, may also be included.

PURPOSE

This Standard Operating Procedure (SOP) is required for the RDT lot-testing coordinator, the Project manager(s) and the staff of the lot-testing laboratories to coordinate the assessment of the laboratories using the EQA LAT and the evaluation of the EQAP RDTs.

SCOPE

This procedure relates to the methods for the preparation of RDT quality control samples and evaluation of malaria RDTs described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the Project Manager.

PROCEDURE

PART 1 - The EQA LAT

1. The lot-testing laboratories controlled by the EQA Programme should be assessed with the External Quality Assurance Laboratory Assessment Tool (EQA LAT) annually. Laboratories collecting samples but not conducting lot testing should ideally be assessed before or right at the start of each sample collection, as much as possible. The procedure for the assessment visits is described in more detail in chapter 6 of the Manual.

   The EQA LAT is an MSExcel-based programme that allows immediate feedback of the assessor's findings. The assessment produces a score for individual categories of laboratory work, and an overall score. Certain items are 'flagged' as high priority.

   The EQA LAT should be available to all laboratories on request.

2. The results should be interpreted according to 2 threshold values:

   - Threshold 1 = 85% of the General EQA Indicator (GEI), Threshold 2 = 65% of GEI
   - Flagged items that reveal non-conformities with the required standard should be noted separately in the assessor's report, and corrective actions to address them should be initiated as soon as possible (immediately if possible) after the assessment visit.

3. Laboratories that achieve GEI results 85% to 100% should continue to be 'Certified' and can continue their lot testing and/or specimen collection activities.

4. Laboratories that achieve GEI results 65% to 84% should be subject to remedial action and be re-assessed with the EQA LAT ideally in 6 months. For laboratories assessed before a sample collection, the re-assessment should be done as soon as possible, and the sample collection activity can be put on hold, upon decision of the project manager, if any of the critical items (flags) need to be addressed beforehand. Verification is by repeat visit or remote assessment based on documented evidence sent by the laboratory.

5. Laboratories that achieve GEI results <65% must cease lot-testing and/or specimen collection immediately. Remedial action must be taken, and testing/specimen collection should not continue until a successful re-assessment result is achieved.

6. The EQA assessor and lot-testing coordinator will report all non-conformities (performance below the above standards) to the WHO programme, and ensure that remedial action is discussed during regular project management meetings. Inadequate performance on flagged items should be discussed by the assessor, lot-testing coordinator and responsible person from the WHO programme, and a separate, specific response instituted.

A full report on EQA assessments will bereviewed by the WHO Lot testing coordinator.

PART 2 – PARALLEL TESTING

On special arrangement between the lot-testing coordinator and the institution requesting RDT evaluation, parallel testing may be instituted at two lot-testing laboratories, and results compared on submission to the lot-testing coordinator. In these cases, the lot-testing coordinator only will receive results, and send a combined report to the result recipient. The lot-testing coordinator will investigate discrepancies in the results and arrange confirmatory testing in a third laboratory as appropriate.

The EQA LAT may also be instituted in response to specific issues regarding performance.

RESULT FEEDBACK, NETWORK CONFERENCES AND ON-SITE VISITS

Feedback of assessments using the EQA LAT is partly performed on-site at the time of assessment and finalized remotely. Results of the EQAP should be available to laboratories within a month of testing, and when all laboratories have submitted results.

All laboratories should participate in scheduled teleconferences with WHO on a regular basis, to discuss general issues, EQA issues, pertaining to the collection of specimens and lot-testing.

On-site visits are organized on a regular basis by the WHO project manager and/or lot-testing coordinator in order to go through the full lot-testing process, and/or sample collection and management, and/or general laboratory quality management.

PROCEDURE HISTORY

Date	Version	Comments	Initials
1 JULY 2008	D	Draft Introduced	KGL
22 JULY 2009	D2	General revision	DB, SI
27 MAY 2009	5a	Minor modifications from 2.4	DB
MARCH-JUNE 2010	6	Modifications to EQA schedule, other changes in assignment of responsibilities.	DB. SI, AA
MAY 2014	7	Completed the EQA visits for the sample collection context, 2 EQAP rounds instead of 3, added on-site visits, parallel testing	DB, SI, NC
JUNE 2019	9	Minor changes, proficiency testing using EQA panel removed	JC, JL

SOP 2.06a RDT Laboratory EQA Programme – Use of Proficiency Testing Panels

AIM

To outline the processes required for the Use of Proficiency Testing Panels of malaria RDTs for Lot-Testing Laboratories

BACKGROUND

To ensure there are high standards in specimen collection/preparation and testing of RDTs it is essential to have a robust and well-documented EQA Programme. Two important components of this QA programme are the EQA LAT and proficiency testing using a set of RDTs of pre-determined antigen detection threshold called an External Quality Assurance Panel (EQAP). A third component, parallel testing of RDT lots, may also be included.

PURPOSE

This Standard Operating Procedure (SOP) is required for the use of proficiency panels of malaria RDTs to confirm concordance between lot-testing laboratories in the WHO malaria RDT evaluation programme.

SCOPE

This procedure relates to the methods for the use of RDT quality control samples and evaluation of malaria RDTs described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the Project Manager.

PROCEDURE

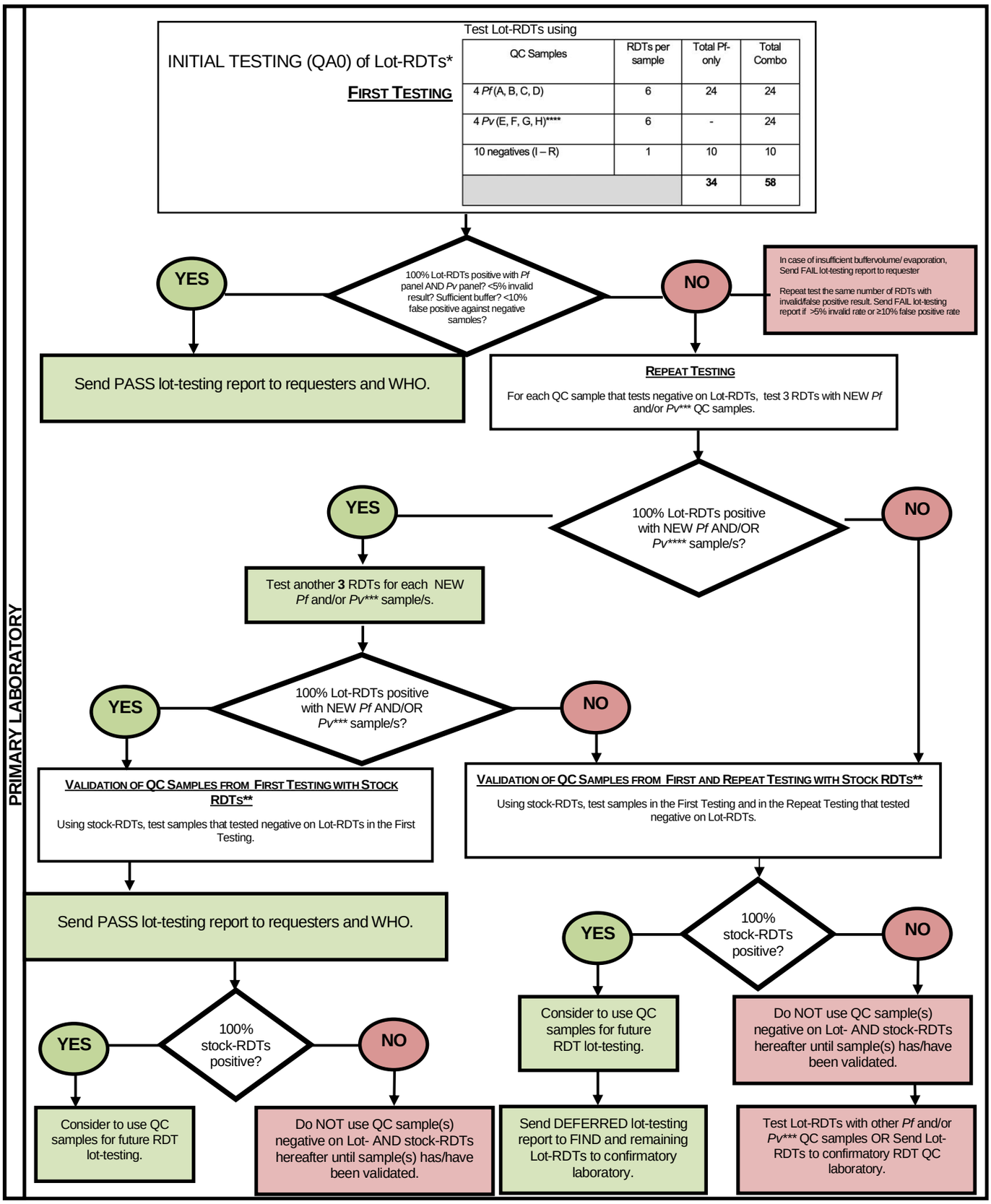
Coordination

Coordination of production of, and use of, proficiency panels will be the responsibility of the WHO lot-testing coordinator.

The coordinator will keep a log of all results, for reporting to the specimen bank steering committee in case of any major concern.

1. RDTs should be managed on receipt in the same way as a normal consignment to be lot-tested.
2. The proficiency test consists of an initial round of testing according to the standard protocol for lot-testing **but excluding confirmatory testing (See Figure below)**.
3. Report results to the WHO lot-testing coordinator, clearly marking the origin, and the identification number allotted to each box by the coordinator.
4. The lot-testing coordinator will record all dates of interaction between the laboratory and the coordinator that are required by the lot-testing SOPs.
5. The lot-testing coordinator will maintain a record of the results of each laboratory in each round of lot-testing. Results should be reported at the next monthly Project Management Meeting of the WHO malaria RDT evaluation project.
6. Discrepant results will be reviewed and acted upon on consultation between WHO and the laboratory concerned.

- Figure 2-7: Algorithm for QC Testing of Pf-only and Combination RDTs



* Lot-RDTs refer to the RDT lot under evaluation;

** Stock-RDTs refer to a RDT lot considered to have high quality and good sensitivity, based on previous QC testing results. They are stored at 4°C.If available, ELISA can be used instead of stock RDTs (see Chapter 5).

*** Pv samples are used for testing of combination (combo) RDTs only

REFERENCES

1. WHO Malaria RDT QC Methods Manual, Version 6, 2008

PROCEDURE HISTORY

Date	Version	Comments	Initials
MARCH-JUNE 2010	6	Minor modifications from 2008 draft 2.4b	DB, AA
MAY 2014	7	Modified flowchart	DB, SI, NC
APRIL 2016	1	Updated figure 2.8 with failure in case of buffer evaporation	SI, NC
JUNE 2019	9	Renamed figure 2.8 (version 8) to figure 2.7 (version 9). Removal of long-term testing information (figure 2.7 updated accordingly). Addition of percentage of invalid results criteria in figure 2.7.	JC, JL
JANUARY 2020	9	This SOP has been suspended due to lack of resources	JC
MARCH 2023	10	Addition of repeat testing instructions for invalid and false positive results on figure 2.07. SOP suspended due to lack of resources	JC, JL

SOP 2.06b RDT Laboratory EQA Programme – Production of Proficiency Testing Panels

AIM

To outline the processes required for production of Proficiency Testing Panels of malaria RDTs for Lot-Testing Laboratories

BACKGROUND

To ensure there are high standards in specimen collection/preparation and testing of RDTs it is essential to have a robust and well-documented EQA Programme. Two important components of this EQA Programme are the EQA LAT and proficiency testing using a set of RDTs of pre-determined antigen detection threshold called an External Quality Assurance Panel (EQAP). A third component, parallel testing of RDT lots, may also be included.

PURPOSE

This Standard Operating Procedure (SOP) is required for the production of proficiency panels of malaria RDTs to confirm concordance between lot-testing laboratories in the WHO malaria RDT evaluation programme.

SCOPE

This procedure relates to the methods for the preparation of RDT quality control samples and evaluation of malaria RDTs described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the Project Manager.

PROCEDURE

Coordination

Coordination of production of, and use of, proficiency panels will be the responsibility of the WHO lot-testing coordinator.

The coordinator will keep a log of all results, for reporting to the specimen bank steering committee in case of major concern.

Production of proficiency panels at Preparatory Laboratory

Sufficient high quality and low quality (heat-stressed) RDTs are produced for two proficiency testing rounds (1 year) in each of the two lot-testing laboratories.

- Figure 2-8: Number of each set of RDTs to be procured and prepared for one year of proficiency testing

Total number of RDTs to be sent directly to the lot-testing coordinator (good RDTs)

Two sites/ two rounds*	Total # of RDTs	Rounded numbers	Detail of the testing carried out
Initial testing at testing lab.	232	240	6 RDTs tested against 4 different QC panels on Pf and Pv at 200p/µl +10 Negatives
Repeat testing at testing lab.	192	200	6 RDTs tested against 4 different QC panels on Pf and Pv at 200p/µl
Spare RDTs	40	40	10 invalid/malfunctioning/red background RDTs, etc.
		480	

Total number of RDTs to be sent to the laboratory in charge of degradation

Two sites/ two rounds*	Total # of RDTs	Rounded numbers	Detail of the testing carried out
Initial testing at testing lab.	232	240	6 RDTs tested against 4 different QC panels on Pf and Pv at 200p/µl +10 Negatives
Repeat testing at testing lab.	192	200	6 RDTs tested against 4 different QC panels on Pf and Pv at 200p/µl
Spare RDTs	40	40	10 invalid/malfunctioning/red background RDTs
RDTs for testing during degradation	56	70	16 for the initial testing +8 for two monthly testing + 16 for four bi-weekly testing + 16 for the final testing
		550	

Total number of RDTs to be sent to each testing laboratory

One site/ one round	Total # of RDTs	Rounded numbers	Detail of the testing carried out
Initial testing at testing lab.	58	60	6 RDTs tested against 4 different QC panels on Pf and Pv at 200p/µl +10 Negatives
Repeat testing at testing lab.	48	50	6 RDTs tested against 4 different QC panels on Pf and Pv at 200p/µl
Spare RDTs	10	10	10 invalid/malfunctioning/red b background RDTs, etc.
		120	

1. A set of 1,030 combination RDTs (HRP2 / pan-pLDH or Pf-pLDH / pan-pLDH or HRP2 / pan-aldolase RDTs) are procured from the same batch (lot #):

   480 are shipped directly to the lot-testing coordinator

   550 are shipped directly to the laboratory in charge of the degradation of RDTs

2. Perform initial lot-testing screening tests using 16 RDTs (*Modified from SOP 2.05, testing only 2 RDTs at each of four different Pf and Pv samples at 200 parasites/µL*).If, at least, one

RDT fails, then stop testing and contact immediately the lot-testing coordinator and Project Manager; a replacement batch will have to be ordered.If, the RDTs are positive with all samples, then the degradation process can start.

3. Degradation of RDTs

Upon receipt of the RDTs by the laboratory

Stabilize incubator for 2 days at 60°C and place all the RDTs in incubator, ensuring air-spaces against at least 2 sides of all boxes. Beforehand, take out 2-3 buffer bottles and keep them at controlled room temperature (20-30°C) for the testing during the incubation period. Use only one bottle for the testing until the bottle is used up; only then start using another one.

For checking of RDTs during incubation:

For checking, please perfrom the following testing:

Remove 4 RDTs from 4 different boxes (one RDT per box, in total 4 RDTs) spread through the batch, and test against one *P. falciparum* QC sample at 200 parasite/μL. Use same QC sample throughout incubation period. Use only one bottle of buffer taken from one box (and not one bottle of buffer from the different boxes where RDTs where withdrawn) to perform the testing. The bottle of buffer is to be kept in a refrigerator in order to be re-used for the next check. When used up, a new bottle of buffer from another box is to be used, which will also have to be kept in the refrigerator when used up.

No mark or information is to be written on the outer box (e.g. identifying the number of RDTs left, etc.).

Start the checking 3 months after the initial testing.

Test every month from month 3 to month 5.

5 months after the initial testing, test, every second week.

<u>If at least one RDT gives a negative result, perform the final testing as described below:</u>

4. Final testing (when at least one RDT has failed):

Continue incubation for 1 week more, then perform the same testing as described for the initial testing above.

Record results and send to Project Manager and Lot-testing coordinator (including QC sample ID used for the testing).

The Project manager and/or Lot-testing coordinator will decide if the degradation rate is sufficient and will advise on the next steps if degradation is to be continued.

5. Preparation of the proficiency panels before shipping to the lot-testing sites

All the degraded RDTs are to be sent to the lot-tesing coordinator

Upon receipt of the RDTs, the Lot testing coordinator is to ensure that each box is complete (with all the accessories and buffer as originally received) and that no specific label or identification is visible on the box and/or on the RDT packs.

The Lot testing coordinator is to withdraw 160 RDTs for each testing laboratory from the degraded RDTs batch and label each box with an identification letter (one letter for degraded RDTs and another letter for the non-degarded RDTs); (A or B or C or D) and is to keep track of the corresponding batch as in the table below.

The Lot-testing coordinator is to also withdraw 160 RDTs from the non-degraded RDTs initially received directly by him/her and label each box with an identification letter (one letter for degraded RDTs and another letter for the non-degarded RDTs), as below:

Testing laboratories	Degraded RDTs	Non-Degraded RDTs	Shipping to the testing laboratory
Lab. 1	120 RDTs labelled "A"	120 RDTs labelled "B"	"A" and "B"
Lab. 2	120 RDTs labelled "C"	162 RDTs labelled "D"	"C" and "D"

The Lot testing coordinator will then ship one btach of each degaded and non-degraded RDTs to each testing laboratory.

The testing laboratory is to test them as a non-routine, initial testing without confirmatory testing.

The results are to be sent to the Project Manager and Lot testing coordinator for checking.

PROCEDURE HISTORY

Date	Version	Comments	Initials
MARCH-JUNE 2010	6	Split from former SOP 2.07b	DB, AA
MAY 2014	7	RDT proficiency testing preparation for one year with revised numbers	DB,SI,NC
APRIL 2016	2	No marking/identification on the outer box- Only one bottle of buffer to be used until it is used up, then a second one can be used.	NC, SI
JUNE 2019	9	Renamed figure 2.10 (version 8) to figure 2.08	JC, JL, CL
JANUARY 2020	9	This SOP has been suspended due to limited resources	JC

Methods Manual for Laboratory Quality Control Testing of Malaria RDTs

Chapter 3: RDT QC SAMPLE PREPARATION

PART 1: Overview, Requirements and Preparatory activities

LIST OF FORMS FOR CHAPTER 3, PART 1:

3.01: Preparatory activities for RDT QC Sample Preparation

3.02: Supplies and Equipments Checklist

3.03: Staff Responsibilities

SOP 3.01 Preparation of Quality Control Samples: Overview and requirements

PURPOSE

This SOP describes an overview of the working steps required for preparing quality control (QC) samples to be used in quality assurance (QA) of malaria rapid diagnostic tests (RDTs), as well as a list of requirements concerning the field collection site, the staff, the equipments and the materials, in order to help ensuring that all conditions are fulfilled for this activity.

BACKGROUND

The mainstay of malaria diagnosis has previously been clinical diagnosis and malaria microscopy. However, parasite-based diagnosis is now recognized as vital for good case management of febrile illness, to reduce anti-malarial drug wastage, and for monitoring of malaria prevalence and the impact of anti-malaria interventions. Rapid diagnostic tests (RDTs) have gained increasing importance in addressing this need, in areas where good-quality microscopy is unavailable [1]. To ensure RDTs contribute to management, it is essential to ensure the accuracy of products prior to disseminating to the field where quality monitoring is often difficult.

An important component of the RDT quality monitoring programme of the WHO malaria RDT evaluation programme is the development and use of panels of standardized samples of malaria parasites, to test product capabilities in the Product Testing programme, and as quality control samples to test the quality of RDTs during lot-testing, or when there are other requirements to confirm that RDTs achive a sufficient threshold of antigen detection.

The standard operating procedures in this chapter describe methods for collection and preparation of wild-type parasites. Wild parasites may have greater variation in antigen production between samples and are likely to be more representative of parasites encountered under field conditions. The preparation of culture-based and recombinant-antigen-based panels, also used in the Product Testing programme, is described elsewhere [2]. Parasite panels should mimic fresh blood infected with wild parasites as closely as possible, as malaria RDTs are designed for use with fresh human blood. When preparing the dilutions, loss of antigen or other changes that may affect RDT performance must be minimized. This requires considerable forethought in choice of the patient recruitment site, organization of the blood samples transport, the field and laboratory staff teams, and anticipated preparation of all required equipments and materials.

The 'Quality Control' (QC) samples described here consist of dilutions of blood of infected patients to a parasite density of 200 parasites/µL. Past WHO expert consultations have recommended that RDTs achieve 95% sensitivity at 100 paraisites/µL. While this recommendation remains, the natural variation in antigen structure and variation in the ratio of antigen concentration to parasite density, and changes in antigen concentration between clinical specimens and preserved specimens, make this impossible to directly measure through the use of standardized panels. In view of this, the lower density of 200 parasites/µL is used in the panels described here, on the basis that tests failing to detect these samples are highly likely to fail to reach the recommended field sensitivity. The higher density of 2,000 parasites/µL is selected as a marker of extreme deterioration or lack of sensitivity and is used for malaria RDT product evaluation only (not for lot testing of RDTs).

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the Project Manager.

PROCEDURE

A. Overview of the preparation of malaria RDT QC samples

The preparation of RDT QC sample aliquots consists in three main steps: i) recruitment of *Plasmodium*-infected patients in the field and collection of venous blood (parasitized blood), ii) procurement of blood from non-infected donors (parasite-free blood), and iii) dilution of the "parasitized blood" with the "parasite-free blood" to low parasite densities (200 p/µL), aliquoting and freezing.

1. Patient recruitment and blood collection

Patients are recruited on the basis of their age, symptoms and history of intake of anti-malarial drugs. Patients are diagnosed for malaria by RDT and/or by microscopy, and certain requirements for the RDT result (strong RDT signal) and/or microscopy result (parasite density over a threshold limit) must be met. Informed consent is required for venous blood collection and screening for viral infections (hepatitis B and C, HIV 1 and 2). Patients may be screened for blood-borne viruses by rapid tests in the field and confirmed later by ELISA, or screening may be by ELISA only after venous blood sampling. Only virus-negative samples are retained for QA testing. Venous blood is collected, slides with thin and thick films are prepared, as well as blood spots on filter paper. All samples are immediately transported to the laboratory in appropriate storage conditions.

2. Preparation of parasite-free blood

For dilution of the *Plasmodium*-infected patient blood, "parasite-free" blood is prepared by centrifugation of O- (preferable) or O+ whole blood and replacement of the O- or O+ plasma by AB+ plasma (ensures compatibility with all patient blood groups). Alternatively, blood from a donor having the same blood group as the patient recruited in step 1 can be used. Whole blood and plasma are obtained from informed and consented volunteer donors or from accredited blood banks. These samples must also be screened for *Plasmodium* parasites, hepatitis B and C and HIV 1 and 2.

3. Dilution of the parasitized blood

Parasites are characterized for species and parasite density by thin/thick film analysis by two microscopists, and the mean parasite density is used for calculating the dilutions A small test mixture of parasite-free blood and parasitized blood is prepared and the absence of red cell agglutination confirmed. Larger volumes of dilutions are then prepared, the absence of agglutination is checked again, and the results obtained with malaria RDTs of each dilution are recorded. The dilutions are then aliquoted in 50 µL volumes in pre-labeled cryotubes and immediately frozen at -70°C ("QC sample aliquots"). Additional "high-volume" aliquots of the

patient blood and of the diluted blood are also prepared for further characterization of the QC sample (parasite species confirmation, antigen content, etc.). If all required characteristics are met, the QC sample aliquots are characterized (usually in other laboratories contracted by WHO and FIND) by PCR and ELISA. Aliquots confirmed as appropriate for use after these steps may be used in the malaria RDT QC testing process as described in the Chapter 2 of this Methods Manual.

B. Requirements for the field collection site

It is recommended to visit eventual field recruitment sites 3-6 months before the planned collection campaign, in order to evaluate the following main decision criteria:

1. Malaria patients:

   The probability of malaria patients satisfying the recruitment criteria (within the allowed age limit, no recent intake of anti-malarial drugs, infections with high parasite densities) should be evaluated beforehand, by discussing with local health staff and checking registration books. The number of daily recruited patients should ideally be between 1 and 3. If the probability of recruitment is lower or higher, then the duration of the collection campaign and the amount of staff in field and laboratory must be adapted accordingly (see below).

2. Delay of blood sample transport:

   To minimize loss of antigen and other changes in the blood sample, the delay between venepuncture and freezing of the final QC sample aliquots must be kept to a minimum. The field collection site should be within easy reach of a laboratory where the blood samples can be processed, allowing transport of samples at 4°C and each day of patient recruitment. The transport delay should ideally be less than 3 hours and should never exceed 6 hours.

3. Cooperation and quality of facilities:

   Local authorities, health staff and the community should be as cooperative as possible, ideally be already familiar with clinical studies. The recruitment facilities should be clean, well organized and provide enough working space for the staff involved in recruitment.

C. Requirements for the laboratory

The quality of the QA-RDT network laboratory is regularly assessed by external quality assessment visits with a detailed questionnaire containing a list of what should ideally be fulfilled by this laboratory. For the QC sample preparation, the main requirements are as follows:

1. The QA-RDT network laboratory should – as much as possible - have a general Quality Assurance (QA) system (such as ISO/CEI 900X), including safety, staff management and training, management of equipments and documents. Microscopists should be enrolled in an active external quality assurance programme.

2. The laboratory should be clean, well organized and provide enough working space for the staff involved in processing the patient blood samples.
3. The requirements for laboratory equipments are listed in Form 3.02. Proper management of these equipments is strongly recommended (life sheet, maintenance, reparation service, eventual disinfections, calibrations, etc.).
4. The laboratory should also be able to perform blood cell counts (automatic blood cell counter, or manual counting) and eventually blood group determinations, either in the laboratory or in a nearby partner laboratory (with a short delay of analysis, as results are required during the QC sample preparation process, ideally within 15-30 min).

If the patient blood samples are processed in another laboratory nearby the field collection site, because of too long transport delays to the QA-RDT laboratory, then this other laboratory should fulfill the conditions 2. to 4., without the need of a strict laboratory QA system.

D. Staff requirements

The staff requirements depend on the expected number of daily recruited patients, the time of blood sample transport to the laboratory, and the capacity of the laboratory staff to process the patient blood samples within the allowed time limit (maximum of 24 hours between the venous puncture and the final freezing of the QC sample aliquots).

1. Staff requirements in the field recruitment site

Staff must be available and sufficiently trained for the following activities (one staff can be responsible for more than one activity if required):

- Interviewer(s) who should be fluent in the local language,
- Technician(s) for diagnosis by malaria RDT and/or malaria microscopy (rapid parasite count),
- Technician(s) for venous puncture, preparing thin/thick films of good quality and eventually blood spots on filter paper,
- Health worker authorized to give malaria treatment according to the national protocol,
- Health worker authorized to provide HIV counseling and obtain the informed and signed consents.

The total staff number can be from 2 persons (one doctor and one technician) if recruitment is done passively in district health facilities, up to a minimum of 4-5 persons if recruitment is done in an active case detection manner.

2. Staff requirements in the laboratory

Staff (technicians and/or scientists) must be available and sufficiently trained for the following activities (one staff can be responsible for more than one activity if required):

- Coordination of the different activities for processing the patient blood samples,
- Two experienced microscopists having followed a competency assessment (pre-qualification) with satisfying results (SOP 6.04) are required,
- Preparation of "parasite-free" blood,
- Dilution calculations using the MS Excel "calculator",
- Dilution of blood,
- Labelling and aliquoting of QC samples,
- Preparation of all required "high-volume" aliquots, eventual blood spots on filter paper, serum for screening of viral infections, performing RDT of all dilutions,
- Completion of forms,
- Supervisor for checking and signing all forms.

If two or more samples are processed per day, there should be one staff entirely dedicated to coordinating the whole team, eventually being also responsible for dilutions calculations and/or completing the forms.

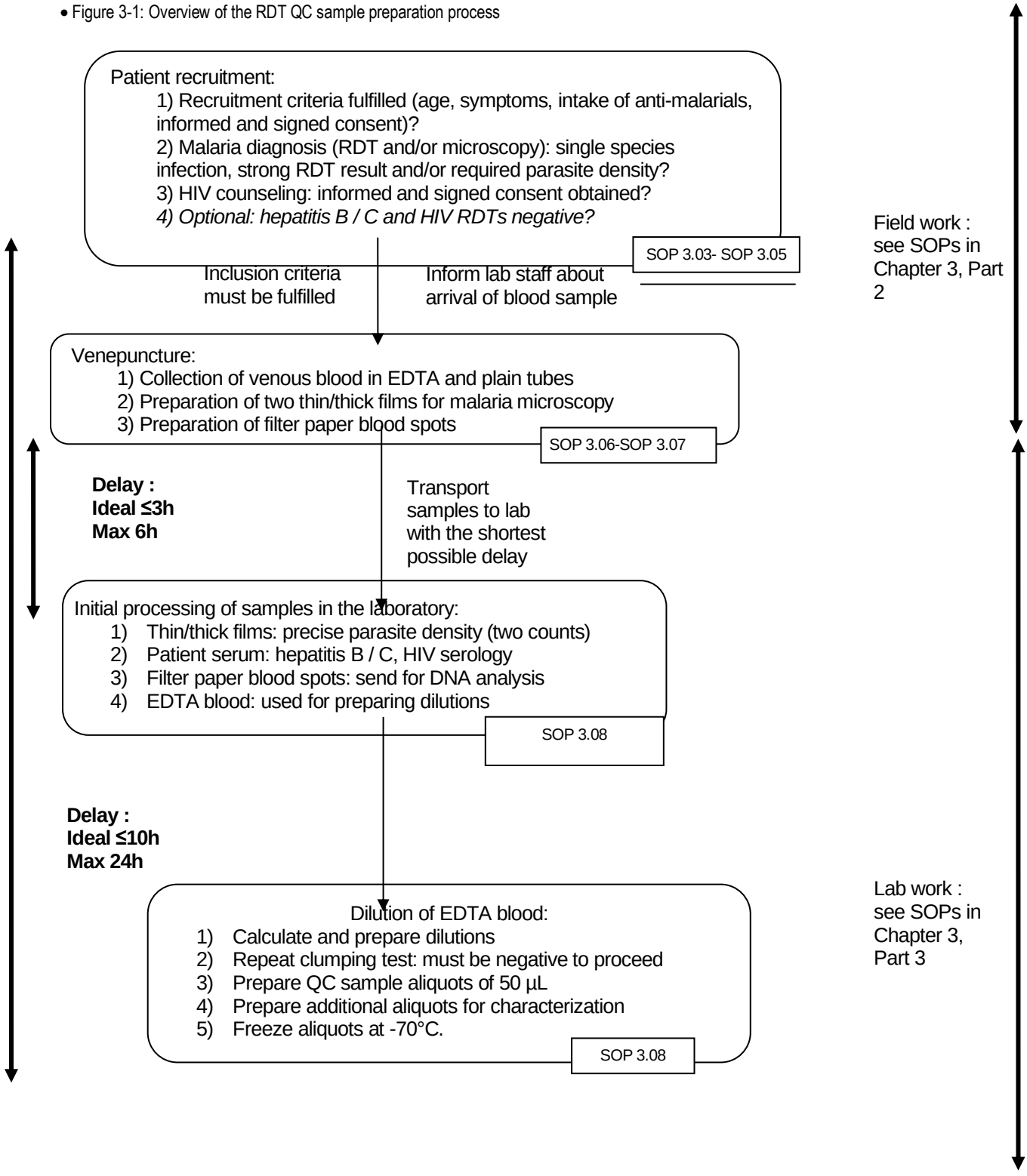
E. Material and equipment requirements

See Form 3.02.

Notes on the equipment and tubes used for mixing blood samples:

1. *The mixing equipment should allow homogenization of blood in tubes of volume 2 mL up to 50 mL, by slowly inverting the tubes. A rocking tray or ideally a sample rotator can be used, by setting a slow rotation speed. The equipment should be installed at 4 ℃ (in refrigerator or cold storage room) for best stability of the antigens in the blood sample, and temperature should regularly be checked (lower temperatures can increase the blood agglutination risk).*
2. *The mixing tubes should have a shape and volume allowing free inversion movement of the whole blood volume. Use tubes with round-shape bottom rather than narrow conical-shape bottom, and do not fill up to more than 4/5th of the tubes volume (e.g. 2 mL round bottom tubes for mixing 1 mL of blood, 15 mL round bottom tubes for mixing 10 mL of blood, or 50 mL conical bottom tubes for mixing 25 mL of blood).*

• Figure 3-1: Overview of the RDT QC sample preparation process



REFERENCES

1. WHO, New Perspectives: Malaria Diagnosis. Report of a joint WHO/USAID informal consultation 25-27 October 1999. 2,000, World Health Organization: Geneva.
2. Methods Manual for Product Testing of Malaria Rapid Diagnostic Tests, Version 5, 2012, Western Pacific Regional Office of the World Health Organization, Manila, Philippines.

PROCEDURE HISTORY

Date	Version	Comments	Initials
01 MAY 2008	5	SOP introduced	DB/JL/PJ/SI/WO
MAY 2014	7	Minor changes	DB, SI, NC
JUNE 2019	9	Formatting changes	JC, JL

SOP 3.02 Preparation of Quality Control Samples: Preparatory activities

PURPOSE

This SOP describes the procedure for all preparatory activities that have to be accomplished before starting the collection of field samples of malaria parasites and the preparation of quality control (QC) samples to be used in quality assurance (QA) of malaria rapid diagnostic tests (RDTs).

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the Project Manager.

PROCEDURE

Three months prior to the collection and preparation of QC samples, some groundwork has to be made to ensure that the RDT QC sample aliquots are prepared in the most efficient and systematic manner. Below is a recommended timetable of preliminary activities, which should at least be accomplished during the month preceding the collection campaign. Some of these activities may not apply to all laboratories.

Fill in Form 3.01 as the different activities are accomplished.

A. Ideally 3 months before the collection campaign

1. Obtain ethical clearance from appropriate institution(s) prior to fieldwork (e.g. national ethics committee), including WHO ERC,
2. Purchase or ensure availability of supplies, reagents and equipments listed in Form 3.02. Complete the form.
3. Perform lot testing or obtain a lot testing report from one of the WHO lot testing laboratories, of the malaria RDT lot to be used for patient screening. If QC test fails, and if failure is confirmed by the confirmatory QA-RDT laboratory, inform immediately the Project Manager and Lot Testing Coordinator.
4. Pre-qualify two microscopists, as per the procedure in chapter 6.
5. Review malaria cases in potential field collection sites and visit sites beforehand if needed. Choose appropriate site(s) based on the criteria described above.
6. Determine availability of sufficiently trained staff for the field and laboratory activities listed above.

The two previous points allow planning the approximate duration and appropriate dates of the collection campaign by considering the probability of patient recruitment (season, daily number of expected patients) and the number of available staff in the field and the laboratory.

B. Ideally 2 months before the collection campaign:

7. Send communication to (or visit) local authorities and clinic/hospital heads of the identified collection site: inform about purpose and needs of the collection campaign, eventually ask for sourcing of malaria cases in the area.
8. If the site is far (requires air or long land travel), and if recommanded transport delays cannot be fulfilled, make arrangements for use of a nearby laboratory where the QC samples can be prepared:
    a) Get in contact with / obtain authorizations from the laboratory head,
    b) Verify if the required equipment is available on site, as listed above,
    c) Book travel tickets and accommodation for the whole team,
    d) Arrange for transport of the frozen QC sample aliquots at ≤ -70°C from this laboratory to the laboratory conducting the collection (dry ice, transport containers).
9. Identify potential volunteer blood donor(s). Alternatively, arrange for availability of *Plasmodium* parasite- and virus-negative (hepatitis B / C, HIV 1 & 2) blood and fresh frozen AB+ plasma from a reliable and accredited blood bank (e.g. National Blood Transfusion Centre).
10. Arrange for blood cell counting and blood group determination of collected blood samples in a haematology service.
11. Arrange for screening of hepatitis B / C and HIV by ELISA. This screening will be performed in advance for eventual donors of « parasite-free » blood, but also retrospectively for the samples of *Plasmodium* infected patients.
12. If these viral infections are frequent in the region, then try to get adequate rapid diagnosis tests, in order to do a rapid on-site screening of hepatitis B / C and HIV I & II infections.
13. Arrange for availability of appropriate HIV diagnostic counseling and management of positive results with national and/or local authorities.
14. Follow-up and verify purchases of lacking materials, reagents and equipments, if required. Complete Form 3.02.
15. Verify if sufficient storage space is available at -70°C for the QC sample aliquots (calculate expected number of cryotubes/cryoboxes). If insufficient, arrange for additional storage space in partner laboratories and/or for purchase/availability of additional -70°C freezers.

During the last month before the collection campaign:

Weeks 1 and 2:

16. Verify that all arrangements with collaborating laboratories and partners have been made.
17. Verify the availability of all materials, reagents and equipments. Complete Form 3.02 if needed.
18. Verify the quality of the Giemsa stain and of the pipettes (calibrate if needed). Ensure that all equipments are working properly.
19. Brief the staff involved in field and laboratory activities on all procedures and forms. Assign tasks/responsibilities, complete the Form 3.03, and distribute copies of the completed form.

Also distribute copies of the relevant procedures and forms, depending on each staff's responsibility.

Weeks 3 and 4:

20. Gather/pack up all materials and equipments in the working area.
21. Secure/purchase the "parasite-free" blood and AB+ plasma from volunteer donors or from a blood bank, and store properly. Perform hepatitis B / C and HIV screening of the blood donors.
22. Print out the required number of all forms.

PROCEDURE HISTORY

Date	Version	Comments	Initials
01 MAY 2008	5	SOP introduced	DB/JL/PJ/SI/WO
MAY 2014	7	Lot testing can be done by another WHO-FIND laboratory.	SI, NC

PART 2: Patient Recruitment and Blood Sample Collection in the field

LIST OF FORMS FOR CHAPTER 3, PART 2:

3.04: Information Sheet and Consent Forms

3.05: Malaria Patient Screening

3.06: Patient Record

3.07: Venepuncture

SOP 3.03 Preparation of Quality Control Samples: Field collection procedure

AIM

To collect field samples of malaria parasites with parasite density sufficiently high to use for the preparation of quality control (QC) dilutions for testing malaria rapid diagnostic tests (RDTs).

BACKGROUND

The preparation of QC dilutions for testing malaria RDTs requires parasite densities sufficiently high to allow preparation of dilutions of 200 to 2,000 parasites/µL. Patients are therefore screened for malaria infections, and inclusion criteria are based on the need of sufficiently high parasitaemia (malaria RDT band intensity and/or parasite density determined by malaria microscopy).

PURPOSE

This SOP describes the procedure for collection of samples of malaria parasites from the field for preparation as QC samples.

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the Project Manager.

REAGENTS/EQUIPMENT

See Supplies - Equipment checklist – Form 3.02

PROCEDURE

The following working steps are summarized in Figure 3-2.

See also Figure 3-1 in SOP 3.01 for a general overview.

A. Malaria patient screening in the field collection site

1. Transport screening RDTs (for malaria infections: panLDH and Pf HRP2- based RDTs, for viral infections: hepatitis B / C and HIV RDTs) to the field in appropriate conditions (SOP 3.04). In regions where HRP2-deleted *P. falciparum* infections are reported, use Pf LDH- and Pan or Pv LDH-based RDTs.
2. Select patients for malaria screening based on the following criteria:
    a) aged 5 years and older (higher age limits are used on some sites, depending on ethics submission and national protocols),
    b) history of malaria symptoms (define locally relevant clinical criteria, e.g. headache, fever, etc.),
    c) no history of anti-malaria treatment in the last week, and preferably in the last month.
3. If malaria screening is based on RDT, and if this is not part of normal clinical practice, then inform the patient about the purpose of the study and obtain signed consent at this stage, using Form 3.04a or adapted version.
4. Complete Form 3.05 and assign a chronological patient number, made of two letters for identification of the recruitment site, and three digits for identifying each screened patient (e.g. PH 001).
5. Complete Form 3.06 with the patient's information, in particular record any intake of anti-malarial drugs in the last 1 to 4 weeks.
6. Screen for malaria infections by one or both of the two following options:
    a) malaria RDTs (see above),
    b) malaria microscopy (rapid parasite count and species identification).

Follow SOP 3.05 for performing malaria RDTs and/or preparing a thin and thick film, using a finger-prick blood sample.

7. Label malaria RDTs and/or blood films with the patient number (keep the desiccant provided in the RDT pouch). Allow blood films to dry free from dust and direct sunlight, then stain and read according to SOP 4.01. Interpret malaria RDT results as described in SOP3.05.
8. Record the results of the malaria RDTs and/or malaria microscopy in Form 3.05 and Form 3.06.
9. Select patients for venepuncture if one or both of the following criteria are fulfilled:
    a) malaria RDT results are strongly positive (band intensity 2+ or 3+),
    b) malaria microscopy indicates a single species infection (no mixed infection) and a parasite density higher than 2,000 parasites per microlitre of blood (2,000 p/µL). Parasite density over 5000 parasites/µL is ideal).
10. Exclude patients if clinically anaemic, and/or if not satisfying the above criteria, and treat them immediately with anti-malarial drugs according to the national protocol.

Note on optional screening of viral infections by RDTs:

Screening of hepatitis B / C and HIV 1 & 2 infections is systematically and obligatorily accomplished by ELISA analysis of patient's serum, obtained after the venepuncture. Optionally, the QA-RDT team can decide to perform an anticipated screening of patients in the field by hepatitis B / C and HIV 1 & 2 RDTs, in order to exclude infected patients and avoid processing infected blood samples.

There exist various options for performing these RDTs.

1. Using a finger-prick sample:

- *using the same finger-prick blood sample as for the malaria screening (paragraph A),*
- *by performing a second finger-prick blood sample before venepuncture (paragraph B).*
- *In both cases, HIV counselling must be provided, and the informed and signed consent of the patient must be obtained before the finger-prick.*

2. Using patients' venous blood or serum:

- *using freshly collected venous blood in the field (paragraph B),*
- *once the blood samples have arrived in the laboratory, using the serum obtained after centrifugation of the plain tube (*SOP *3.08).*

    RDT results must be recorded in Form 3.05 and Form 3.06.

    *If any of the RDTs is positive, the patient is excluded or the blood sample is not used for preparing QC sample aliquots. If all RDTs are negative, the process is continued, but hepatitis B / C and HIV 1 & 2 infections must still be screened by ELISA (*SOP *3.08).*

B. Blood sample collection

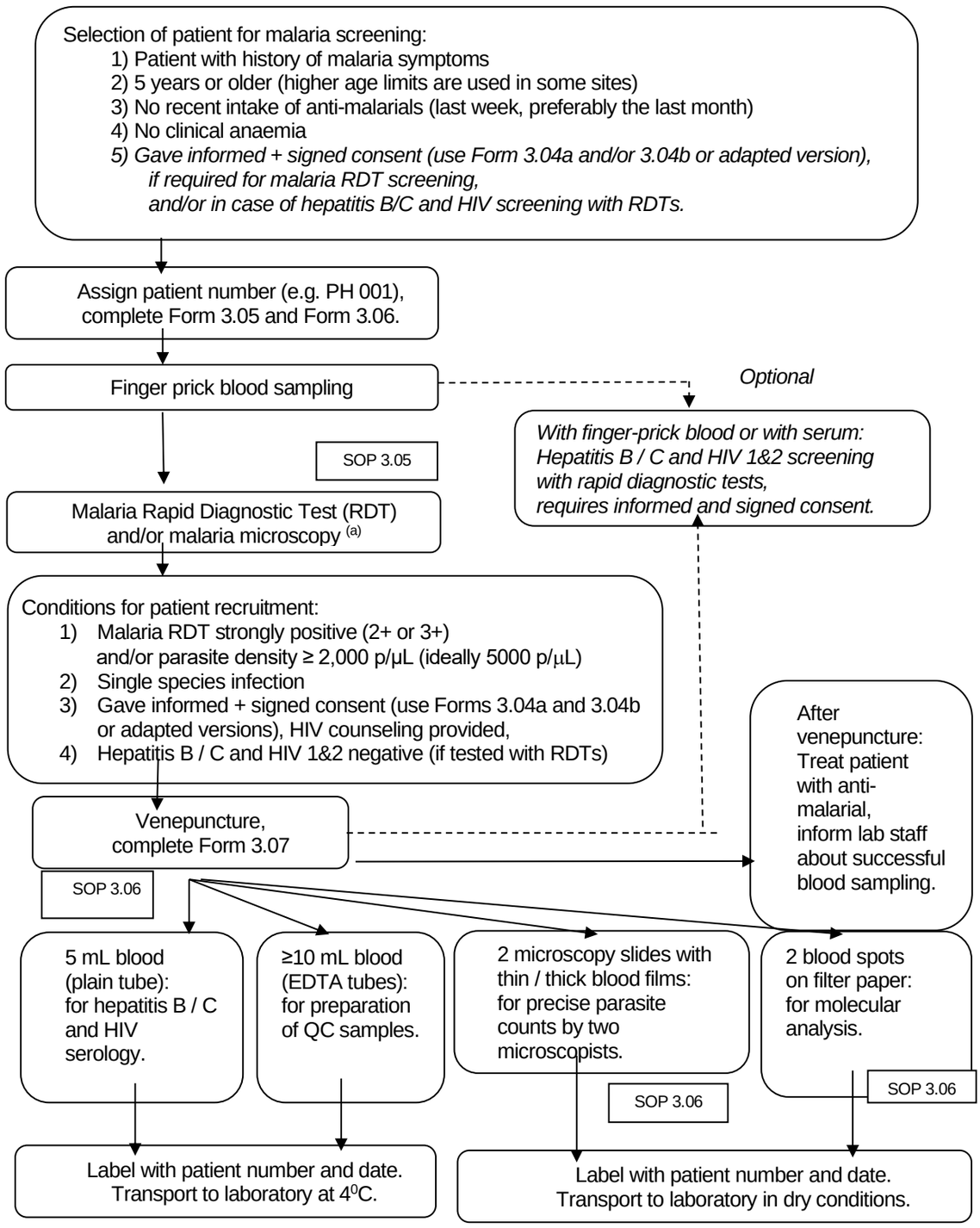
1. If the above described screening step did not require informed and signed consent for study participation, then obtain this informed and signed consent at this stage, using form 3.04a or adapted version.
2. Provide HIV counselling and obtain the informed and signed consent for HIV screening and venepuncture. Use Form 3.04b or adapted version.
3. Apply a tourniquet, clean skin with alcohol swab, extract at least 10 mL blood in EDTA tubes and 5 mL blood in plain tube, and gently agitate EDTA tubes. Label tubes with date and patients' number, and store immediately at 4°C (refrigerator or cooler box with ice packs).
4. Collect 2 drops of blood on a piece of pre-labelled (date and patients' number) filter paper (Whatman 3M) and allow to dry free from dust and insects. See SOP 3.07 for detailed instructions.

5. Prepare two thick and thin blood films from fresh venous blood (not EDTA) for malaria microscopy and allow drying free from dust and insects (see SOP 3.06 for detailed instructions). Label blood films with date and patient's number.
6. Complete Form 3.07 – Venepuncture.
7. After venepuncture, treat patient with anti-malarial drugs according to national protocol.
8. Inform the laboratory staff that a blood sample has been obtained (allows laboratory staff to be prepared for processing the sample). Provide all useful information, in particular the parasite density (if known) and the volume of blood collected in EDTA tubes.
9. When completely dry, keep blood films inside a box. Transport and stain in the lab.
10. When completely dry, package the filter paper in individual plastic envelopes and include desiccant (from opened RDT package) to reduce any possible remaining moisture.
11. Transport samples to laboratory, ideally within 6 hours of venepuncture: blood samples at 4°C, blood films in a box, and filter paper blood spots with desiccant in plastic envelopes.

NOTES

1. The two thin and thick films prepared in step B. 4. should be of good quality, as they are used for precise parasite counting and species determination. They should be made with fresh **venous** blood, as films made with EDTA blood may dry poorly.
2. A blood film should be prepared and screened rapidly at the site, if possible, to avoid loss of samples with high parasite density but poor RDT response (exact parasite count is determined later under controlled conditions).
3. Recent drug use must be noted on the patient record form (Form 3.06) to allow later interpretation of results. It is noted that accurate information can not always be obtained.

- **Figure 3-2: Organization of Blood Collection from the Field**



(a) The parasite count performed in the field on the fingerpick specimen is only used as a guide for patient recruitment. Precise parasite counts are determined subsequently in the laboratory, using the two thin/thick films prepared with fresh venous blood.

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	2	Routine Revision: changes made to include requirements of African sites	RG
28 MARCH 2006	4	Modification to parasite screening and HIV screening	DB
01 MAY 2008	5	Re-numbered from SOP 3.1 (version 4) to SOP 3.03 (version 5). Revised.	DB/JL/PJ/SI/WO
MAY 2014	7	Updated forms and process for patient consent, PfLDH RDTs in areas of HRP2 deletion.	NC/SI

SOP 3.04 Transport and storage of RDTs in the field

AIM

To maintain quality of malaria (and blood-borne virus) RDTs while transporting, storing, and using in the field.

BACKGROUND

Malaria rapid diagnostic tests, and rapid tests for other diseases including HIV and Hepatitis B and C, are biological tests that deteriorate on exposure to high temperatures and deteriorate rapidly on exposure to high humidity. They may also deteriorate through freeze-thawing. To maintain sensitivity, it is important to store in as close as possible to the conditions specified by the manufacturer.

PURPOSE

This SOP describes the procedure for transporting, storing, and using malaria (and blood-borne virus) RDTs in the field.

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the “Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests” and is not to be modified except by the Project Manager.

EQUIPMENT

Electronic temperature monitors (optional)

Thermometers (optional)

PROCEDURE

1. Keep RDTs in controlled temperature storage (within manufacturer’s specifications) at a central location when not required in the field. Refrigerated storage will maintain high sensitivity more effectively. Do not freeze.
2. Maintain a ‘cool chain’ during transport to the field. Hand-carriage on aircraft will reduce heat exposure on the tarmac. Transport in air-conditioned vehicle where possible. Avoid leaving in vehicles parked in direct sunlight. Maintain in shaded position at field site. Consider storage of thermometer or electronic temperature monitor with RDTs if prolonged exposure to high temperatures is unavoidable, to assist in later interpretation of results.
3. Do not use RDTs if the moisture-proof envelope is damaged, or the desiccant colour indicates exposure to moisture.
4. Open moisture-proof envelope immediately prior to use; do not open multiple RDTs and leave exposed before use.
5. Note transport conditions and storage conditions on unused boxes returned from field for future use.

NOTES:

Suspicious negative results should be investigated by performing laboratory-based screening of the sample and returning the box of RDTs for laboratory-based quality-control testing and cross-checking with microscopy (for malaria parasites).

PROCEDURE HISTORY

Date	Version	Comments	Initials
INTRODUCED	1	SOP introduced	DB
01 MAY 2008	5	Re-numbered from SOP 3.8 (version 4) to SOP 3.04 (version 5)	DB/JL/PJ/SI/WO

SOP 3.05 Finger-prick blood collection and preparation of malaria RDTs and blood films

PURPOSE

This SOP describes the procedure for performing a malaria RDT and preparing a blood film, using a freshly collected finger-prick blood sample.

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the Project Manager. Procedures for finger-prick blood collection, preparation, staining and reading of blood films may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP as a minimum standard.

EQUIPMENT

Malaria RDTs

Cleaned and wrapped slides

Absorbent cotton wool

Alcohol

Sterile lancets

Lint free clean cotton cloth

Sharps container

Marker pen

Pencil

Slide box (or a cover to protect slides)

PROCEDURE

A. Preparation of the malaria RDTs

Carefully study the manufacturers instructions provided in the RDT kits.

Approximately 30 minutes before testing, bring RDTs to room temperature (20-30°C) BEFORE OPENING the package. This applies only to RDTs stored under different conditions than room temperature (20-30°C) (e.g. at 4°C).

1. Remove the RDT packaging.
2. Check integrity of RDT packaging when opening. If signs of moisture are present, DO NOT use the RDTs.
3. Check desiccant for any colour changes (e.g. blue to white). If present, discard RDTs and use another kit for testing.

4. Label the RDTs with the patient number and date of blood collection (DD/MM/YY), using a marker pen.

B. Finger-prick blood collection

1. Universal precautions for handling and disposal of human blood should be followed (see SOP 6.01).
2. With the patient's hand, palm upwards; select the third or middle finger (the big toe can be used for infants). The thumb should not be used. With a pledge of cotton wool lightly soaked in alcohol, clean the finger, using firm strokes to remove dirt and grease from the ball of the finger. With the clean cotton towel dry the finger, using firm strokes to stimulate blood circulation.
3. With a sterile lancet puncture the ball of the finger using a quick rolling action. By applying gentle pressure to the finger express the first drop of blood and wipe it away with a dry pledget of cotton wool. Make sure no strands of cotton remain on the finger to contaminate blood.
4. Dispose of the dirty lancet and cotton wool in a sharps container.
5. Apply gentle pressure to the finger until a new blood drop appears.

C. Performing the malaria RDTs

1. Test the RDTs as per manufacturer instructions BUT use a micropipette to transfer the specified blood volume to the RDT.

   If a micropipette is not available, use the device provided in the RDT kits, but take care to transfer the exact blood volume as described in the manufacturers instructions.
2. Use a timer to record all steps exactly as per manufacturer instructions.
3. Read RDT results within the manufacturer recommended time.Refer to the standard color chart provided by WHO for rating the band intensity from 0 (negative) to 4+.

4. Record the results in SOP 3.05 and SOP 3.06.

D. Preparation of a blood film for malaria microscopy

1. Working quickly and holding a clean slide by the edges, collect the blood as follows: apply gentle pressure to the finger and collect a single small drop of blood (about 2 mm in diameter) on to the middle of the slide. This is for the thin film. Apply further pressure to express more blood and collect two or three large drops, about 2 mm in diameter, on to the slide about 1 cm from the drop intended for the thick film. Wipe the remaining blood away from the finger with a pledget of cotton wool. Dispose the dirty cotton wool in a sharps container.
2. Thick film: When making a thick film always handle the slides by the edges or by a corner. Using the corner of the spreader, quickly join the drops of blood and spread them to make an even, thick film. The blood should not be excessively stirred but can be spread in a circular or rectangular form with 3 to 6 movements. The circular film should be about 1 cm in diameter.

3. Thin film: Using another clean slide as a "spreader", touch the small drop with the spreader and allow the blood to run along its edge. Firmly push the spreader along the slide, keeping at an angle of 45 degrees. Make sure that the spreader is in even contact with the surface of the slide at all times the blood film is being prepared.
4. Label the dry thin film with the soft lead pencil by writing across the thicker portion of the film the patient number and date (DD/MM/YY). Do not use ball pen for labelling the slide. Allow the thick film to dry in a flat, level position protected from flies, dust, and extreme heat.

E. Staining and reading

1. This slide is intended for a rapid analysis in the field, in order to guide patient inclusion (determination of the precise parasite density will be done at a later stage, using blood films prepared with fresh venous blood).
2. Follow SOP 4.01 for Giemsa staining and reading (determination of the parasite species and the parasite density).
3. Record the results in Form 3.05 and Form 3.06.

PROCEDURE HISTORY

Date	Version	Comments	Initials
01 MAY 2008	5	SOP introduced: Adapted from former SOP 2.3 (performing RDT), with changes listed above (Chapter 2, SOP 2.05), and made specific for RDT programme. Includes parts of former SOP 4.2 (finger-prick blood collection and blood film preparation).	DB/JL/PJ/SI/WO
MAY 2014	7	Standard color chart for rating of RDT band intensities	NC/SI

SOP 3.06 Venous blood collection and preparation of blood films

PURPOSE

This SOP describes the procedure for collecting venous blood and preparing thick and thin films for malaria microscopy.

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP as a minimum standard.

EQUIPMENT

Cleaned and wrapped slides

Slide box (or a cover to protect slides)

Pencil

Absorbent cotton wool

Alcohol

Lint free clean cotton cloth

Vacutainer or 5- or 10-mL syringes (21/23 gauge needles)

Tourniquet

EDTA tubes (5 and/or 10 mL)

Sharps container

PROCEDURE

A. Venous blood collection

1. Universal precautions for handling and disposal of human blood should be followed (see SOP 6.01).
2. Apply a venous tourniquet, clean skin with alcohol swab, and proceed to venepuncture according to standard protocols (vacutainer or "butterfly" needles are preferable, rather than syringes).
3. Collect at least 10 mL of blood into tubes containing EDTA. The use of anticoagulant other than EDTA may yield misleading results. Fill the tubes with the correct volume (i.e. 5 mL or 10 mL depending on the tubes format). Under-filling will result in a higher ratio of anticoagulant to blood and will falsely lower parasite counts.
4. Mix the specimen thoroughly by gentle inversion of the EDTA tubes.
5. Collect 5 mL of blood in a plain tube.
6. Label the tubes with the patient number and date of blood collection (DD/MM/YY), using a marker pen, and place immediately at 4°C (refrigerator or cooler box with ice packs).

7. Prepare two thick and thin films with fresh venous blood, immediately after blood collection (see hereunder).
8. Complete Form 3.07.

B. Preparation of thick and thin films

See SOP 4.01 for details.

1. Fresh venous blood can be transferred from the blood collection device or from the plain tube (before coagulation) to the slides by using an applicator stick, capillary tube or a micropipette.
2. Alternatively, blood drops can be allowed to fall directly from the blood collection device on to the slides.
3. Prepare two slides as follows: transfer a single small drop of blood (about 2 mm in diameter) on to the middle of the slides (for the thin film) and two or three large drops (about 2 mm in diameter) about 1 cm from the drop intended for the thick film. Wipe the remaining blood away from the finger with a cotton wool ball. Dispose the dirty cotton wool in a sharps container.
4. Thick film: When making a thick film always handle the slides by the edges or by a corner. Using the corner of the spreader, quickly join the drops of blood and spread them to make an even, thick film. The blood should not be excessively stirred but can be spread in a circular or rectangular form with 3 to 6 movements. The circular film should be about 1.2 cm in diameter.
5. Thin film: Using another clean slide as a “spreader”, touch the small drop with the spreader and allow the blood to run along its edge. Firmly push the spreader along the slide, keeping at an angle of 45 degrees. Make sure that the spreader is in even contact with the surface of the slide at all times the blood film is being prepared.
6. Label the dry thin films with the soft lead pencil by writing across the thicker portion of the film the patient number and date (DD/MM/YY). Do not use ball pen for labelling the slide. Allow the thick films to dry in a flat, level position protected from flies, dust, and extreme heat.
7. Place the slides in a slide box for transport to the laboratory (ensure there is no contact between slides during transport). The slides are now ready for staining.

PROCEDURE HISTORY

Date	Version	Comments	Initials
01 MAY 2008	5	SOP introduced: Adapted from parts of former SOP 4.2, made specific for the field collection procedure	DB/JL/PJ/SI/WO

SOP 3.07 Preparation of blood spots on filter paper, using fresh finger-prick or venous blood

AIM

To preserve dried blood samples suitable for PCR analysis for malaria parasite species identification and genetic diversity.

BACKGROUND

DNA is stable for long periods if in dried sample and protected from moisture. Samples should be used to exclude mixed infection and to analyze genetic diversity of target antigen in all QC samples, as mixed infection and variant antigens affect RDT results.

PURPOSE

This SOP describes the procedure for preparing dried blood spots suitable for DNA analysis.

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the “Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests” and is not to be modified except by the Project Manager.

EQUIPMENT

Alcohol Prep

Cotton wool

Lancet

Filter paper (Whatman No.1 or No. 3, or equivalent)

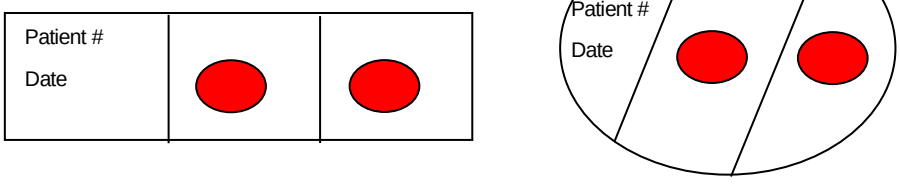
Plastic bags

Desiccant

PROCEDURE

1. Prepare the filter paper to 10cm x 3 cm strips or to 8cm diameter disks and draw 3 squares (3cm x 3cm each).
2. Write patient number and date of collection (DD/MM/YY) in one of the squares (Figure 3-3)

• Figure 3-3: Filter paper blood spots for DNA analysis



Direct from finger prick:

3. For instructions on finger-prick blood sampling, see SOP 3.05.
4. Allow 1 drop of blood (~20 – 50 µL) to fall onto one of the squares on a filter paper directly from the finger. The finger must not touch the filter paper.
5. Allow another drop of blood to fall onto the other square on the filter paper directly from the finger. The finger must not touch the filter paper.

From syringe or pipette from venous blood sample:

6. For instructions on venous blood sampling, see SOP 3.06.
7. Place 2 drops (~20 – 50 µL each) on paper as above, from syringe tip or pipette.
9. Dry the filter-paper samples completely in air and place them in small, separate plastic bags.
10. During the drying process, avoid contact between filter papers prepared from different patient blood samples (and ensure proper labelling).
11. Add desiccant to each plastic bag (left-over desiccant from recently opened RDT pouches can be used, but it must be ensured there is no colour change, or desiccant can be purchased commercially).
12. Store and transport the dried filter-paper samples at room temperature (20-30°C), with desiccant included.
13. Ensure completely dry storage conditions (regularly check the desiccant for any colour change indicating moisturizing and change if needed).

PROCEDURE HISTORY

Date	Version	Comments	Initials
AUGUST 2006	1	SOP introduced	QC/DB
01 MAY 2008	5	Re-numbered from SOP 3.9 (version 4) to SOP 3.07 (version 5). Removed storage at -20°C, updated list of equipment. References to other SOPs added	DB/JL/PJ/SI/WO
JUNE 2019	9	Formatting changes	JC, JL

PART 3: Preparation of RDT QC sample dilutions in the laboratory

LIST OF FORMS FOR CHAPTER 3, PART 3:

3.08: Parasite-free blood preparation

3.09: Malaria Microscopy Record (microscopist 1, first read)

3.10: Malaria Microscopy Record (microscopist 2, first read)

3.11: Malaria Microscopy Record (microscopist 1, second read)

3.12: Malaria Microscopy Record (microscopist 2, second read)

3.13: Parasite density & Dilution Calculations

3.14: Dilution Preparation

3.15: RDT Results Sheet

3.16: QC Sample Preparation Checklists

3.17: Negative Control Samples

SOP 3.08 Preparation of Quality Control Samples: Dilution Procedure

AIM

To prepare quality control samples from wild parasites to be used in Quality Assurance (QA) of malaria rapid diagnostic tests (RDTs). The sample should simulate fresh clinical specimens of parasites in blood but have a parasite density close to the lower limit of detection of RDTs. It must be stored with minimal deterioration, allowing qualitative detection of loss of sensitivity.

BACKGROUND

Published trials and experience in various countries has demonstrated a wide variability in the sensitivity of malaria RDTs, both within and between products trials [2-11]. Sensitivity is particularly variable at lower parasite densities. The 1999 and 2003 WHO expert consultations recommended 95% sensitivity at 100 parasites/µL (p/µL) as a reasonable target for RDT performance [12-13]. However, due to variation in the relationship between parasite density and antigen concentration, and unavoidable small measurement error within the dilution procedure, samples with 100 p/µL may frequently have inadequate antigen to test RDTs. Low density samples for QC testing are therefore prepared at 200 p/µL, giving an appropriate margin of error [14]. Higher density samples at 2,000 p/ µL are also prepared for malaria RDT product evaluation.

The procedure also takes into account that the condition of blood should be as close as possible to fresh blood when dilutions are prepared, to minimize loss of antigen or other changes, which may affect RDT performance.

PURPOSE

This Standard Operating Procedure (SOP) describes the procedure for preparing dilutions (quality control samples) of wild parasite samples to be used for quality assurance of malaria RDTs.

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the “Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests”. The SOP is only to be modified with agreement of the Project Manager.

REAGENTS/EQUIPMENT

See Supplies - Equipment checklist (Form 3.02) and requirements described in SOP 3.01.

PROCEDURE

Important notes concerning communication between field and laboratory staff

1. *The laboratory staff is informed by the field staff about the successful collection of a blood sample, once venepuncture of a recruited patient has been completed in the field (*SOP *3.03,*

step B. 7.). In particular, the lab staff is already informed about the approximate parasite density of the sample (if known) and the volume of blood collected in EDTA tubes.

2. *While the samples are transported to the laboratory, the laboratory staff must already prepare the required volume of parasite-free blood (see* SOP *3.10). The required volume should be estimated to 50 mL for processing one patient blood sample. If the approximate parasite density is known in advance, the required volume can be estimated with the MS Excel Dilution_Calculator (see* SOP *3.10).*
3. *Once the samples arrive in the laboratory, the lab staff should therefore be ready to process the blood sample as quickly as possible.*

Important notes concerning organization of laboratory staff and activities

1. *Rapid processing of the blood samples is essential, so that the delay between blood sample arrival in the laboratory and freezing of the final QC sample aliquots is kept to a minimum. A good organization is also required to avoid errors and confusions, particularly when more than one patient sample are processed in parallel.*
2. *All documents (forms, results of laboratory analyses etc.) related to a patient blood sample should be kept together, and the patient number should immediately be written on each page. All samples, aliquots, tests etc. should immediately be labelled with the patient number. Once the definitive QC sample ID has been assigned, this ID should be written on each document and labelled on all samples, aliquots, and tests.*
3. *Staff organization depends on the size of the team and the number of patient samples processed in parallel. Ideally, one staff could entirely be dedicated to coordinating the different activities.*

The following working steps are summarized in Figure 3-4.

See also Figure 3-1 in SOP 3.01 for a general overview.

A. Samples arrival in the laboratory

Once the samples arrive in the laboratory, the following four steps should ideally be done in parallel by different staff, with a large priority on **steps 1** (EDTA tubes) and **4** (Thin/thick films).

1. EDTA tubes

(a) Once in the laboratory, maintain blood at 4°C (refrigerator).

(b) Perform white cell counts (manually or with automated cell analyzer), either in the laboratory or in a partner laboratory/haematology service. Results (number of white blood cells per microlitre of blood) must be obtained as soon as possible, as they are needed for the calculation of the parasite density.

Note: If this patient blood is expected to be diluted with donor blood of a matched blood group, then the patient blood group must be determined.

(c) Before starting the dilution, the blood must be gently mixed on a rocking tray/sample rotator for at least 30 min at 4°C.

2. Plain tube

(a) Centrifuge at 2500g for 20 minutes. Keep the serum and immediately send an aliquot for screening of hepatitis B / C and HIV 1&2 by ELISA, or store at -20°C until testing.

(b) Once the results become available, record in Form 3.14. If positive for hepatitis B, hepatitis C or HIV, discard the corresponding QC sample aliquots.

3. Filter paper blood spots, individually packed with desiccant

Note: If the filter paper blood spots have not been prepared in the field, then they must be prepared in the laboratory with the venous EDTA blood. Using a micropipette, prepare two spots of 20 µL-50µL of blood on a filter paper labeled with date and patient number (see SOP *3.13), and allow them to dry.*

(a) Store at room temperature (20-30°C) until shipment for molecular analysis. Ensure completely dry storage conditions (regularly check the desiccant for any colour change indicating moisturizing and change if needed).

(b) Shipment will be coordinated with the Project Manager. The filter papers should be shipped at room temperature (20-30°C), in their individual plastic envelope, with fresh desiccant included just before the shipment.

4. Thin/thick films (see SOP 4.01).

(a) Stain the slides with Giemsa (SOP 4.01).

Note: if more than one patient blood sample have been collected, prioritize samples based on a strong RDT result (obtained in the field), and on the parasite species required.

(b) Malaria microscopy must be performed in a strictly blinded manner by two expert microscopists, which should have previously been pre-qualified by following a competency assessment as described in SOP 6.04. The two microscopists can either read the two slides in parallel (less time consuming), or the same slide one after the other (if second slide wants to be used for internal long-time archiving). After reading, the two slides should be kept in dry conditions for at least 6 months.

(c) Parasite species must be determined carefully (SOP 4.01). If a mixed infection is detected (more than one *Plasmodium* species), the blood sample can not be used for preparation of RDT QC samples (discard the sample).

(d) The parasite density must be determined carefully (SOP 4.01). The two microscopists must record their results on two separate forms (Form 3.09 and Form 3.10). The previously obtained white cell count is used for calculating the parasite density (number of parasites per microlitre of blood).

(e) The discrepancy between the two parasite densities is calculated by using Form 3.13.

(f) If the discrepancy is equal or below 20%, calculate the mean parasite density using the same form.

(g) If the discrepancy between the two parasite density results is above 20%, then repeat the two blinded readings by the two microscopists. Use Form 3.11 and Form 3.12 for recording the results of the second reading.

(h) If the discrepancy is equal or below 20% after the second readings, calculate the mean parasite density using Form 3.13.

(i) If the discrepancy is still above 20% after the second readings, the sample is not used for preparing RDT QC samples (discard the sample).

B. Clumping test

Before preparing dilutions of the patient blood sample (parasitized blood), its compatibility with the parasite-free donor blood must be checked. A small clumping test mixture is therefore prepared.

1. In a sterile round-bottom tube of 2 mL volume, mix 100 µL of patient EDTA blood and 900 µL of parasite-free donor blood.

   Note that the parasite-free blood must have been previously prepared according to SOP 3.10 and mixed during at least 1 hour. The patient EDTA blood must have been mixed during at least 30 min.

2. Mix gently on a rocking tray or sample rotator, during at least 15 min at 4°C.
3. Check for red blood cell clumping, according to SOP 3.15.
4. If clumping occurs, and if an alternative parasite-free donor blood is available, repeat steps 1. to 3. with this other parasite-free blood. If no other parasite-free blood is available, do not process the patient blood sample any further. Record the result on Form 3.14.
5. If no clumping occurs, continue processing (in this case, only the result of the clumping test of the large dilution will be recorded in Form 3.14, see the description in step E. 3 below.).

C. RDT QC sample aliquots to be prepared

1. Dilutions:

   Prepare one high parasite density dilution at 2,000 parasites per microlitre of blood (p/µL), and one low parasite density dilution at 200 p/µL.

2. Aliquots:

   The number of aliquots to prepare for each dilution usually consists in at least 600 aliquots at 200 p/ul and 100 aliquots at 2,000 p/ul, but these should be confirmed wth WHO.

D. Calculation of dilutions using the MS Excel calculator

Note: Dilution calculations can also be done manually. In this case:

- all calculations must be double-checked by a second person,
- for each dilution step, the dilution factor must be between 2 and 10. Depending on the initial parasite density of the patient blood, this will oblige to perform the appropriate intermediate dilution steps (e.g. initial parasite density = 30000p/µL, prepare first an intermediate dilution at 5000 p/µL, and use this for preparing serial dilutions at 2,000 p/µL and 200 p/µL).

1. Use the up-to-date **Calculator** file provided by the Project Manager for this purpose (check most recent version, available from WHO). Study the detailed "Instructions" worksheet before first use.
2. Fill in or verify the values in the red-bordered cells only, as indicated in the worksheets:
   a) the mean parasite density, as calculated in Form 3.13,
   b) the volume of venous blood collected in EDTA tubes (default value 10 mL),
   c) The volumes of venous EDTA blood used for other purposes than preparing QC sample aliquots:
   d) the volume used for performing blood cell counts and eventual blood group testing (default value 0,1 mL),
   e) the volume used for "high-volume" aliquots for malaria antigen ELISA (obligatory value 0,6 mL),
   f) the volumes used for two 1 mL aliquots of whole blood (one kept as whole blood, one kept as serum and pellet (obligatory value 1 mL each),
   g) the volume used for the agglutination test mixture (obligatory value 0,1 mL),
   h) the cell "others" allows to adjust, if extra-volume of patient blood is required for additional tests.
3. The calculator will indicate the number of the "applicable situation", depending on the mean parasite density. The volumes to be used for dilutions can be found in the corresponding column (don't consider the other columns).
4. The calculator also indicates the volume of venous EDTA blood available for preparing QC sample aliquots (total volume collected, minus volumes used for other purposes), as well as the volume of venous EDTA blood actually required for preparing a specified number of aliquots.
5. The number of aliquots is set to default values (minimum number of 600 aliquots at 200 p/µL and 100 aliquots at 2,000 p/µL).

6. If the available volume is higher than the required volume, then the number of aliquots can be increased: type in larger numbers in the corresponding red-bordered cells, by keeping the same ratio 1:2 between the high/medium and the low parasite density aliquots numbers.
7. If the available volume is lower than the required volume, then the sample cannot be used for preparing QC sample aliquots (except specific arrangements with the Project Manager).
8. The volumes to be pipetted for the dilutions are indicated as Vp and V-Vp. A mention in red indicates which blood / which dilution is to be used for pipetting Vp.
9. The volume to be aliquoted is indicated as VA. The remaining volume VR is used for further dilutions, for "high-volume" aliquots for malaria antigen ELISA (for 200 p/µL dilutions only) and/or corresponds to a margin volume of 1 mL.
10. Complete the Form 3.13 with the volumes indicated in the calculator.

E. Preparation of dilutions and QC sample aliquots

1. Prepare the first dilution as indicated on top of the applicable situation column in the dilutions calculator. The parasitized EDTA blood and the parasite-free donor blood must have been mixed during at least 30 min and 1 h, respectively.
2. Take note of the following rules:
    - all blood must be kept at 4°C during the pipetting process at the bench (use ice-filled tray)
    - chose sterile tubes of adapted shape and volume, as described in SOP 3.01,
    - label the tubes with the patient number and the parasite density of the dilution, before starting to pipette blood
    - for pipetting volumes V_p and V-V_p, use a combination of the two following methods: i) sterile disposable plastic pipettes for pipetting large volumes (e.g. > 1 mL), with SLOW aspiration and dispensing, ii) micropipettes for pipetting smaller volumes to a +/- 1 µL precision, by using SLOW reverse pipetting according to SOP 3.14
    - change disposable pipettes and/or pipette tips for every volume of blood dispensed,
    - mix blood at 4°C on an adapted rocking tray/sample rotator, as described in SOP 3.01
    - time of mixing depends on the total blood volume to be mixed: at least 15 min for volumes ≤ 2 mL, at least 30 min for volumes ≤ 10 mL, at least 1 h for volumes > 10 mL
    - when dilutions are not used for any pipetting, they should be kept at 4°C and gently rotated
    - record time and temperature at start and end of each mixing step in Form 3.14
3. After mixing of the first dilution, perform a clumping test according to SOP 3.15 and record the result in Form 3.14. Also record the number of the "parasite-free" blood on the same form.
4. If clumping occurs, do not process any further. If no clumping occurs, the blood sample can definitively be used for preparing QC sample aliquots.
5. Assign a QC sample ID, according to the following scheme:

AA = **ISO** Country code, e.g. CO for Colombia*

= round of collection†

A = species (i.e. F for *P. falciparum*) F, V, O, M

= unique specimen identifier‡

= dilution (e.g. 200)

Example: **CO 05 F 14**
2,000

Fifth collection round from Colombia
14th QC sample prepared for *P. falciparum*,
diluted to 2,000 parasites/µL

* List of "ISO Country Codes":

Country	**ISO country Code**
ASIA	
Philippines	PH
Cambodia	KH
Myanmar	MM
AFRICA	
Tanzania	TZ
Kenya	KE
Nigeria	NG
Central African Republic	CF
Ethiopia	ET
Madagascar	MG
Senegal	SN
AMERICAS	
Colombia	CO
Peru	PE
OTHER	
Australia	AU
USA	US
United Kingdom	GB

† "Round of collection" indicates a field trip to collect samples. Where sample collection extends continuously over a longer period, the lab needs to determine an appropriate way of distinguishing collections (e.g. numbering by transmission season).

‡ "Unique specimen identifier" corresponds to the successive numbers of the cases prepared for that particular species during that particular collection campaign.

6. Subsequent dilutions, as indicated in the "applicable situation" column in the dilutions calculator, should only be prepared after sufficient mixing of the previous dilutions, by taking note of the rules listed above.
7. The QC sample dilution at 2,000 and 200 p/µL must be tested with HRP2- and pLDH-based RDTs, according to SOP 3.12, and results must be recorded in Form 3.15.
8. For each QC sample dilution, prepare 2 thick and thin blood films for malaria microscopy (SOP 4.01). These may be Earl-Perez films or standard thick blood films, depending on experience and local policy. Label slides with QC sample ID, date, and dilution. Slides may take 24 hours to dry. Once dry, stain with Giemsa (see SOP 4.01).
9. Label low absorption cryotubes with external screw cap with the QC sample ID and the dilution to be aliquoted. Use different colours for each dilution, e.g. blue for 2,000 p/µL and black for 200 p/µL, to reduce the possibility of error. Note that there is no formal colour coding of the specimen bank samples, each site may choose a preferred set. If more than one

patient blood sample is processed in parallel, use different working areas for each blood sample.

10. For each dilution, prepare aliquots of 50 µL in the pre-labeled cryotubes. Use multi-dispense pipette with adapted 'combitips', or micropipette with SLOW reverse pipetting. Change the 'combitips' or pipette tips for every dilution.
11. For each dilution, freeze the QC sample aliquots at -70°C as soon as possible, placing tubes upright in a freezing rack or in cryoboxes (to allow easier sampling later). For more detail on storage instructions, see SOP 3.16.
12. Record the freezing times and number of aliquots in Form 3.14.
13. Discard contaminated waste as per safety SOP 6.01.

F. Preparation of high-volume aliquots

1. Prepare the following High-Volume aliquots to be used for antigen quantitation by ELISA:
   a) Using the undiluted EDTA patient blood: 6 aliquots of 100 µL,
   b) Using the low parasite density dilution at 200 p/µL: 8 aliquots of 250 µL,
   c) Using the high parasite density dilution at 2,000 p/ µL: 4 aliquots of 250 µL,
2. Prepare two 1mL aliquots of the undiluted EDTA patient blood:
   a) save one as 1 mL whole blood
   b) the other should be centrifuged, separated and the red cell pellet and plasma saved in two separate tubes,
3. Label these High-Volume aliquots with the QC sample ID, and an additional mention specifying the type of High-Volume aliquot: “ELISA”, “whole blood”, “plasma”, “pellet”. Freeze at -70°C as soon as possible. For more details on storage instructions, see SOP 3.16.
4. Record the list of High-Volume aliquots prepared, the volumes, the freezing times and freezing temperatures in Form 3.14.
5. Discard contaminated waste as per safety SOP 6.01.

G. Final check at the end of each day

1. Check-up if all tests and aliquots have been prepared, adequately labelled and stored (Table 3-1). Fill in Form 3.16.
2. Check if all forms have been filled in with all required information (Table 3-2). Write the QC sample ID on each form and on each document (laboratory results, etc.), and obtain the signature of the supervisor. Fill in Form 3.14.
3. File all forms in a designated “QC sample preparation” folder, arranged by date and by patient number / QC sample ID.

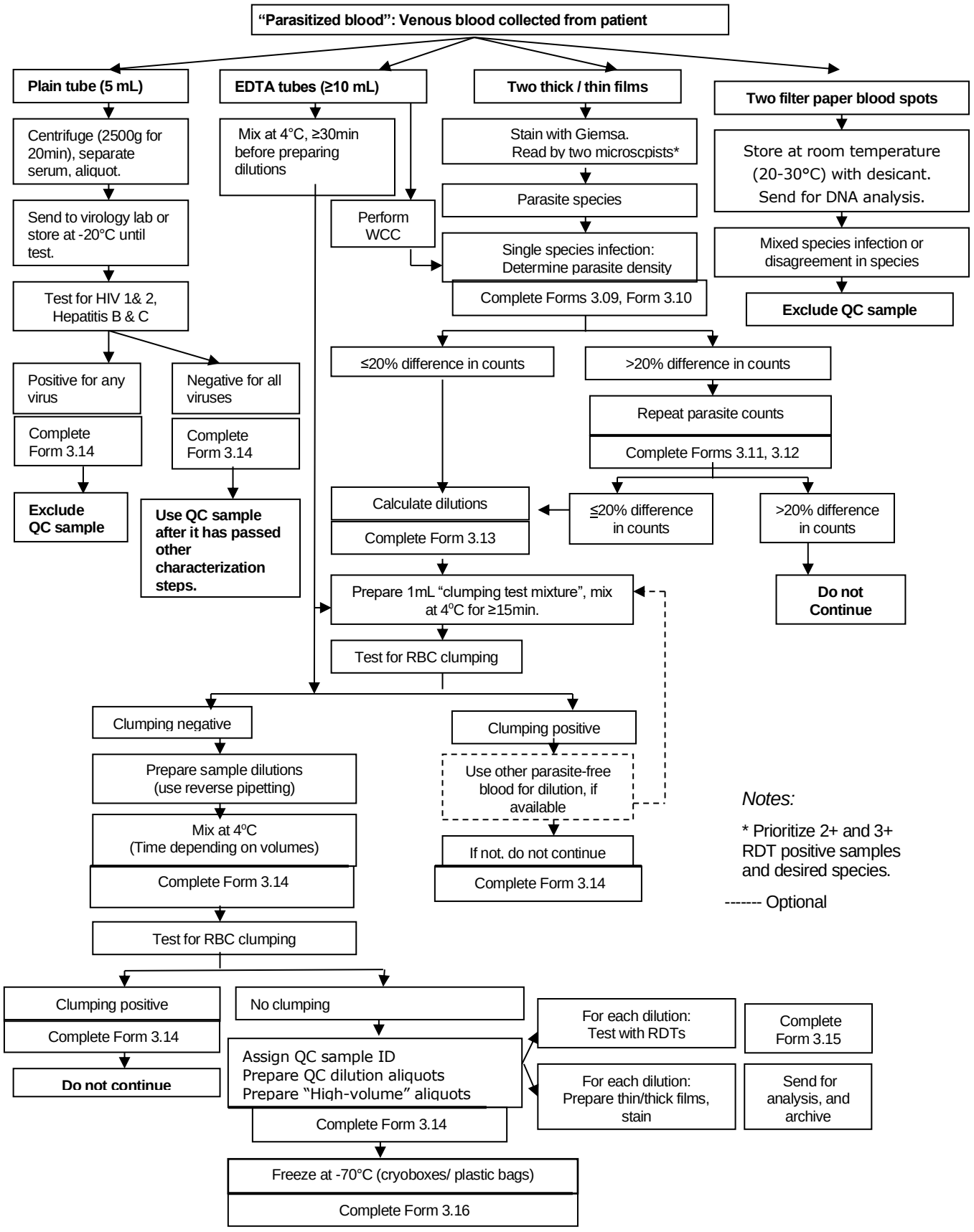
H. During and after the sample collection campaign

1. Follow-up for results of the hepatitis B / C and HIV 1&2 serology for each patient. Once results become available, record in Form 3.14. If positive for hepatitis B, hepatitis C or HIV,

discard the corresponding QC sample aliquots. Send laboratory results back to responsible staff at the field recruitment site for information of the patient.

2. QC sample aliquots will partly be used in the lot testing laboratories for as described in Chapter 2 of this Manual, partly at the central malaria specimen bank (at the National Centers for Disease Control and Prevention (CDC), Atlanta, USA), for malaria RDT product testing.
3. Table 3-1 indicates the requirements of sample shipment, for further characterization and/ or for inclusion in the central malaria specimen bank at the CDC. For more detail and arrangements for shipment of samples, contact the designated Coordinator at FIND. See also SOP 3.16 to SOP3.19 for storage and transport of QC sample aliquots.
4. Fill in the QC samples information sheet with all required information, according to the guidelines provided for this. Once completed, send the files to the designated Coordinator at WHO.

- Figure 3-4: Summary of the QC sample dilution process in the laboratory



- **Table 3-1: Summary of aliquots and tests**

	Aliquots / tests to prepare	Purpose / use	Labeling	Storage	Shipment
Patient blood (plain tube)	serum	hep. B/C, HIV serology	patient number	-20°C (until test)	yes (virol. lab)
Patient blood (EDTA tubes)	2 thick/thin films	microscopy	patient number, ID	RT, dry	no
	2 filter paper blood spots	molecular analysis	patient number, ID	RT, dry	yes (AMI)
	1 aliquot 1 mL whole blood	pharmacology	ID "whole blood"	-70°C	no
	1 aliquot 1 mL centrifuged:				
	- pellet	molecular analysis	ID "pellet"	-70°C	no
	- plasma	pharmacology	ID "plasma"	-70°C	no
	6 aliquots 100 µL	ELISA (antigen content)	ID "patient" "ELISA"	-70°C	yes (HTDL)
Dilution 2000 p/µL	≥ 100 aliquots 50 µL	QC of RDTs	ID "2000"	-70°C	yes (CDC)
	1 Thick film (maybe Earle-Perez)	microscopy (density)	ID "2000"	RT, dry	yes (CDC)
	1 Thick/thin smear (maybe Earle-Perez)	microscopy (archive)	ID "2000"	RT, dry	no
	1 pfHRP2 RDT	RDT result to record	ID "2000"	NA	NA
	1 pLDH RDT	RDT result to record	ID "2000"	NA	NA
	4 aliquots of 250 µL	ELISA (antigen content)	ID "2000" ELISA	-70°C	yes (HTDL)
Dilution 200 p/µL	≥ 600 aliquots 50 µL	QC of RDTs	ID "200"	-70°C	yes (CDC)
	1 Thick film (maybe Earle-Perez)	microscopy (density)	ID "200"	RT, dry	yes (CDC)
	1 Thick/thin smear (maybe Earle-Perez)	microscopy (archive)	ID "200"	RT, dry	no
	1 pfHRP2 RDT	RDT result to record	ID "200"	NA	NA
	1 pLDH RDT	RDT result to record	ID "200"	NA	NA
	8 aliquots of 250 µL	ELISA (antigen content)	ID "200" "ELISA"	-70°C	yes (HTDL)

RT = room temperature (~25°C), dry = with dessicant
NA = not applicable
AMI = Australian Army Malaria Institute, Queensland, Australia
HTDL = Hospital for Tropical Diseases, London, UK
CDC = National Center for Disease Control and Prevention, Atlanta, USA

- **Table 3-2: Summary of forms**

Patient screening	3.05
Patient record	3.06
Venepuncture	3.07
"Parasite-free blood" preparation	3.08
Microscopy (microscopist 1, read 1)	3.09
Microscopy (microscopist 2, read 1)	3.10
Microscopy (microscopist 1, read 2)	3.11
Microscopy (microscopist 2, read 2)	3.12
Parasite Density	3.13
Serology results	3.14

NOTES

A. Antibiotics

As quality control samples were prepared using aseptic technique, adding antibiotics to the dilutions is not required. Whenever possible, prepare dilutions inside a clean hood (biosafety cabinet).

B. Parasite concentrations for QC testing

Two WHO expert consultations have recommended 95% sensitivity at 100 p/µL as a reasonable target for RDT performance [1, 13]. For quality assurance of RDTs, quality control samples of 200 p/µL are

prepared to test the lower limit of detection. Samples at 100 p/µL were not chosen as sufficient antigen concentration could not be guaranteed for a fair evaluation of RDTs due to the following reasons:

1. *Some variability in malaria microscopy during preparation of dilutions is unavoidable, and exact parasite densities will vary around the designated value.*
2. *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.*

Preparation time (venepuncture to freezing) should be minimized, and ideally less than 24 hours. Samples should be kept at 4℃ at all times.

C. Reverse pipetting

Reverse pipetting is recommended for pipetting of viscous fluids. See SOP3.14.

D. 50 µL aliquots

As part of the quality assurance testing of RDTs, each dilution will be used to test 2 RDTs; one RDT requires approximately 5 to 10 µL of blood.

E. Earle Perez slides

An Earle-Perez film allows accurate assessment of parasite density after the dilution process, as white cells will also be heavily diluted. One Earle-Perez film, or a standard thick film, will be included with the samples sent to the global specimen bank, for future cross-checking if required.

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PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	2	Routine Revision: dilution steps expanded, quantity of QC panels to be prepared modified	RG
19 DECEMBER 2005	2	Minor revision	DB
01 MAY 2008	5	Re-numbered from SOP 3.2 (version 4) to SOP 3.08 (version 5) Modifications: see separate list	DB/JL/PJ/SI/WO/CS
01 APRIL 2010	6	500 parasite/µL aliquotes made optional	DB, AA
MAY 2014	7	Removed aliquots at 500 p/µL Updated numbers of aliquots for QC and for ELISA	DB/NC/SI
JUNE 2019	9	Formatting changes	JC, JL

SOP 3.09 Preparation of Quality Control Samples: Negative Control Samples

PURPOSE

This Standard Operating Procedure (SOP) describes the procedure for preparing negative control samples to be used for quality assurance of malaria RDTs.

BACKGROUND

Malaria RDTs are designed for the use with fresh human blood. Negative control samples for the quality control of malaria RDTs should therefore mimic fresh human blood as closely as possible and must be exempt from *Plasmodium* parasites. The absence of blood-borne viruses must also be ensured for safety reasons.

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the Project Manager.

PROCEDURE

A. General principle

The blood for preparation of negative control samples can be obtained from a blood bank or from a volunteer donor.

For "clean" negative control samples, any blood bag or any donor with low probability of infection by *Plasmodium* and blood-borne viruses can be chosen (e.g. donors with no history of fever and no exposure to malaria in the past year).

For negative control samples having other characeristics (e.g. positive for Leishmaniosis, Dengue fever, etc.), appropriate screening tests have to be performed for choice of the appropriate donor. Screening tests should be performed according to Standard and/or National protocols. Test results and eventually recommended treatment in case of positive results should be provided to the donor through the channels agreed with the Ministry of Health or appropriate agency.

B. Use of whole blood from individual donors

1. Counsel donor(s) on HIV and other proposed tests and obtain a written (signed) informed consent before venous blood collection (use Form 3.04 or adapted version, by changing the blood volume to be collected from donors).
2. Donor(s) must be screened for Hepatitis B surface Antigen, Hepatitis C and HIV 1 & 2. Collect 5mL of venous blood in a plain tube, centrifuge for 20 min at 2500g, separate serum and send for hepatitis B / C and HIV 1&2 serology.

3. If any of the above tests are positive, the donor cannot be used for obtaining "parasite-free" blood.
4. Provide virus screening results to the donor through the channels agreed with the Ministry of Health or appropriate agency. If virus screening tests are negative, arrange with the donor for being available on the planned day for preparing the negative control samples.
5. On the day of blood collection (ideally 2-3 hours before negative control samples are prepared), collect a finger-prick blood sample: perform pLDH and HRP2 RDTs, and prepare a thick and thin film for malaria microscopy.
6. Refer to SOP 3.05 for blood collection and malaria diagnosis by RDT and microscopy.
7. Donors with a positive RDT result or positive thick film must be excluded.
8. If negative by malaria RDT and microscopy, record all required information on Form 3.17.
9. Collect venous blood, either in EDTA tubes in case of small volumes (e.g. 50 mL), or in citrate blood bags in case of larger volumes (e.g. 450 mL).
10. Assign the Negative Control Sample ID, according to the following scheme:

AA = **ISO** Country code, e.g. KH for Cambodia*

= Lot number†

N = Negative Control Sample

= unique sample identifier‡

Example: **KH 01 N 04** — 4th negative control sample prepared in Cambodia, Lot number 1.

* The list of "ISO Country Codes" can be found in SOP 3.08, paragraph E.

† The "Lot number" is assigned by the laboratory preparing the negative control samples, e.g. it can correspond to one year or to one sample preparation campaign. It can also be fixed to 01, and successive numbers are assigned to all negative control samples prepared over an indefinite time.

‡ "Unique sample identifier" corresponds to the successive numbers of the negative control samples prepared for that particular lot.

11. Label all tubes / the bag with the Negative Control Sample ID and the expiry date (1 month after blood collection), and store blood immediately at 4°C.
12. Complete the required information on Form 3.17.
13. Expired blood must be discarded as per safety SOP 6.01.

C. Use of whole blood from a blood bank

1. Blood should be obtained from a reliable and/or accredited blood bank (e.g. National Blood Transfusion Centre). The blood bank should be contacted beforehand, informed about the project and the specific needs of blood (blood groups, volumes, dates).
2. On the day of blood procurement (ideally 2-3 hours before negative control samples are prepared), obtain information for each blood bag procured: number of blood bag, collection and expiry dates, refrigeration delay and temperature, blood bank screening tests for

hepatitis B, hepatitis C, HIV 1 & 2 viruses (by ELISA), and for malaria (by microscopy): test results, dates of testing, lab having performed the tests.

3. Record all required information on Form 3.17.
4. Eventually lacking screening tests must be performed and results must be negative before using the blood for QC sample dilution (screen for hepatitis B / C and HIV 1&2 infections by ELISA, screen for malaria by microscopy and RDT, record results on Form 3.17).
5. Blood bags must immediately be stored at 4°C and be used before the expiry date (indicated by the blood bank, usually 1 month after blood collection).
6. Expired blood must be discarded as per safety SOP 6.01.

D. Preparation of aliquots

1. The “parasite-free” blood must be gently mixed at 4°C during at least 15 min (transfer in sterile tubes for mixing if blood obtained in blood bags). Refer to SOP 3.01 for appropriate mixing equipments and tubes.
2. Label low absorption cryotubes with external screw cap with the Negative Control Sample ID, according to the scheme above. Use different colours for labelling tubes of different control samples, if prepared from different blood donors / blood bags on the same day.
3. Prepare aliquots of 50 µL, by using multi-dispense pipette with adapted 'combitips', or micropipette with SLOW reverse pipetting. Change the 'combitips' or pipette tips for aliquoting blood of different blood donors / blood bags.
4. Freeze the Negative Control Sample aliquots at -70°C as soon as possible, placing tubes upright in a freezing rack or in cryoboxes (to allow easier sampling later). For more detail on storage instructions, see SOP 3.16.
5. Record freezing times, number of aliquots and all other required information in Form 3.17.
6. Discard contaminated waste as per safety SOP 6.01.

PROCEDURE HISTORY

Date	Version	Comments	Initials
01 May 2008	5	SOP introduced. Adapted from former SOP 3.2, by retaining paragraphs on negative control samples preparation and use of blood from volunteer donors.	DB/JL/PJ/SI/WO

SOP 3.10 Preparation of Quality Control Samples: Parasite-free blood for dilution

AIM

To prepare blood exempt from *Plasmodium* parasites, which can be used for diluting blood from *Plasmodium* infected patients, in order to prepare quality control (QC) samples for the quality assurance (QA) of malaria rapid diagnostic tests (RDTs).

BACKGROUND

The samples for quality control of malaria RDTs should mimic fresh blood infected with wild parasites as closely as possible. It is therefore essential to use human blood, exempt of *Plasmodium* parasites ("parasite-free blood"), for dilution of the blood from patients naturally infected with *Plasmodium* parasites ("parasitized blood"). ABO incompatibility between the parasite-free and the parasitized blood must be prevented, as clumping of red blood cells would modify the properties of the blood dilutions to be used for QC of RDTs. Clumping of blood should be avoided for the following reasons:

1. It may prevent accurate confirmation of parasite density;
2. Quality control samples should be as close as possible to the quality of fresh blood (for which the products were designed); and
3. Clumping may possibly influence the ability of the lysed blood products to pass through the pores of the nitrocellulose strip in RDTs.

It is therefore recommended to use either blood from donors having the same blood group as the *Plasmodium* infected patient, or to use blood from a O+ or O- donor after having replaced the plasma with AB+ plasma.

PURPOSE

This Standard Operating Procedure (SOP) describes the procedure for preparing "*Plasmodium* parasite-free" donor blood which is to be used for dilution of blood from *Plasmodium* infected patients.

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the Project Manager.

REAGENTS/EQUIPMENT

See Supplies - Equipment check-list – Form 3.02.

PROCEDURE

A. General principle

The "parasite-free" donor blood used for dilution must be compatible with the "parasitized" patient blood, in order to minimize the risk of clumping of red blood cells. There are two options:

a) **Universal blood mixture:** For universal compatibility, O+ or O- blood (preferably O-) is centrifuged and the O+ / O- plasma is replaced by AB+ plasma. O+ or O- blood can be obtained

from individual volunteer donors, or from a blood bank. The AB+ plasma can be directly obtained from a blood bank or from a commercial supplier. It can also be prepared from AB+ blood obtained from a blood bank or from a volunteer blood donor.

b) **Matched blood group:** The blood group of the parasite-free blood can also be matched to the patient blood group. In this case, the patient blood group must be rapidly determined when the patient blood sample arrives in the laboratory, and parasite-free blood stocks of different blood groups (according to local prevalences) should be available. These bloods can be obtained from volunteer donors or from a blood bank.

All parasite-free blood must be exempt from *Plasmodium* parasites (malaria RDTs and microscopy) and Hepatitis B / C and HIV 1 & 2 viruses (ELISA).

B. Required volumes of parasite-free blood

1. Required volumes for the collection campaign (advanced planning of blood procurement): Estimates for the whole QC sample collection campaign should be based on 50 mL of parasite-free blood for processing one patient blood sample (e.g. for an expected number of 20 patient blood samples, plan for 1 L of blood and plasma). An additional spare margin (~25%) should be planned, in case of eventual problems like blood clumping etc.
2. Required volume for processing one patient blood sample (planning on the day of dilutions preparation): The precise volume for dilution of one patient blood sample depends on the initial parasite density and the number of QC sample aliquots to prepare. It can be calculated using the MS Excel Dilution Calculator, by entering the parasite density (approximate value when parasite density is known in advance during patient recruitment, and/or precise value after calculation of the mean parasite density in Form 3.13) and adjusting the number of QC sample aliquots. See SOP 3.08 for precise instructions on the MS Excel Dilution Calculator.

C. Use of whole blood from individual donors

1. Recruit donor(s) at least 4 weeks before the proposed period for QC sample collections.
2. Counsel donor(s) on HIV and other proposed tests and obtain a written (signed) informed consent before venous blood collection (use Form 3.04 or adapted version, by changing the blood volume to be collected from donors).
3. Perform A, B, O blood typing. For preparation of a universal blood mixture, donor(s) must be O negative or O positive. O negative is preferred as likelihood of blood clumping are reduced. For preparation of matched blood group blood, donors can be of any blood group (preferably the most common blood group in the area).
4. Donor(s) must be screened for Hepatitis B surface Antigen, Hepatitis C and HIV 1 & 2. Collect 5mL of venous blood in a plain tube, centrifuge for 20 min at 2500g, separate serum and send for hepatitis B / C and HIV 1&2 serology.
5. If any of the above tests are positive, the donor cannot be used for obtaining “parasite-free” blood.
6. Provide virus screening results to the donor through the channels agreed with the Ministry of Health or appropriate agency. If virus screening tests are negative, arrange with the donor for being available during the sample collection campaign for collecting blood.
7. On the day of blood collection (ideally 2-3 hours before blood is needed for preparing dilutions), collect a finger-prick blood sample: perform pLDH and HRP2 RDTs, and prepare a thick and thin film for malaria microscopy.
8. Refer to SOP 3.05 for blood collection and malaria diagnosis by RDT and microscopy.
9. Donors with a positive RDT result or positive thick film must be excluded.
10. If negative by malaria RDT and microscopy, record all required information on Form 3.08.
11. Collect venous blood, either in EDTA tubes in case of small volumes (e.g. 50 mL), or in citrate blood bags in case of larger volumes (e.g. 450 mL).

    Note: a number of donors will be required, see paragraph b above.
12. Assign a number to the donor blood (eg. X ##, where “X” would stand for the blood group A, B, AB or O, and ## for numbering the donor). Label all tubes / the bag with the number and the expiry date (1 month after blood collection), and store blood immediately at 4°C.
13. Complete the required information on Form 3.08.

14. Expired blood must be discarded as per safety SOP 6.01.

D. Use of whole blood from a blood bank

1. Blood should be obtained from a reliable and/or accredited blood bank (e.g. National Blood Transfusion Centre). The blood bank should be contacted at least one month before the planned QC sample collection campaign, informed about the project and the specific needs of blood (blood groups, volumes, dates of collection campaign).
2. On the day of blood procurement (ideally 2-3 hours before blood is needed for preparing dilutions), obtain information for each blood bag procured: number of blood bag, collection and expiry dates, refrigeration delay and temperature, blood bank screening tests for hepatitis B, hepatitis C, HIV 1 & 2 viruses (by ELISA), and for malaria (by microscopy) : test results, dates of testing, lab having performed the tests.
3. Record all required information on Form 3.08.
4. Eventually lacking screening tests must be performed and results must be negative before using the blood for QC sample dilution (screen for hepatitis B / C and HIV 1&2 infections by ELISA, screen for malaria by microscopy and RDT, record results on Form 3.08).
5. Blood bags must immediately be stored at 4°C and be used before the expiry date (indicated by the blood bank, usually 1 month after blood collection).
6. Expired blood must be discarded as per safety SOP 6.01.

E. Preparation of AB+ plasma from whole blood

1. AB+ plasma can be prepared from blood obtained from a donor or from the blood bank.
2. If AB+ blood is obtained from a volunteer donor, follow the procedure described above (paragraph C). If obtained from a blood bank, follow the procedure of paragraph D).
3. Prepare the AB+ plasma as soon as possible (immediately) after AB+ blood collection. Note that sterile tubes must be used at all times in this procedure.
4. Transfer the AB+ blood in sterile 50 mL conical tubes (approximate volume needed for processing one patient sample) or in 25 mL conical tubes and centrifuge at 2500g for 20 minutes. Use other tube formats if the centrifuge is not adapted.
5. Carefully pipette the AB+ plasma (without aspiration of AB+ blood cells) and transfer in sterile 15 mL or 25 mL or 50 mL tubes.
6. Eventually repeat centrifugation and pipetting as in steps 3. and 4. above. *This is to ensure that there are no left-over AB+ cells to prevent subsequent clumping problems during sample dilutions.*
7. Aliquot the obtained AB+ plasma into 25mL or 50mL sterile containers. *This permits the use of only the required volume of AB+ plasma for dilutions*.
8. Assign a number (ex. AB ##, with “AB” standing for the blood group and ## for numbering the plasma). Label all tubes with the number, the preparation date and the expiry date (2 years after plasma preparation).
9. Store immediately at -20°C or -70°C, and/or the required volume at 4°C if it is to be used on the same day.
10. Record all required information on Form 3.08.
11. Expired plasma must be discarded as per safety SOP 6.01.

F. Use of AB+ plasma from a blood bank or a supplier

If the fresh frozen plasma is provided in a bag:

1. Thaw according to SOP 3.11.
2. Clean the porthole with an alcohol-soaked cotton wool and using a 21-gauge syringe and 10 mL needle make aliquots of 15 mL, 25 mL or 50 mL in sterile tubes.
3. In all cases:
4. Label the tubes with the AB+ plasma number and expiry date (indicated by blood bank or supplier).

5. Store immediately at -20°C or -70°C, and/or the required volume at 4°C if it is to be used on the same day.
6. Record all required information on Form 3.08.
7. Expired plasma must be discarded as per safety SOP 6.01.

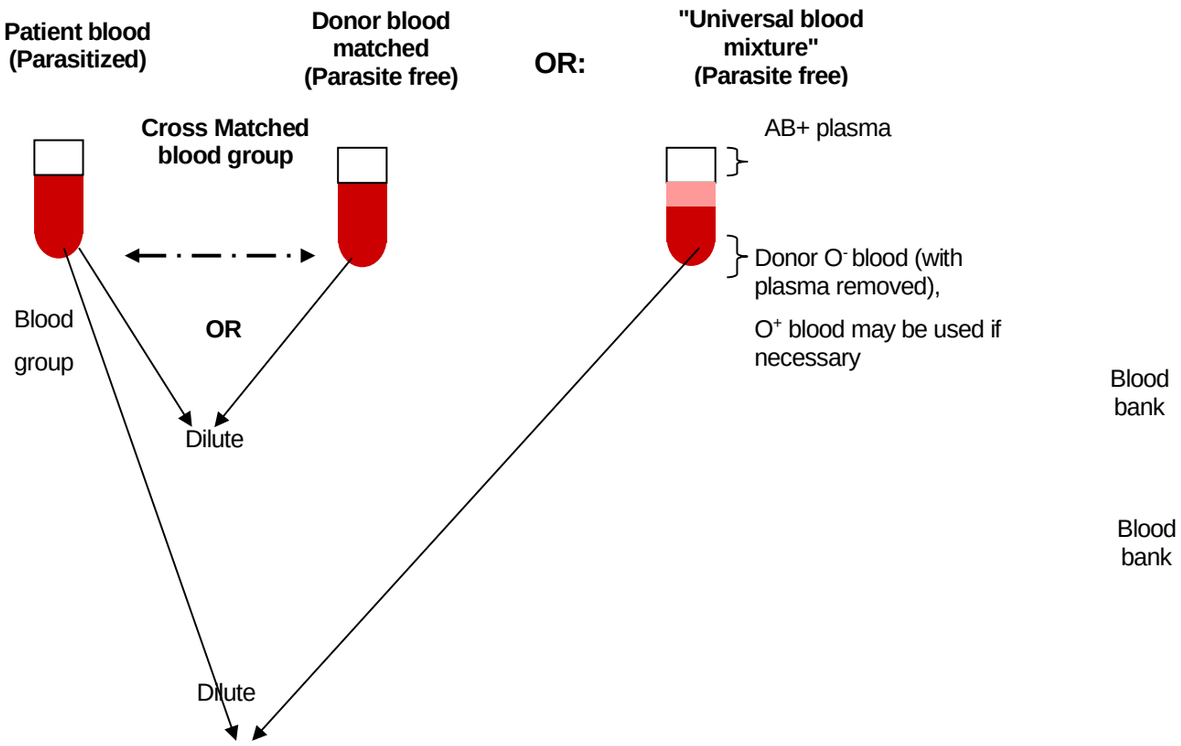
G. Preparation of the Universal blood mixture (Plasma Replacement)

1. Remove the required number of AB+ fresh frozen plasma tubes from the –20 or -70°C freezer and start thawing in a 37°C water bath (see SOP 3.11). This is ideally to be done one to two hours before blood is needed for dilutions preparation.
2. Once thawed, store at 4°C until use.
3. Centrifuge O- / O+ blood in EDTA tubes at 2500g for 20 minutes.
4. OR
5. If O- / O+ blood is in a blood bag, clean the porthole of the blood bag using an alcohol-soaked cotton wool, and aseptically transfer the blood into sterile 15mL, 25mL or 50mL conical tubes. Centrifuge the blood at 2500g for 20 minutes.
6. The sample will now be separated into a deposit (blood cells) and supernatant (plasma). With a marking pen, mark the outside of the tube at the top of the plasma.
7. Remove and discard plasma from each EDTA or conical tube with a sterile plastic pipette.
8. Eventually repeat centrifugation and pipetting as in steps 3. to 5. above. *This is to ensure that the O+/O- blood plasma is properly removed to prevent subsequent clumping problems during sample dilutions.*
9. Take out the thawed AB+ plasma from 4ºC. Using a sterile pipette, aseptically fill up the EDTA or conical tubes containing the centrifuged blood cell pellet to the previously marked line.
10. Assign a number to the parasite-free blood mixture (eg. N ##, with “N” standing for “negative” blood, and ## for numbering the negative blood mixture). Label all tubes with the number and the expiry date and time (24 hours after plasma replacement).
11. Mix gently at 4°C on a rocking tray / sample rotator, during at least 1 hour.
12. Record all required information in Form 3.08.
13. Store at 4°C until use and use within 24 hours.
14. Expired blood must be discarded as per safety SOP 6.01.

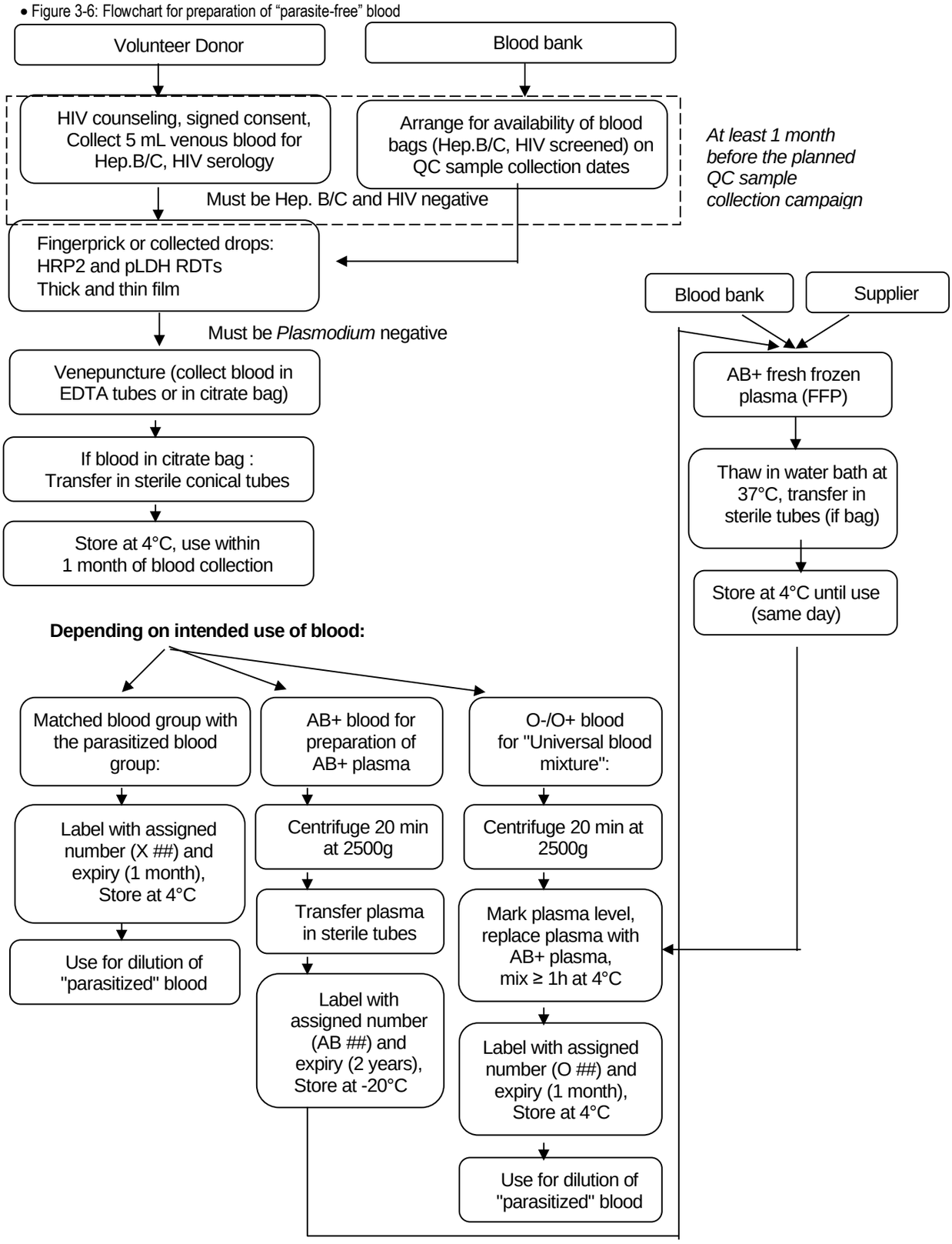
H. Organization of documents related to parasite-free blood

1. Each time that any parasite-free whole blood, plasma or universal blood mixture is prepared, fill in a Form 3.08 and file in a designated **parasite-free blood folder**.
2. Each time that a patient blood sample (parasitized blood) is diluted with a particular parasite-free blood (universal blood mixture or matched blood group), the identification number of this parasite-free blood must be recorded in the “Dilution preparation” - Form 3.14, which is filed in the “QC sample preparation folder", together with all other forms related to this particular patient blood sample.

- Figure 3-5: Different options for the procurement and preparation of “parasite-free” blood



• Figure 3-6: Flowchart for preparation of "parasite-free" blood



PROCEDURE HISTORY

Date	Version	Comments	Initials
01 MAY 2008	5	SOP introduced. Adapted from former SOP 3.2. Modifications: see separate list.	DB/JL/PJ/SI/WO/CS
MAY 2014	7	Modified the figure	NC/SI
JUNE 2019	9	Formatting changes	JC, JL

SOP 3.11 Thawing of Fresh Frozen Plasma

PURPOSE

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

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the Project Manager.

EQUIPMENT

Water bath

Thermometer (0°C to 100°C)

Plastic bag

PROCEDURE

1. Set the water bath at 37°C.
2. Remove the bag or tubes with fresh frozen plasma (FFP) from the freezer.
3. If FFP is provided in a bag: put the FFP bag 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.
4. If FFP is provided in tubes: place the tubes in a metal rack, and place in the water bath, making sure that the water level is not reaching the tubes caps to avoid water contamination.
5. Keep the FFP in 37°C water bath for 20-30 minutes. Invert occasionally.
6. Store the thawed FFP at 4°C in the refrigerator prior to use.
7. At a temperature range of 1-6°C, thawed FFP can be used up to 24 hours.

REFERENCES

1. Bontempo, F. Fresh Frozen Plasma. Transfusion Medicine Update. August 1992. (www.itxm.org date accessed: 17-10-02).
2. Technical Manual.12th edition. Maryland, American Association of Blood Banks Press, 1996.

3. The Clinical Use of Blood Handbook. Geneva, World Health Organization, 2001 (unpublished document).
4. Truizi D. Use and Abuse of Fresh Frozen Plasma. Transfusion Medicine Update. March 1997 (www.itxm.org date accessed: 17-10-02).

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
01 MAY 2008	5	Re-numbered from SOP 3.3 (version 4) to SOP 3.11 (version 5) Changed to include FFP in tubes.	DB/JL/PJ/SI/WO

SOP 3.12 Performing an RDT with freshly prepared QC sample dilutions

PURPOSE

This SOP describes the procedure for performing a Rapid Diagnostic Test, using freshly prepared QC sample dilutions, before aliquoting into QC sample aliquots for RDT QC.

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the “Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests”. The SOP is only to be modified with agreement of the Project Manager.

PROCEDURE

1. Carefully study the RDT manufacturer instruction sheet provided in the RDT kits. Approximately 30 minutes before testing, bring RDTs to room temperature (20-30°C) (~25°C) BEFORE OPENING the package. This applies only to RDTs stored under different conditions than room temperature (20-30°C) (e.g. incubator, fridge).
2. Remove the RDT packaging.
3. Check integrity of RDT packaging when opening. If signs of moisture are present, DO NOT use the RDT.
4. Check desiccant for any colour changes (e.g. blue to white). If present, discard RDT and use another kit for testing.
5. Label the RDT with QC sample ID, dilution, and date of test (DD/MM/YY), using a marker pen.
6. Test the RDTs with the QC sample as per manufacturer instructions, BUT use a micropipette to transfer the specified blood volume to the RDT.
7. Change the pipette tip for testing of each QC sample and each dilution.
8. Use a timer to record all steps exactly as per manufacturer instructions.
9. Read RDT results within the manufacturer recommended time.
10. Refer to the standard color chart provided by WHO for rating the band intensity from 0 (negative) to 4+.
11. Record the results in Form 3.15.

PROCEDURE HISTORY

Date	Version	Comments	Initials
01 MAY 2008	5	SOP introduced: Adapted from former SOP 2.3 (performing RDT), with changes listed above (Chapter 2, SOP 2.05), more specific for RDT programme	DB/JL/PJ/SI/WO
MAY 2014	7	Standard color chart for rating of RDT band intensities	DB/NC/SI
JUNE 2019	9	Formatting changes	JC, JL

SOP 3.13 Preparation of blood spots on filter paper, using venous EDTA blood

(Only when they were not made using fresh blood in the field)

AIM

To preserve dried blood samples suitable for PCR analysis for malaria parasite species identification and genetic diversity.

BACKGROUND

DNA is stable for long periods if in dried sample and protected from moisture. Samples should be used to exclude mixed infection and to analyse genetic diversity of target antigen in all QC samples, as mixed infection and variant antigens affect RDT results.

PURPOSE

This SOP describes the procedure for preparing dried blood spots suitable for DNA analysis.

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the “Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests” and is not to be modified except by the Project Manager.

EQUIPMENT

Micropipette and tips

Filter paper (Whatman No.1 or No. 3, or equivalent)

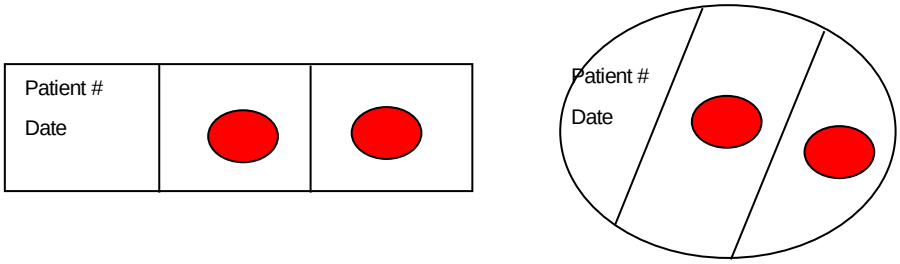
Plastic bags

Desiccant

PROCEDURE

1. Prepare the filter paper to 10cm x 3 cm strips or to 8cm diameter disks and draw 3 squares (3cm x 3cm each).
2. Write patient number and date of collection (DD/MM/YY) in one of the squares (Figure 3-7).

- Figure 3-7: Filter paper blood spots for DNA analysis



3. Using a micropipette, transfer 20 µL-50µL of patient blood from the EDTA tube to one of the squares on the filter paper.
4. Repeat the same step for the other square on the filter paper.
5. Dry the filter-paper samples completely in air and place them in small, separate plastic bags.
6. During the drying process, avoid contact between filter papers prepared from different patient blood samples (and ensure proper labeling).
7. Add desiccant to each plastic bag (left-over desiccant from recently opened RDT pouchs can be used, but it must be ensured there is no colour change, or desiccant can be purchased commercially).
8. Store and transport the dried filter-paper samples at room temperature (20-30°C), with desiccant included.
9. Ensure completely dry storage conditions (regularly check the desiccant for any colour change indicating moisturizing and change if needed).

PROCEDURE HISTORY

Date	Version	Comments	Initials
01 MAY 2008	5	SOP introduced Adapted from former SOP 3.9 (Preparation of filter paper blood spots), removed storage at -20°C, updated list of equipment, minor changes.	DB/JL/PJ/SI/WO
FEBRUARY 2020	9	Minor changes, renamed figure 1 to figure 3-7	JL, CAL

SOP 3.14 Reverse Pipetting Technique

PURPOSE

This Standard Operating Procedure (SOP) describes the reverse pipetting technique, to be used for pipetting viscous liquids such as human blood.

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP as a minimum standard.

PROCEDURE

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.

1. Fit the appropriate tip.
2. Set the volume on the pipette counter.
3. Depress the pipetting button up to the second stop (i.e. when the first stop is felt, which is used for standard pipetting, press further until the second stop).
4. Immerse the tip around 2 to 3 mm in the sample, making sure that the pipette is held vertically.
5. Allow the button to retract slowly, observing the filling operation. The optimum speed for drawing depends of the sample. Also note that a larger volume enters the tip than the set value.
6. When dispensing, the pipetting button is only pressed up to the first stop. This ensures that the correct volume (set on the pipette counter) is dispensed.
7. Quickly wipe off the tip against the side of the container.
8. Discard tip with remaining sample, as per safety SOP 6.01.

REFERENCES

1. Farnell, H. Good Pipetting Practice. International Labmate XXV (V), 2002. (www.internationallabmate.com, date accessed: 17-10-02)
2. Ylatupa. S. Liquid Handling Application Notes. European Clinical Laboratory, October 1996.

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
01 MAY 2008	5	Re-numbered from SOP 6.5 (version 4) to SOP 3.14 (version 5) Explanations of the pipetting and discarding infectious waste modified.	DB/JL/PJ/SI/WO

SOP 3.15 Light Microscopy for Red Blood Cell Clumping

PURPOSE

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

BACKGROUND

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

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the “Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests”. The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP as a minimum standard.

EQUIPMENT

Microscopy slides

Cover slips

Saline solution (0,9% w/v NaCl)

Pipette tips

PROCEDURE

A. Performing the clumping test

1. Use a frosted-ended glass slide.
2. Label with patient number and dilution.
3. Add a drop of saline solution (0,9% w/v NaCl) to the slide.
4. Touch the blood sample dilution with a pipette tip, add to the saline and mix gently using the same pipette tip.
5. Add a cover slip.
6. Look under a light microscope, and check for presence of red cell clumping at 10x and 40x power.
7. If no clumping occurs, red blood cells are scattered in a relatively uniform distribution, and cells can be observed unattached to others. Record as negative clumping.
8. If clumping occurs, cells are attached to each other and form cell packs. Record as positive clumping.

B. Recording of results and decision on further processing

1. *First clumping test with the small clumping test mixture:*

   If no clumping occurs, results are not recorded and the patient blood sample is further processed as per SOP 3.08.

   If clumping occurs, there is incompatibility between the parasitized patient blood and the parasite-free donor blood. For decision on further processing of the patient blood sample (with a different “parasite-free” blood), see SOP 3.08.

2. *Second clumping test with the patient blood dilution (large volume for preparing QC sample aliquots):*

   If no clumping occurs, record result in Form 3.14, and continue preparation of QC sample aliquots as per SOP 3.08.

   If clumping occurs, there is incompatibility between the parasitized patient blood and the parasite-free donor blood. This dilution can not be used for preparing QC sample aliquots. Record result in Form 3.14 and stop processing the patient blood sample.

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
01 MAY 2008	5	Re-numbered from SOP 4.3 (version 4) to SOP 3.15 (version 5) Added background and equipment, detail on saline, description of clumping results, results record and decisions.	DB/JL/PJ/SI/WO

PART 4: Storage and Transport of RDT QC Samples

LIST OF FORMS FOR CHAPTER 3, PART 4:

3.18: Internal Movements of RDT QC Samples

3.19: QC Sample Referral Log

SOP 3.16 Storage and Transport of RDT QC samples

PURPOSE

This Standard Operating Procedure (SOP) describes how to store and transport RDT QC sample aliquots.

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the Project Manager.

PROCEDURE

A. Organization of the RDT QC sample aliquots storage

1. RDT QC sample aliquots must always be stored at -70°C (ensure that enough space is always available).
2. Exceptionally, if there is a lack of storage space at -70°C, QC sample aliquots can be kept at -20°C. Nevertheless, the time of storage at -20°C should not exceed two weeks.
3. The storage space should be labelled or indicated on organization charts. Labelling and/or organization charts should allow to quickly find the required QC sample aliquots depending on their QC sample ID.
4. The -70°C freezers should be connected to electricity with stabilizers and with a system switching to an emergency generator in case of shortage. Sufficient back-up storage space should be available in a different -70°C freezer.
5. Calibrated thermometers with the appropriate temperature range have to be used for daily recording of the temperature, either by using the SOP 6.08 and the Form 6.07 of the Methods Manual or by using equivalent procedures and forms of the eventually pre-existing general laboratory QA system.
6. The staff responsible for temperature recording must be clearly defined, and replacement staff must be identified in the case of absence.

B. Internal movements of QC sample aliquots

1. If QC sample aliquots have to be moved from one storage area to another (different -70°C freezer, or exceptional movement to -20°C), the movement must be registered in Form 3.18.
2. During manipulation and transfer of the QC sample aliquots, extreme care must be taken to avoid thawing. Prepare foam boxes with ice packs for quick transfers and handle the aliquots as quickly as possible. If possible, work in a cool air-conditioned room.

C. Transport of QC sample aliquots to other laboratories

1. Details of the samples shipment (number of samples, adress, date, etc.) will be arranged with the Project Manager.

2. As soon as possible (once the shipment requirement and destination are known), study SOP 3.16, 3.17 and 3.18, to know about packaging of the QC sample aliquots, transport documentation, and organization of the transport. Enquire the Project Manager if the samples packaging is done by the laboratory staff or by staff from a contracted carrier company.

PROCEDURE HISTORY

Date	Version	Comments	Initials
01 MAY 2008	5	SOP introduced	DB/JL/PJ/SI/WO

SOP 3.17 Packaging of Quality Control Samples for Transport

PURPOSE

This Standard Operating Procedure (SOP) describes methods for proper packaging prior to transport of QC samples.

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the Project Manager.

BACKGROUND

Proper packaging and labeling of the material being shipped is vital to maintaining the integrity of the specimens, preventing accidents, and ensuring that there are no delays due to violations of regulations. The packaging requirements for various types of laboratory materials are subject to international and national regulations. There are a number of licensed agencies worldwide that provide training for personnel who need to know how to package materials in compliance with international regulations.

The international regulations for the transport of infectious materials by any mode of transport are based upon the Recommendations of the United Nations Committee of Experts on the Transport of Dangerous Goods (UN). International organizations such as the Universal Postal Union (UPU), the International Civil Aviation Organization (ICAO), and the International Air Transport Association (IATA) have incorporated these recommendations into their respective regulations. The World Health Organization serves in an advisory capacity to these bodies.

The regulations specify five types of materials that must meet the requirements for safe transport. The requirements differ depending on which category of material is being shipped:

Infectious Substances: Those substances known or reasonably expected to contain pathogens. Pathogens are defined as microorganisms (including bacteria, viruses, rickettsiae, parasites, fungi) or recombinant microorganisms (hybrid or mutant), that are known or reasonably expected to cause infectious disease in animals or humans.

Diagnostic Specimens: Any human or animal material including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluids being transported for diagnostic and investigation purposes, but excluding live infected animals.

Biological Products: Those products derived from living organisms, which are manufactured and distributed in accordance with the requirements of national governmental authorities which may have special licensing requirements, and are used either for prevention, treatment, or diagnosis of disease in humans or animals, or for related development, experimental or investigational purposes. They include, but are not limited to, finished or unfinished products such as vaccines and diagnostic products.

Genetically Modified Micro-organisms and Organisms: Micro-organisms and organisms in which genetic material has been purposely altered through genetic engineering in a way that does not occur naturally.

Clinical Waste and Medical Waste: Clinical Waste and Medical Waste are wastes derived from the medical treatment of humans or animals or from bioresearch, where there is a relatively low probability that infectious substances are present.

Carbon Dioxide, Solid (Dry Ice): Dry ice is classified as a dangerous good by IATA. The product does not contain oxygen and may cause asphyxiation. Exposure may cause nausea and respiratory problems, and contact may cause frostbite.

Other Dangerous Goods: Under this classification are cryogenic liquids, ethanol solutions, methanol, pyridine, strong formaldehyde solutions, hypochlorite solutions, aviation regulated liquids, and iodine.

In general, all of the above categories of materials should be shipped using the basic triple packaging system, in addition to the specific requirements necessary for that category (see sections below for category specific instructions). Packaging materials for this system should be manufactured in compliance with the Dangerous Good Regulations. There are a number of manufacturers who can provide containers manufactured to these specifications. The packaging system is (Figure 3-8)

Primary receptacle: A labeled primary watertight, leak-proof receptacle containing the specimen.

Secondary receptacle: A second durable, watertight, leak-proof receptacle (Ex Plastic bag) to enclose and protect the primary receptacle(s). Several wrapped primary receptacles may be placed in one secondary receptacle. Sufficient additional absorbent material must be used to cushion multiple primary receptacles. Specimen data forms, letters, and information to identify the specimen, the sender, and the receiver should be placed in a waterproof bag and taped to the outside of the secondary receptacle.

Outer shipping package: The secondary receptacle is placed in an outer shipping package that protects it and its contents from outside influences such as physical damage and water while in transit.

Currently, IATA regulations classify materials for shipping based on establishing a “risk group” for the material. A risk group is characterized by the pathogenicity of the organism, the mode and relative ease of transmission, the degree of risk to both an individual and a community, and the reversibility of the disease through the availability of known and effective preventative agents and treatment. The criteria for each risk group according to the level of risk are as follows:

PROCEDURE

Preliminary note on applicable instructions:

For the purpose of transport, malaria RDT quality control (QC) samples are treated as Biological substance Category B. Packing instructions therefore fall under IATA dangerous goods regulations packing instructions 650: Infectious substances in category B.

The use of dry ice requires a declaration of Dangerous Goods class 9, UN1845, and must comply with packing instruction 904. The instructions given here comply with all these rules.

Packaging and transport is usually overseen by a professional courier company when shipping between countries. In such cases, the courier's instructions on packaging should be followed. These should comply fully with the relevant IATA regulations, and regulations of the countries of the consigner and the destination and through which the package is transiting. Laboratory personnel involved in the process should familiarize themselves with this SOP prior to the courier's arrival, to facilitate rapid packaging and transfer. In cases of in-country transport by the specimen collecting institution, staff should fully familiarize themselves with the SOP and with national regulations, and liase beforehand with the airline concerned.

1. All quality control (QC) sample aliquots must be packaged in sealed cryovials (e.g. screw-cap tubes with O-ring) supplied by the WHO programme, and labeled with the complete ID code (e.g. PH01 F04 2,000).

2. During manipulation and transfer of the QC sample aliquots, extreme care must be taken to avoid thawing. Prepare foam boxes with ice packs for quick transfers and handle the aliquots as quickly as possible. If possible, work in a cool air-conditioned room.

3. The sealed tubes must be placed in a suitably sized plastic bag together with a small amount of absorbent material, for example cotton wool. The bag must be sealed, either using a bag heat-sealer or waterproof adhesive tape (Figure 3-8).

4. Aliquots of different sample ID and different dilutions should never be sealed in the same bag.

5. The plastic bags must be labeled with the ID code of the QC sample aliquots and with the Biological Substances Category B.

6. The plastic bags may then be placed in sealable paper bags, labeled with the relevant ID codes and the UN 3373.

7. To ensure samples remain frozen during transport, the bags must be placed in a container (foam box) with cooling material (dry ice). Ensure that all bags are well covered with dry ice, and that the amount if dry ice is sufficient for the expected transport time.

8. The foam box must then be placed in an outer packaging. The outer packaging must conform to *IATA Dangerous Goods Regulations Packaging Instruction 650*. The box must have the appropriate markings on the outside.

   An extra label is required on the outside of the over pack stating:

“INNER PACKAGES COMPLY WITH PACKING INSTRUCTIONS 650”

9. The outer packaging must be labeled with the following information (Figure 3-9):

- The sender's name, address and contact telephone/fax numbers.
- The UN Classification numbers and proper shipping names:

UN 3373

BIOLOGICAL SUBSTANCE CATORGORY B

[MALARIA Vol. X mL]

UN 1845 DRY ICE

- The total volume X of QC sample aliquots contained in the package.
- The weight of dry ice included in the package at commencement of shipment.
- The receiver's name, address and contact telephone/fax numbers.
- ***UN 3373 label and Biological Substance Category B***
- ***Miscellaneous label class 9*** (if dry ice is being used).

•

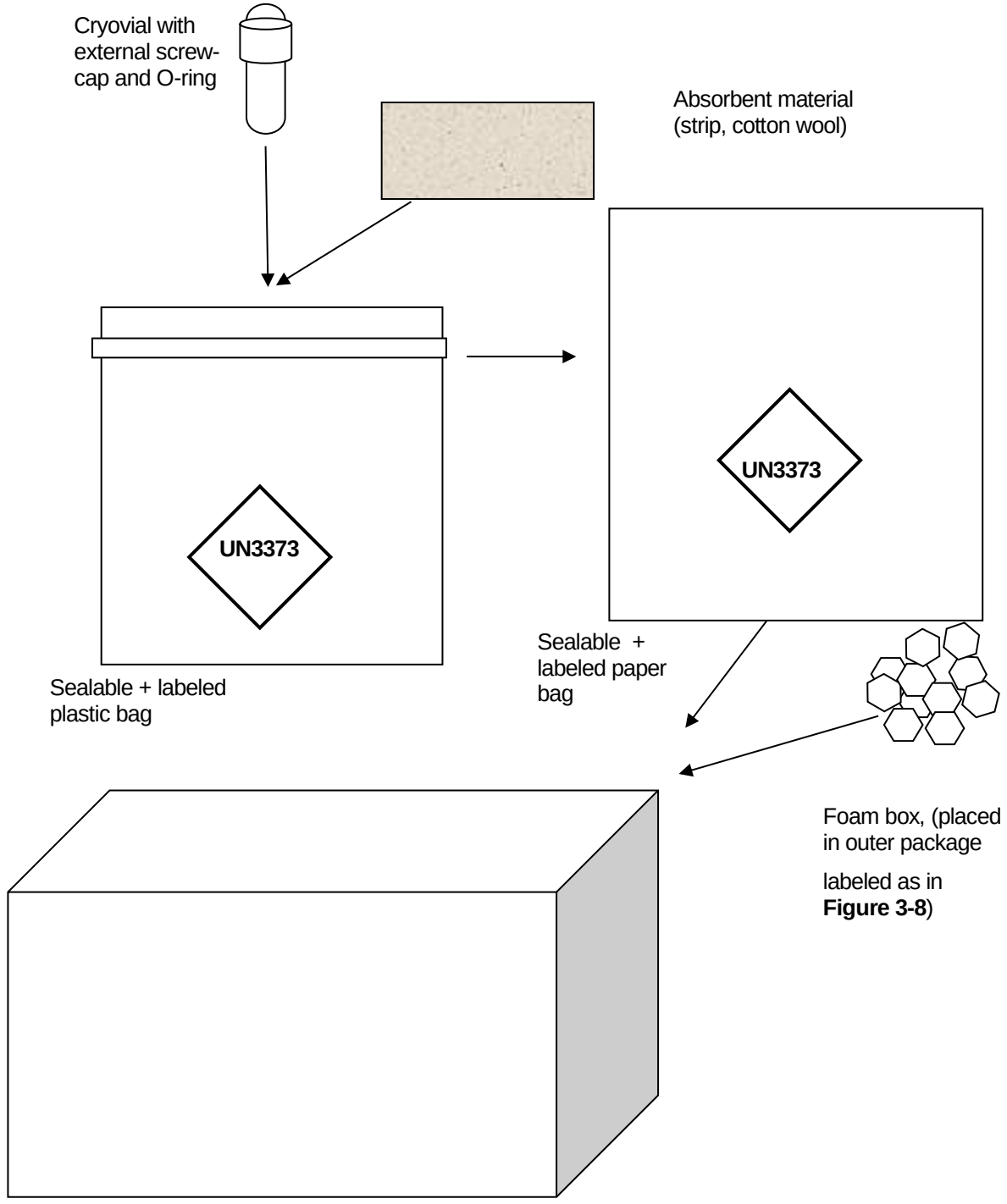
10. It may be of benefit to include an additional label requesting: "Keep package at -70^{o}C ". The box should be sealed using wide sealing tape, taking care not to obscure the labels with the tape and leaving a gap for venting of the dry ice.

11. All infectious substances must be accompanied by a **Sender's Declaration for Dangerous goods**, indicating shipment of infectious substances and the use of dry ice in the shipment.

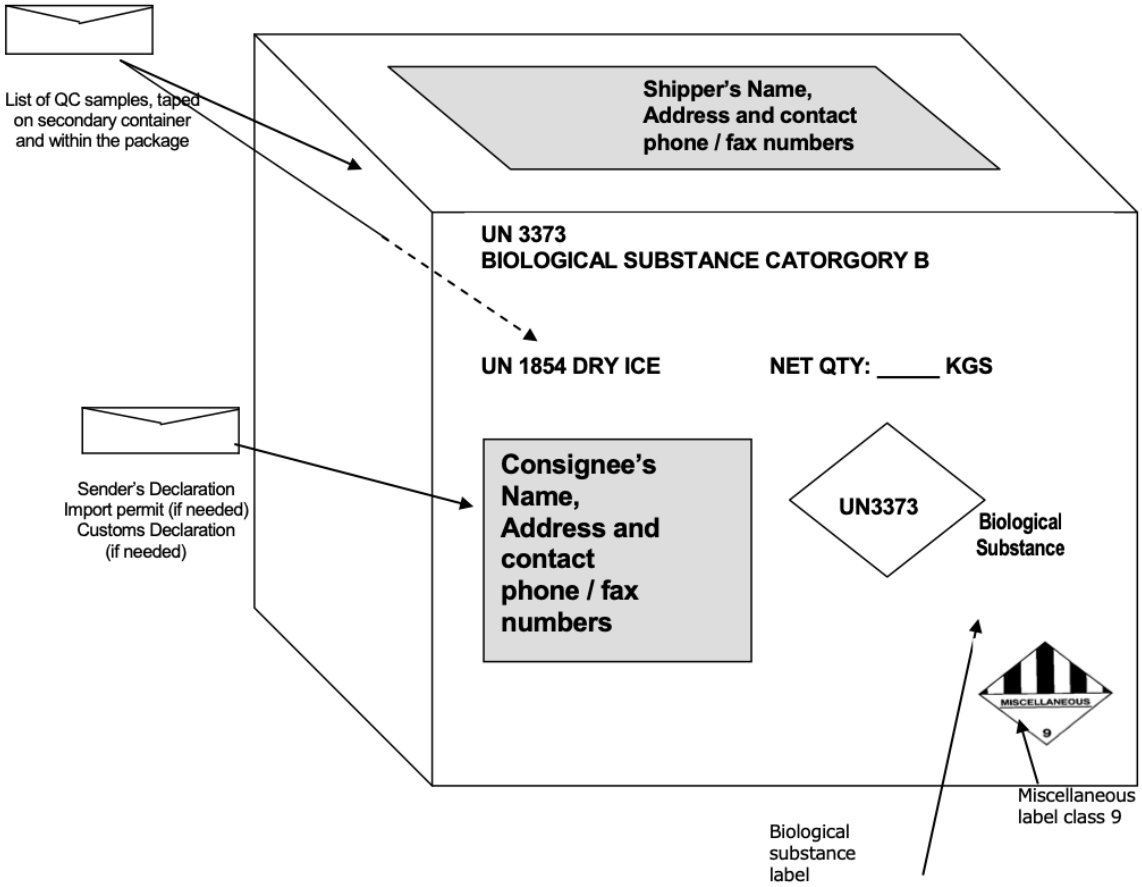
    A list of quality control samples contained in the package should be included in an envelope within the outer container, and taped on the outside of the outer packaging. For more detail on transport documentation, see SOP 3.18**.**

12. Commercial couriers may elect to transport samples under a higher IATA classification.

- Figure 3-8: Packaging of quality control sample aliquots



- Figure 3-9: Example of correct labeling for a shipment of infectious substances (QC samples, dangerous goods label class 6) chilled with dry ice (dangerous goods label class 9).



REFERENCES

1. World Health Organization. *Requirements and Guidance for External Quality Assessment for Health Laboratories.* Geneva, World Health Organization, 1989 WHO/DIL/LAB/99.2).
2. Victoria Infectious Diseases Reference Laboratory. Standard Operating Procedure Manual for WHO Polio Laboratory – Chapter 9: Specimen and Isolate Transport. Victoria Infectious Diseases Reference Laboratory, 2001
3. Infectious Substances Shipping Guidelines, The Complete Reference Guide for Pharmaceutical and Health Professionals: 7th Edition, International Air Transport Association (IATA), 1st Jan 2006

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB/AS
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
5 APRIL 2004	2	Routine review, format, typo changes, material classifications addition	DB/RGD
15 OCTOBER 2004	2	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	2	Routine Revision, minor changes only	RG
23 MAY 2008	5	Re-numbered from SOP 3.5 (version 4) to SOP 3.17(version 5). Changed classification of QC samples and packaging instructions, with reference to IATA Guidelines.	DB/JL/SI/WO
FEBRUARY 2020	9	Minor changes, renamed figures 3-7 and 3-8 to 3-8 and 3-9 respectively	JL, CAL

SOP 3.18 Documentation of Quality Control Samples for Transport

PURPOSE

This Standard Operating Procedure (SOP) describes the essential documents required when transporting quality control samples, in addition to documentation required by consignee and consignor countries for transport of human blood products.

SCOPE

This procedure is part of the methods for the preparation of RDT quality control samples described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP is only to be modified with agreement of the Project Manager.

PROCEDURE

For the transport of RDT quality control samples, the following documents need to be prepared:

A. Documents completed within the Sending Institution (Consignor)

1. Complete the QC Sample referral log (Form 3.19) with all required information.
2. Complete the QC Samples database, then send the files to the Designated Database Coordinator at FIND and to the Consignee. Guidelines for filling in the database and for sending the information are provided separately.
3. Any problems occurring during the packaging, transport, or at receipt should be recorded in Form 3.19, by attaching eventually relevant documentations.
4. As soon as receipt has been confirmed by the consignee, record the receipt date in Form 3.19.
5. All records should be archieved for at least five years. Refer to SOP 6.11 for documents storage.

B. Documents to attach to package for transport

1. Sender's Declaration of Dangerous Goods: It is recommended to include 6 copies for international shipments and 2 copies for domestic packages. See Figure 3-10 for an example.

2. A packing list: which includes shipping name, the receivers address, the number of packages, detail of contents (UN 3373, Biological Substance Category B), source, weight, value (required for international shipping only – Figure 3-10).

3. Customs declaration with appropriate information for national authorities including UN 3373 (Biological Substance Category B) declaration.

4. 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.

5. Airway bill (Figure 3-11): The airway bill should be marked with the following information:

- Name, address, telephone/fax of receiver

- Number of specimens
- "Highly perishable"
- "Telephone receiver upon arrival" (include telephone number)
- Airway bill handling information

- "URGENT: DO NOT DELAY:
- Biological specimens -- highly perishable -- store at -70ºC"

6. Export/import documentation e.g. waiver letter. These vary with national government regulations, and the onus is on consignee and consignor to ensure regulations are fulfilled prior to transport.

• Figure 3-10: Example of Packing list for attachment to outside of shipment

1 November 2003

TO WHOM IT MAY CONCERN:

This shipment contains Biological Substance Category B in accordance with IATA packing instruction 650. These samples are to be used for research and laboratory testing purposes only. These samples have no commercial value and are not for resale. For customs purposes only we place a nominal value of US $10.

Contents:

Full scientific name: Human blood containing dead malaria parasites
Volume per vial: 0.05 mL
Number of vials: 2,000
Country of origin: The Philippines

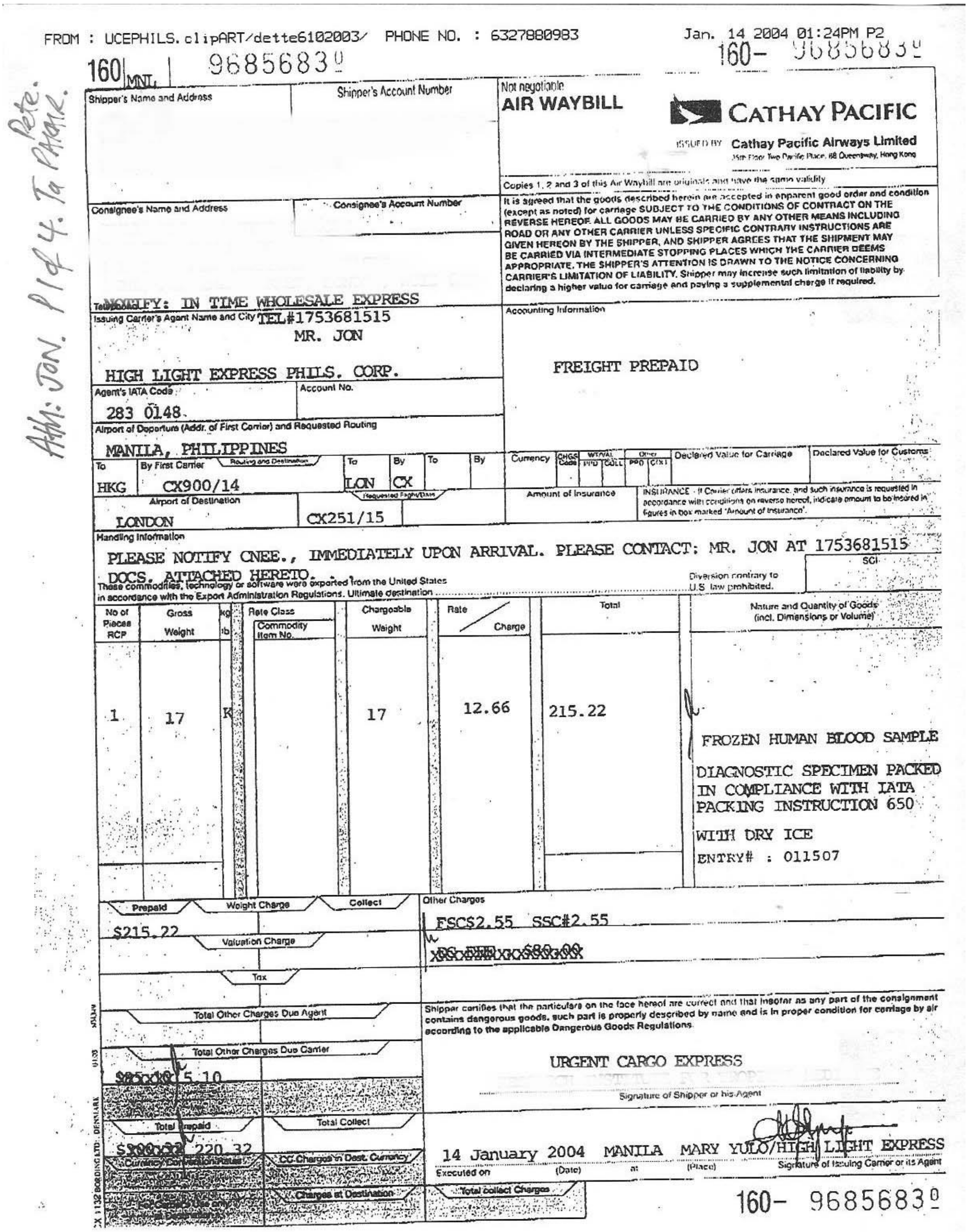
From: <Consigner>
<Address>

To: <Consignee>
<Address>

Value - US$10.00

e.g.
World Health Organization
Regional Office for the Western Pacific
UN Avenue
1000 Manila
PHILIPPINES

- Figure 3-11: Example of a completed dangerous goods form for the transportation of an infectious substance on dry ice. (The statement "over pack used" need only be included if such a packaging system was used).



FROM : UCEPHILS.clipART/dette6102003/ PHONE NO. : 6327880983 Jan. 14 2004 01:24PM P2

160 MNL 96856830 160- 96856830

Att: JON. P1 of 4. To PATRICK. Pete.

Shipper's Name and Address | Shipper's Account Number

Not negotiable
AIR WAYBILL
CATHAY PACIFIC
ISSUED BY Cathay Pacific Airways Limited

Copies 1, 2 and 3 of this Air Waybill are originals and have the same validity

It is agreed that the goods described herein are accepted in apparent good order and condition (except as noted) for carriage SUBJECT TO THE CONDITIONS OF CONTRACT ON THE REVERSE HEREOF. ALL GOODS MAY BE CARRIED BY ANY OTHER MEANS INCLUDING ROAD OR ANY OTHER CARRIER UNLESS SPECIFIC CONTRARY INSTRUCTIONS ARE GIVEN HEREON BY THE SHIPPER, AND SHIPPER AGREES THAT THE SHIPMENT MAY BE CARRIED VIA INTERMEDIATE STOPPING PLACES WHICH THE CARRIER DEEMS APPROPRIATE. THE SHIPPER'S ATTENTION IS DRAWN TO THE NOTICE CONCERNING CARRIER'S LIMITATION OF LIABILITY. Shipper may increase such limitation of liability by declaring a higher value for carriage and paying a supplemental charge if required.

Consignee's Name and Address | Consignee's Account Number

NOTIFY: IN TIME WHOLESALE EXPRESS
TEL#1753681515
MR. JON

Issuing Carrier's Agent Name and City: HIGH LIGHT EXPRESS PHILS. CORP.

Accounting Information: FREIGHT PREPAID

Agent's IATA Code: 283 0148 | Account No.

Airport of Departure (Addr. of First Carrier) and Requested Routing: MANILA, PHILIPPINES

To: HKG | By First Carrier: CX900/14 | To: LON | By: CX

Currency | CHGS Code | WT/VAL PPD COLL | Other PPD COLL | Declared Value for Carriage | Declared Value for Customs

Airport of Destination: LONDON | Requested Flight/Date: CX251/15 | Amount of Insurance | INSURANCE - If Carrier offers insurance, and such insurance is requested in accordance with conditions on reverse hereof, indicate amount to be insured in figures in box marked 'Amount of Insurance'.

Handling Information
PLEASE NOTIFY CNEE., IMMEDIATELY UPON ARRIVAL. PLEASE CONTACT: MR. JON AT 1753681515
DOCS. ATTACHED HERETO.

These commodities, technology or software were exported from the United States in accordance with the Export Administration Regulations. Ultimate destination ... Diversion contrary to U.S. law prohibited.

No of Pieces RCP	Gross Weight	kg lb	Rate Class Commodity Item No.	Chargeable Weight	Rate / Charge	Total	Nature and Quantity of Goods (incl. Dimensions or Volume)
1	17	K		17	12.66	215.22	FROZEN HUMAN BLOOD SAMPLE DIAGNOSTIC SPECIMEN PACKED IN COMPLIANCE WITH IATA PACKING INSTRUCTION 650 WITH DRY ICE ENTRY# : 011507

Prepaid / Weight Charge / Collect: $215.22

Other Charges: FSC$2.55 SSC#2.55

Valuation Charge

Tax

Total Other Charges Due Agent

Shipper certifies that the particulars on the face hereof are correct and that insofar as any part of the consignment contains dangerous goods, such part is properly described by name and is in proper condition for carriage by air according to the applicable Dangerous Goods Regulations.

URGENT CARGO EXPRESS
Signature of Shipper or his Agent

Total Other Charges Due Carrier: 5.10

Total Prepaid: 220.32 | Total Collect

Currency Conversion Rates | CC Charges in Dest. Currency

14 January 2004 MANILA MARY YULO/HIGH LIGHT EXPRESS
Executed on (Date) at (Place) Signature of Issuing Carrier or its Agent

For Carrier's Use only at Destination | Charges at Destination | Total Collect Charges

160- 96856830

REFERENCES

1. World Health Organization. *Requirements and Guidance for External Quality Assessment for Health Laboratories.* Geneva, World Health Organization, 1989 WHO/DIL/LAB/99.2).
2. Victoria Infectious Diseases Reference Laboratory. Standard Operating Procedure Manual for WHO Polio Laboratory – Chapter 9: Specimen and Isolate Transport. Victoria Infectious Diseases Reference Laboratory, 2001

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format, and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
26 MAY 2008	5	Re-numbered from SOP 3.6 (version 4) to SOP 3.18 (version 5). Changed classification of QC samples (Biological Substance Category B, UN3373), more detail on documents at Sending Institution (form, database), corrected references to figures.	DB/JL/SI/WO
FEBRUARY 2020	9	Formatting changes, renamed figures 3-9 and 3-10 to 3-10 and 3-11 respectively	JL, CAL

SOP 3.19 Coordination of Transport of Quality Control Samples

PURPOSE

This Standard Operating Procedure (SOP) describes guidelines for proper shipment of quality control samples for testing malaria RDTs. The transport of quality control samples requires careful planning and coordination between the consignor, the carrier, and the consignee.

SCOPE

This procedure applies to the WHO malaria rapid diagnostic test quality assurance initiative. The SOP is only to be modified with agreement of the Project Manager.

PROCEDURE

A. Transport Planning

It is the responsibility of the sender 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 consignor or the shipper), the carrier, and the receiver (the consignee or receiving laboratory), 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.

Overall coordination of the transport arrangements will be performed by WHO and FIND.

1. Advance arrangements with the consignee
    a) Once it has been decided that materials need to be shipped from the laboratory, the receiver should be contacted and informed of the nature of the materials to be sent.
    b) The receiver must be notified beforehand of QC samples to be sent for shipping, and acknowledgement of preparedness for receipt must be sent back to the sender.
    c) The sender should inquire about any import permits or other documents required by the receiving laboratory's national government. If permits are needed, the receiving laboratory will need to obtain the CURRENT permit and send it (usually a faxed copy) to the sending laboratory so that the permit can be given to the carrier.
    d) The sender and receiver should then make advance arrangements for a mutually convenient time for shipment to ensure that the appropriate staff are available to receive the shipment. It is recommended to plan to avoid weekend arrivals.

2. Advance arrangements with the carrier

a) Once a shipment is necessary, a carrier familiar with handling infectious substances and diagnostic specimens should be contacted, and arrangements should be made to ensure that:

- The shipment will be accepted.
- The shipment is undertaken by the most direct routing, avoiding weekend arrival.
- Archives and documentation of the shipment progress will be kept.
- The conditions of the shipment while in transit will be monitored.
- The sender and consignee will be notified of any delays.

b) The sender should request any necessary shipping documents that the carrier may require or any specific instructions necessary to ensure safe arrival of the shipment. The carrier may also provide advice on packaging.

c) In cases of delays, the consignor must arrange for both the consignee and consignor to be notified immediately by the carrier and advised on expected arrival arrangements.

B. Notification of the consignee of departure

IATA guidelines require that once the package has been sent, the receiver (consignee) should be immediately notified of the following:

- Type and number of QC sample aliquots (nature and quantity)
- Flight details (airline, flight number, arrival date and time)
- Airway bill number
- "Please inform if not received"

C. Notification of the consignor

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

REFERENCES

1. World Health Organization. Requirements and Guidance for External Quality Assessment for Health Laboratories. Geneva, World Health Organization, 1989 WHO/DIL/LAB/99.2).
2. Victoria Infectious Diseases Reference Laboratory. Standard Operating Procedure Manual for WHO Polio Laboratory – Chapter 9: Specimen and Isolate Transport. Victoria Infectious Diseases Reference Laboratory, 2001.
3. IATA Regulations Handbook, 2003.

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	KGL/DB/SUP
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
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26 MAY 2008	5	Re-numbered from SOP 3.7 (version 4) to SOP 3.19 (version 5), added mention on assistance by Project Manager, minor changes.	DB/JL/SI/WO

Methods Manual for Laboratory Quality Control Testing of Malaria RDTs

Chapter 4: MICROSCOPY

SOP 4.01 Malaria Microscopy: Blood film Preparation, Staining and Reading

PURPOSE

This Standard Operating Procedure (SOP) describes the process for preparing, staining and reading blood films for malaria microscopy performed as part of the preparation of QC samples for assessing malaria RDTs.

BACKGROUND

Conventional light microscopy is the established method for the laboratory confirmation of malaria. The careful examination by an expert microscopist of a well-prepared and well-stained blood film remains currently the accepted standard for detecting and identifying malaria parasites. In most settings the procedure consists of collecting a finger-prick blood sample; preparing a thin and thick blood smear; staining the smear (most frequently with Giemsa) and examining the smear through a microscope (with 100x oil immersion objective) for the presence of malaria parasites.

The feathery edge of the thin film consists of a single layer of red cells and is used to assist in the identification of the malaria species, after the parasites have been seen in the thick film. The thick film is made up of large numbers of dehaemoglobinised red blood cells. Any parasites present are concentrated in a smaller area than in the thin film and are more quickly seen under the microscope.

When parasites are found, they can be characterised in terms of their species (*P. falciparum*, *P. vivax*, *P. ovale*, and/or *P. malariae*) and the circulating stage (e.g. trophozoites, schizonts, and gametocytes). In addition, the parasite density can be quantified (usually from the ratio of parasites per number of leukocytes).

Good preparation and staining of the film are vital for high quality microscopy.

SCOPE

This procedure is part of the methods for malaria microscopy described in the “Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests”. The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

PROCEDURE

Preliminary note:

The purpose of this SOP is to provide more technical background and detail for staff having to prepare, stain and read blood films during the process of QC sample preparation.

The staff can also refer to the WHO Manual of Basic malaria microscopy, Part 1, Learner’s Guide (see reference).

The actual procedures for the different working steps of QC sample preparation, with references to sample labeling, recording results, documents etc. are described in Chapter 3 of this Methods Manual, and must be studied by the relevant staff working in the field recruitment site (Chapter 3, Part 2) and the laboratory (Chapter 3, Part 3).

A. Blood film preparation

Reagents and equipments:

Cleaned and wrapped slides

Sterile lancets

Vacutainer or 5- or 10-mL syringes (21/23 gauge needles)

Tourniquet

Sharps container

Alcohol and water

Absorbent cotton wool

Slide box (or a cover to protect slides)

Micro-pore tape (or bandaids)

Lint free clean cotton cloth

Pencil

i. Preparation of blood films with finger-prick blood

Universal precautions for handling and disposal of human blood should be followed (see SOP 6.01).

1. With the patient's hand, palm upwards, select the third or middle finger (the big toe can be used for infants). The thumb should not be used. With a pledge of cotton wool lightly soaked in alcohol, clean the finger, using firm strokes to remove dirt and grease from the ball of the finger. With the clean cotton towel dry the finger, using firm strokes to stimulate blood circulation.
2. With a sterile lancet puncture the ball of the finger using a quick rolling action. By applying gentle pressure to the finger express the first drop of blood and wipe it away with a dry pledget of cotton wool. Dispose of the dirty lancet and cotton wool in a sharp's container. Make sure no strands of cotton remain on the finger to contaminate blood.
3. Working quickly and holding a clean slide by the edges, collect the blood as follows: apply gentle pressure to the finger and collect a single small drop of blood (about 2 mm in diameter) on to the middle of the slide. This is for the thin film. Apply further pressure to express more blood and collect two or three large drops, about 2 mm in diameter, on to the slide about 1 cm from the drop intended for the thick film. Wipe the remaining blood away from the finger with a pledget of cotton wool. Dispose the dirty cotton wool in a sharp's container.
4. Thick film: When making a thick film always handle the slides by the edges or by a corner. Using the corner of the spreader, quickly join the drops of blood and spread them to make an even, thick film. The blood should not be excessively stirred but can

be spread in a circular or rectangular form with 3 to 6 movements. The circular film should be about 1.2 cm in diameter.

5. Thin film: Using another clean slide as a "spreader", touch the small drop with the spreader and allow the blood to run along its edge. Firmly push the spreader along the slide, keeping at an angle of 45 degrees. Make sure that the spreader is in even contact with the surface of the slide at all times the blood is being prepared.
6. Label the dry thin film with the soft lead pencil by writing across the thicker portion of the film the patient number and date (dd/mm/yy). Do not use ball pen for labelling the slide. Allow the thick film to dry in a flat, level position protected from flies, dust, and extreme heat.
7. Transport and store slides in appropriate boxes, avoiding direct contact between slides. For long-term storage (several months), store in a dry area or in tight-closed bags / boxes with dessicant.

ii. Preparation of blood films with fresh venous blood

Universal precautions for handling and disposal of human blood should be followed (see SOP 6.02).

1. Apply a venous tourniquet, clean skin with alcohol swab, and proceed to venepuncture according to standard protocols (vacutainer or "butterfly" needles are preferable, rather than syringes).
2. After collecting venous blood for other purposes in EDTA / plain tubes, small drops of fresh venous blood can be transferred from the blood collection device or from the plain tube (before coagulation) to the slides by using an applicator stick, capillary tube or a micropipette. Alternatively, blood drops can be allowed to fall directly from the blood collection device on to the slides.
3. Prepare the thick and thin film as described above.

B. Preparation of reagents for Giemsa staining

1. Preparation of Giemsa stain (if not using commercially available Giemsa stain)

Stain formula

Giemsa Powder	3.8 g
Methanol	250 mL
Glycerol	250 mL
Glass beads	50
Large dark bottle	

Preparation

1. An amber bottle is preferred but if one is not available use a chemically dry, clear, hard glass or polyethylene bottle of suitable size. You will need about 50 solid glass beads of about 5 mm in diameter.

2. Put the glass beads in the bottle, pour in the measured amount of methanol and add the stain powder.
3. Tightly stopper the bottle. Allow the stain powder to sink slowly through the methanol until it settles to the bottom. Shake the bottle with a circular motion for 2-3 minutes.
4. Add the measured amount of glycerol and repeat the shaking process. Continue to shake for 2-3 minutes at half-hourly intervals at least six times.
5. Leave the bottle unused for 2-3 days; shaking it 3-4 times each day until the stain is thoroughly mixed. Keep a small amount of this stock solution in a small bottle for routine use to avoid contamination of stock solution.
6. Each newly prepared batch of stain should be properly labelled, including date of preparation, and should be tested for optimal dilution and staining time. Always keep the bottle tightly stoppered, in a cool place, away from direct sunlight. Clear glass stock bottles can be covered with a thick dark paper jacket to keep out the light.

2. Preparation of Buffered Water (pH 7.2)

Reagents

Potassium dihydrogen phosphate (anhydrous) (KH_2PO_4), 0.7g

Sodium phosphate dibasic (anhydrous) (Na_2HPO_4), 1.0 g

Distilled or de-ionised water, 1000 mL

Two wooden spatulas

One beaker, capacity 250 mL

One conical flask, capacity 1000 mL

Two filter papers, 11 cm in diameter

Analytical balance

Preparation

1. Measure the 0.7 g potassium dihydrogen phosphate (KH_2PO_4) and 1.0 g of sodium phosphate dibasic anhydrous (Na_2HPO_4) using an analytical balance and filter paper. See Operation of Analytical Balance (see SOP 6.06) for instructions on how to use an analytical balance.
2. Add the 0.7 g potassium dihydrogen phosphate (KH_2PO_4) to the beaker and add about 150 mL of water. Stir with the spatula until the salt is dissolved.
3. Add the 1.0 g of sodium phosphate dibasic anhydrous (Na_2HPO_4) to the beaker and stir with the spatula until the salt is dissolved.
4. When the salt is dissolved, add the fluid from the beaker to the conical flask until it is made up to 1L.
5. Note: The pH of the buffered water must be checked weekly using a pH meter (see SOP 6.09). To alter the pH you will need to add small quantities of one of the correcting fluids, 2% Na_2HPO_4 if the pH is below 7.2 (too acid) or 2% KH_2PO_4 if the pH is above 7.2 (too alkaline).

3. Preparation of the 2% correcting fluids

Reagents and equipment

Analytical balance

Two filter papers 11 cm in diameter

Two glass-stoppered bottles (amber coloured)

Potassium dihydrogen phosphate (anhydrous) (KH_2PO_4), 2 g

Sodium phosphate dibasic (anhydrous) (Na_2HPO_4), 2 g

Distilled or de-ionised water, 200 mL

Two wooden spatulas

Two beakers, capacity 250 mL

One measuring cylinder, capacity 100 mL

Labels

Preparation

1. Weigh 2 g of disodium hydrogen phosphate and add it to 100 mL of water in the beaker; stir with the wooden spatula until the salt has dissolved.
2. Pour the solution into one of the glass bottles and label the bottle “2% disodium hydrogen phosphate”.
3. Repeat steps 1 and 2 above, this time weighing out 2 g of potassium dihydrogen phosphate. Pour the solution into the second glass bottle and label it correctly.
4. Note: When not being used, the bottles should be stored in a cool place, away from sunlight.

C. Giemsa staining

Reagents and equipment

Giemsa stain

Methanol

Absorbent cotton wool

Test tubes, capacity 5 mL

Buffered water (pH 7.2)

Pasteur pipette

Curved plastic staining tray or plate

Slide-draining rack

Timing clock

Rapid Method (suitable for individual slides)

1. Allow the thick film to dry thoroughly; if really rapid results are required, drying may be hastened by fanning or briefly exposing the slides to gentle heat such as from the microscope lamp or hot air from a hair dryer. Care should be taken to avoid overheating; otherwise, the thick film will be heat-fixed.
2. Fix the thin film by dipping it a container of methanol for a few seconds. To permit dehaemoglobinisation, the thick film should not be fixed; therefore, avoid methanol or methanol vapour touching the film.
3. Prepare a 10% Giemsa solution in buffered water (pH 7.2); if a small quantity is being used, 3 drops of stain to each millilitre or buffered water will give the correct concentration of Giemsa solution. One slide requires about 3 mL of made-up stain.
4. Gently pour the stain on the slide; a pipette can be used for this purpose. Alternatively, slides can be placed face down on a concave staining plate and the stain introduced underneath the slide.
5. Stain for 5-10 minutes.
6. Gently flush the stain off the slide by adding drops of clean water. DO NOT tip off the stain and wash, as this will leave a deposit of scum over the smear.
7. Place the slide in the rack, film side downwards, to drain and dry, making sure that the film does not touch the slide rack.

Regular technique (suitable for 20 slides or more)

1. Fix each film by dipping it in a container of methanol for a few seconds. With prolong fixation it may be difficult to demonstrate Schuffner's dots and Maurer's spots. To permit dehaemoglobinisation, the thick film should not be fixed; therefore, avoid methanol or methanol vapour touching the film
2. Place the slides back-to-back in a straining trough.
3. Prepare a 3% Giemsa solution in pH 7.2 buffered distilled or de-ionised water in sufficient quantity to fill the number of troughs being used. Mix the stain well.
4. Pour the stain gently into the trough until the slides are totally covered.
5. Allow to stain for 30-45 minutes out of the sunlight.
6. Pour clean water gently into the trough to float off the iridescent scum on the surface of the stain. Alternatively, gently immerse the whole trough in a vessel filled with clean water.
7. Gently pour off the remaining stain and rinse again in clean water for a few seconds.
8. Remove the slides one by one and place them in a rack to drain and dry, film side downward, making sure that the film does not touch the slide rack.

D. Quality Control

This paragraph is only an outline of recommended procedures for internal and external quality assurance of malaria microscopy. Refer to appropriate QA Manuals (e.g. WHO Basic Malaria Microscopy Training Manual) for detail.

i. Internal Quality Control of blood films:

The quality of each prepared slide is assessed at the time of microscopic examination. Where possible, any slide that is inadequately spread should be prepared again until a slide of an acceptable standard is produced.

1. Evaluation of a well-stained thin film

   The film should be checked macroscopically for correct length, colour and thickness.

   The film should also be examined microscopically (after staining):

   - A film of ideal thickness will display some overlap of red cells throughout much of the film length, but separation and lack of distortion towards the tail of the film. The white cells should be easily recognizable throughout the length of the film.
   - The background should be clean and free from debris, the colour of the erythrocytes is pale green pink.
   - Neutrophils have deep purplish red and well-defines granules.
   - The chromatin of malaria parasites is a deep purplish red and cytoplasm a clear purplish blue.
   - Stippling shows us as Schuffner's dots in erythrocytes containing *P. vivax* and *P. ovale* and Maurer's spots in erythrocytes containing the larger ring forms of P. falciparum.

2. Evaluation of a well-stained thin film

   - The thick film should be examined microscopically (after staining) for correct thickness. An ideal thickness will allow enough white cells to be present but parasites can still be easily seen.
   - The background should be clean and free from debris, with a pale-mottles-grey colour derived from the lysed erythrocytes.
   - Leukocytes nuclei are a deep, rich purple.
   - Malaria parasites are well defined with deep-red chromatin and pale purplish-blue cytoplasm. In *Plasmodium vivax* and *P. ovale* infections the presence of Schuffner's stippling in the "ghost" of the host erythrocyte can be seen easily, especially at the edge of the film.

3. Internal quality control of Giemsa stain:

   The quality of Giemsa stain should be evaluated:

   - Each time that a fresh Giemsa stock solution is prepared,
   - At an appropriate frequency for routinely used Giemsa stain. This frequency should be fixed according to frequency of malaria slide staining in the lab (ideally: at least weekly).

   Refer to standard QA procedures for detailed protocols of Giemsa stain IQC.

ii. <u>External Quality Control:</u>

1. A proportion of malaria blood films should be reviewed blindly by an external institution experienced in malaria microscopy. This should include reviewing of parasite species identification and parasite counts. The frequency of this EQC should be fixed according to frequency of malaria blood film reading in the lab (ideally: at least annually).

2. The external QA Institution can be a National Reference Laboratory for Malaria Microscopy, a recognized QA company or laboratory located in another country, or a laboratory of the RDT-QA network recommended by the Project Manager.
3. The EQC results should be recorded in a dedicated folder.
4. If results are not satisfying, refresher trainings for malaria microscopists should be organized as soon as possible.

iii. Training of malaria microscopists:

1. Microscopists should regularly participate in training workshops to improve their skills (recommended frequency: at least every three years).
2. Prior to preparation of QC samples for malaria RDT QA, it is necessary to pre-qualify two microscopists which will be responsible for performing the precise parasite counts for dilution calculations, except if they have recently participated in such a workshop (see SOP 6.05).
3. All training should be recorded in the appropriate documents, according to SOP 6.03.

iv. Maintenance of the microscope: (refer to SOP 6.04).

E. Blood film reading

Equipment:

Microscope with x100 oil immersion objective

Tally counter

Immersion Oil

i. Thin film examination

Routine examination of thin film is not used as it gives a different nparasite densitiy to quantiation on the thick film:

ii. Thick film examination for *Plasmodium* species identification

Routine examination of a thick film is based on examination of 100 good fields. That is, a slide can be pronounced negative only after no parasites have been found in 100 fields of the blood film. If parasites are found, a further 100 fields should be examined before a final identification of species is made. This ensures that there is little possibility of a mixed infection (more than one species present in the blood film) being overlooked.

The technique for thick film examination is as follows:

1. Using the x40 objective, scan the film for any microfilariae that may be present. At the same time, select a part of the film that is well stained, free of staining debris, and well populated with white blood cells. If the film is well made or even thickness, this should present no problems; poorer quality films may need to be quite extensively searched.
2. Place the immersion oil on the thick film.

3. Swivel the x100 oil immersion objective over the selected portion of the blood film.
4. Confirm that the portion of the film selected is acceptable and continue to examine the slide for 100 immersion fields. Move the blood film by one oil immersion field each time, following the pattern described for the thin film.
5. Use a tally counter to count the fields as they are examined.

iii. Thick film examination for parasite counts

ROUTINE malaria parasite counts are performed and are expressed as parasites seen per microlitre of blood (n/µL). For the preparation of quality control samples, accurate malaria counts are crucial as the initial malaria parasite count of the blood is used to calculate and prepare the blood dilutions.

Parasite count per 500 white cells

1. The number of parasites relative to the 500 first white cells is counted.
2. If more than 150 parasites are counted for the first 200 white cells, then calculate parasite count by 200 white cells instead.
3. Count all the species present and record separately the gametocytes of *P. falciparum* and the asexual parasites.
4. White cell counts (number of leukocytes per µL of blood) will be determined by a haematology analyser and used in the parasite density calculation below:
5. (Number of counted parasites, divided by number of counted leukocytes) x (number of leukocytes per µL of blood, determined by haematology analysis) = number of parasites per µL of blood
6. Malaria smears are to be read by two microscopists, who are both blinded to RDT result and the other reader's result.
7. The two microscopists should previously have been pre-qualified according to SOP 6.04.
8. The discrepancy between the two parasite counts will be calculated as follows:
9. ("Difference between the two counts", divided by "mean of the two counts") x 100.
10. If there is a discrepancy of 20% or less, then the mean of the two parasite counts will be used for calculation and preparation of blood dilutions.
11. If the discrepancy is greater than 20%, then the blood films should be read again by the two microscopists who should still be blinded.
12. If the discrepancy between the two repeated parasite counts is 20% or less, then the mean of the the two repeated parasite counts is used for calculation and preparation of blood dilutions.
13. If the discrepancy between the two repeated parasite counts is again greater than 20%, then the sample will NOT be used to prepare dilutions.

F. Results reporting

Results are always expressed as the stage/s of malaria parasite seen, the species seen, and the number seen per microlitre of blood.

Asexual (rings, trophozoites and schizonts) and sexual (gametocytes) are reported separately.

Example:

Mixed infection (*P. falciparum* and *P. vivax*)

Asexual (rings) of *P. falciparum*, count= 10000/ µL

Sexual (gametocytes) of *P. falciparum* seen, count=1000/ µL

Asexual (rings, trophozoites and schizonts) of *P. vivax*, count=2,000/ µL

Sexual (gametocytes) of *P. vivax* seen, count=1000/ µL

REFERENCES

1. Basic malaria microscopy. Part 1. Learner's Guide. Geneva, World Health Organization, 1991 (unpublished document LF.Q.AZ.1991pt.1)
2. Peripheral Blood Film Preparation and Staining Standard Operating Procedure. Brisbane, Australian Army Malaria Institute, 2,000 (unpublished report).
3. Malaria Screening Standard Operating Procedure. Brisbane, Australian Army Malaria Institute, 2,000 (unpublished report).
4. New perspectives: Malaria diagnosis. Geneva, World Health Organization, 2,000 (unpublished document WHO/MAL/2,000.1091).
5. Basic Malaria Microscopy, Part 1, Learner’s Guide, WHO, Geneva, 1991

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	2	Routine review: Earl Perez methods added	RG/KGL/DB
15 OCTOBER 2004	2	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	2	Routine Revision, minor changes only	RG
AUGUST 2006	2	Minor changes only	DB
26 MAY 2008	5	Re-numbered from SOP 4.2 (version 4) to SOP 4.01 (version 5). Moved “Earl-Perez” procedures to a different SOP 4.2, more detail on slide storage, revised venous blood collection, other minor changes	DB/JL/PJ/SI/WO/CS

SOP 4.02 Malaria Microscopy: Preparation and Reading of Earl-Perez slides

PURPOSE

This Standard Operating Procedure (SOP) describes the process for preparing and reading blood films according to the Earle-Perez method, to determine the parasite density of freshly prepared QC sample dilutions.

BACKGROUND

An Earle-Perez film allows more accurate assessment of parasite density after the dilution process, as white cells will also be heavily diluted. One Earle-Perez film will be included with the samples sent to the global specimen bank, for future cross-checking if required.

SCOPE

This procedure is part of the methods for malaria microscopy described in the Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests. The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

PROCEDURE

A. Blood film preparation

Reagents and equipments:

Cleaned and wrapped slides

Slide box (or a cover to protect slides)

Pencil

Silver tipped pen

Cleaned and wrapped engraved Earl Perez slides

1-20 µL Pipette

Pipette tips (1-20 µL capacity)

Earl Perez slide Preparation:

1. Prepare slides by drawing a 6 x 15 mm rectangle on a slide (using a pencil) and engraving with a silver tipped pen.
2. Label the slide with date, QC sample ID and dilution, using the silver tipped pen.
3. Using a 1-20 µL capacity micropipette, transfer 5 µL of the freshly prepared QC sample dilution to the engraved 6 x 15 mm rectangle.
4. Using the pipette tip spread this blood evenly in the 6 x 15 mm rectangle.
5. Allow the thick film to dry in a flat, level position protected from flies, dust and extreme heat.

B. Giemsa staining

Stain the Earl-Perez slides with Giemsa, according to the standard protocol (see SOP 4.01).

C. Storage and transport of blood films

1. Store the slides in an appropriate box, avoiding any contact between slides, at room temperature (20-30°C) and in dry storage conditions (if needed, store in a tightly closed box or plastic envelopes, with dessicant).
2. If slides are to be shipped, shipment can be done at room temperature (20-30°C), in dry conditions (e.g. in box or plastic envelopes with dessicant), or together with the shipment of frozen QC sample aliquots (i.e. with dry ice). For shipment arrangements and organization, refer to SOP 3.08, paragraph H, as well as SOP 3.16 to 3.19.

D. Earl-Perez slide examination

This examination will be done at the Centers for Disease Control, Atlanta, USA.

Equipment:

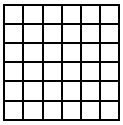
Microscope

Immersion oil

Tally counter

Grid for the ocular of the microscope

Calibration

a) Thick film = 5µL blood in rectangle measuring 6 mm x 15 mm area = 90 mm2
b) For thick film, volume of blood per mm2 = 5 µL/90 mm = 0.055µL/ mm2
c) Counting reticule (6 x 6) in ocular
d) 
Measure grid with stage micrometer. If width=0.1 mm, then grid area=0.01 mm2
e) Thick film is 6 mm wide, or 60 grids wide (6 mm/0.1 mm)
f) Volume of blood under 1 grid = 0.055 µL/ mm2 x 0.01 mm2 = 5.55 x 10-4 µL
g) Volume of blood per band = 5.55 x 10-4 x 60 = 0.033 µL/band
h) Number of parasites in 1µL of blood = 1/0.033 = 30.3

1 parasite per band = 30.3 parasites per µL blood

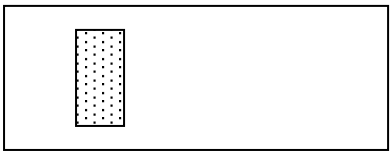
1 parasite per 2 bands = 15.15 parasites per µL blood

The table below is a simplified version of how to calculate parasites/ µL using one band.

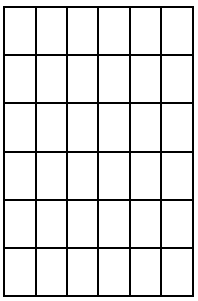
Width	0.1 mm	0.1
Area	0.1 x 0.1	0.01
Number of grids in width	6 mm/0.1	60
Volume of blood under 1 grid	0.055 µL/mm^2 x 0.01	0.00055
Volume of blood per band	0.000285 x 83.3333	0.033
Number of parasites in 1µL blood	1/0.033	30.30303

Counting

a) Parasites are counted (using an oil immersion lens) on a rectangular thick film that measures 6 x 15 mm and contains 5 µL of blood spread evenly within the rectangle.



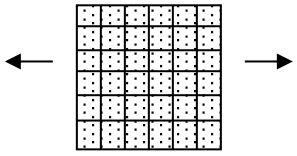
b) The grid in the ocular of the microscope is a square divided into 36 smaller squares (6 x 6), and a calibration factor has been calculated by using an ocular micrometer to measure the actual size of the grid.



c) By counting all the parasites under the grid, while moving horizontally across the width of the thick film (right to left, or left to right), a parasite count per microliter of blood can be calculated by using a chart or by multiplying the number of parasites counted in one band by the factor determined from the calibration of the grid. The factor for the parasite count in one band using the grid in a typical Nikon or Olympus microscope is ~30 at 100 x oil immersion.

d) For high parasite counts (≥6000 µL) count only 1 band (this equates to about 200 parasites in one band)

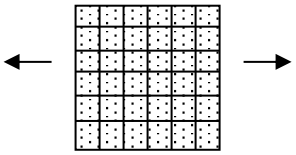
Parasites counted in 1 band (across the width of the thick film) X 30.3 = no. of parasites/ µL



1 band

e) For low parasite counts (<6000) count 2 bands (this equates to less than 200 parasites in one band).

Parasites counted in 1 band (across the width of the thick film) X 15.15 = no. of parasites/ µL



2 bands

E. Reporting of results

Results should be recorded on Form 3.11.

REFERENCES

Earle, W.S. and Perez, M. (1932). Enumeration of parasites in the blood of malarial patients. J. Lab. & Clin. Med. 17:1124.

PROCEDURE HISTORY

Date	Version	Comments	Initials
28 MAY 2008	5	SOP introduced, Adapted from former SOP 4.1 (version 4), Made specific for QC sample dilutions only, mentioned storage and shipment conditions, then examination and results records at CDC.	DB/JL/PJ/SI/WO

Methods Manual for Laboratory Quality Control Testing of Malaria RDTs

Chapter 5: SAMPLE CHARACTERIZATION

FORMS FOR CHAPTER 5:

5.01: ELISA Dilution Form

5.02: ELISA Reporting Form

SOP 5.01 Cellabs Pty HRP2 ELISA Kit Procedure

PURPOSE

This standard operating procedure (SOP) describes the materials, equipment, and procedures required to correctly and safely use the Cellabs malaria antigen HRP2 ELISA kit to diagnose malaria using blood samples. Protocol includes:

- Setting up dilutions of recombinant HRP2 antigen for a standard calibration curve
- Preparation of blood samples for use in the assay
- Running of the ELISA in a 96 well plate format and interpretation of results

SCOPE

This procedure is part of the methods for malaria antigen ELISA described in the Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests. It has been developed for the training of laboratory personnel using the Cellabs malaria antigen HRP2 ELISA kit for malaria diagnosis in clinical and research settings. For the WHO malaria RDT evaluation programme, this SOP describes the use of this assay in the assessment of HRP2 antigen content within patient blood samples that form part of the global specimen bank.

PROCEDURE

A. PRINCIPLE OF THE TEST

Cellabs malaria antigen ELISA is suitable for the detection, in a blood sample, of the antigen HRP2 solely expressed in Plasmodium falciparum. HRP2 contained within the test specimen is bound to wells of an anti-HRP2 plate by monoclonal antibodies directed against the HRP2 protein. Antibodies conjugated with horseradish peroxidase enzyme then bind the HRP2 antigen at a different epitope. Unbound material is removed with a wash step, a substrate solution of TMB is added to the wells and the reaction product is subsequently quenched using an acid stop solution. The colour intensity of the resulting product is directly proportional to the HRP2 concentration and is measured as ΔOD 450/620 nm.

B. ASSAY AND SPECIMEN REQUIREMENTS

NB: All reagents are allowed to equilibrate to room temperature (20-30°C) for 15 min before use.

Sarstedt tubes
Vortex
Anti-HRP2 coated test plate*
Recombinant PfHRP2
Enzyme conjugate 200x (MAPO)*
Conjugate diluent (MACD)*
Substrate chromagen 20x (TMB) (MASC)*
Substrate buffer (MASB)*
Stopping solution (MASS)*
1 X PBST
Plate lid

Micropipettes (50 – 200 µl and 100-1000 µl)
Multichannel micropipette (50 – 250 µl)
Automated plate washer
37 °C incubator
Spectrophotometer

*Contained within Cellabs HRP2 kit boxes.

C. INSTRUCTIONS FOR PERFORMING THE ASSAY

1. Preparation of standards and test samples

Eight standards are used as a reference positive and prepared in serial dilution for this assay. Human blood that has been frozen and thawed is used as a diluent for the standards and as a negative control. A purified recombinant form of HRP2 is used to produce the standards that are diluted in human blood, see Table 1. Human blood is used to dilute the recombinant and is pipetted into sarstedt tubes. Stock antigen should then be added to the first tube at an appropriate dilution to provide a starting concentration of 20 ng/ml. Doubling dilutions made thereafter form the 8 reference points that will generate the standard curve. Between transfers from one tube to another, blood containing recombinant HRP2 should be pipetted up and down several times then a vortex used to mix each tube. A fresh pipette tip should be used between each transfer.

Depending on the concentration of HRP2 added to the specimen being tested, dilution may be appropriate in order for the test samples to fit within range of the calibration curve. If necessary, this should be done using human blood and a conversion factor applied during data analysis.

• Table 5-1. Recombinant PfHRP2 (x ng/ml)* varies depending on aliquots being used

Conc required (ng/ml)	20	10	5	2.5	1.25	0.625	0.313	0.156
Working stock (ng/ml)	x	20	10	5	2.5	1.25	0.625	0.313
Volume stock (µl)	x	500	500	500	500	500	500	500
Volume diluent (µl)	x	500	500	500	500	500	500	500
Total volume (µl)	1000	1000	1000	1000	1000	1000	1000	1000

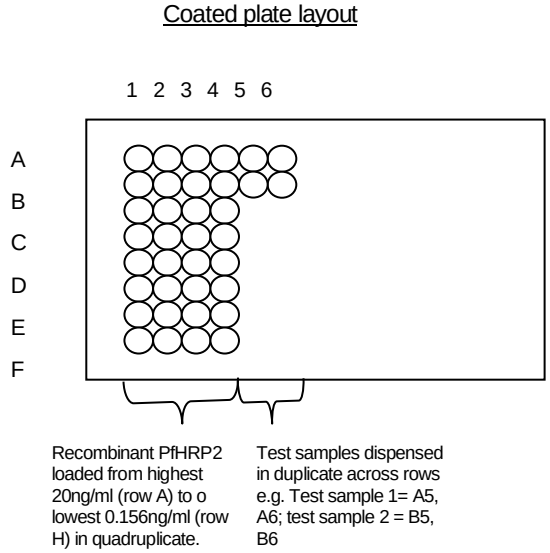
2. Preparation of the coated plate

With a standard micropipette, 100 µl of each of the 8 pre-prepared standards (20-0.156 ng/ml) should be added to the wells of column one to four (A-H) in parallel.

Test samples should then be added to each of the wells consecutively from A5 as far as G12. Each test sample should be duplicated in the adjacent row i.e. test sample 1 will be dispensed into wells A5 and A6. H11 and H12 should contain 100 µl of human blood used as the negative control (see figure

5-1). For one plate, 31 specimens can be tested. When all samples have been added to the coated plate, cover the plate with a plastic lid and leave the plate for 1 hour min at 37ºC.

- Figure 5-1. Loading format for coated plates



3. Wash steps and preparation of enzyme conjugate

The wash solution contained within the assay kit is 10x PBST. A 1 Litre stock of 1 X PBST should be made up and used to wash the wells of the coated plate 3x with an automatic plate washer set to fill the wells with 350 µl solution.

Working strength enzyme conjugate should be made up fresh. Per plate, 55 µl enzyme conjugate 200x should be diluted in 11 ml conjugate diluent and mixed thoroughly. Using a multichannel pipette, 100 µl working strength enzyme conjugate should be dispensed to all test wells. The plate should then be covered with a plastic lid and incubated for 1 hour at 37 ºC.

4. Wash steps and development of substrate

Working strength substrate should be made up fresh. Per plate, 550 µl substrate chromagen should be diluted in 11 ml substrate buffer. Using a multichannel pipette, 100 µl of working substrate should then be dispensed into all test wells. The plate should then be covered with a plastic lid and incubated at room temperature (20-30°C) for 15 mins in the dark.

The assay should then be quenched by dispensing 50µl of stopping solution into all test wells. The endpoint absorbance of the wells should be read at 450 nm with a reference wavelength 620 nm.

5. Interpretation of results

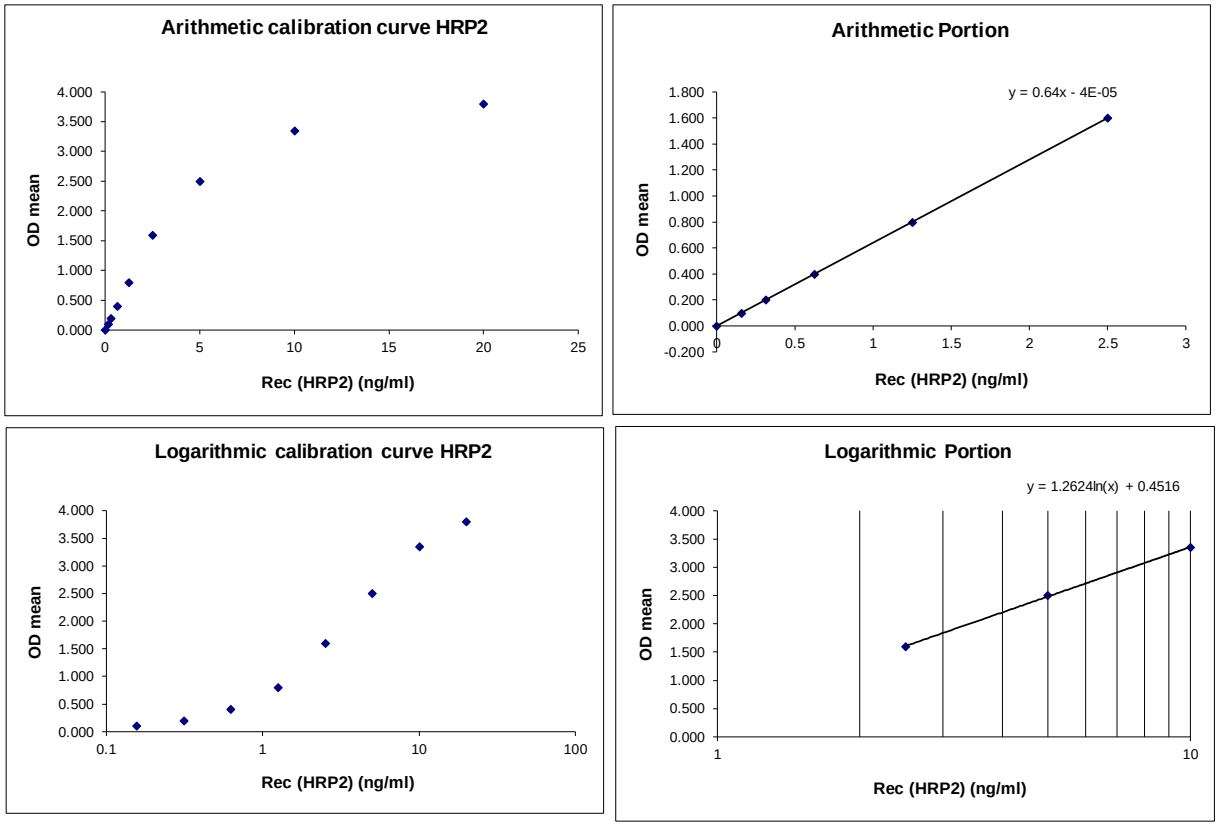
The spectrophotometer will make a print out of ΔOD 450/620 nm results and these should then be transcribed to another PC. Each point comprising the standard curve has been replicated 4 times therefore a mean OD for each point should be calculated in EXCEL. The specimens are in duplicate and also need to be averaged. Mean ODs for the standards, specimens and negative control should all be entered into EXCEL to make a scatter graph (calibration curve). Both arithmetic and logarithmic curves are plotted.

The arithmetic calibration curve will be parabolic in shape. The logarithmic calibration curve should be sigmoidal in shape. ODs that are > 20 ng/ml standard on the logarithmic curve will begin to plateau on the curve. Test samples with ODs higher than the 20 ng/ml standard should not be interpreted because the assay begins to saturate with HRP2 antigen at this point. The negative specimen should have an OD < 0.100.

Separate plots for selected points from the logarithmic and arithmetic curves are used for fitting trend-lines and obtaining equations which are used to calculate concentrations from OD for each test sample. The calculation uses a re-arrangement of the algebraic expression,

y = mx + c. ODs are converted to HRP2 concentration in ng/ml. The result on a test specimen is adjusted for any pre-dilution of the specimen.

• Figure 5-2. Generation of logarithmic and arithmetic trend lines for data interpretation



D. HEALTH AND SAFETY

1. Hazardous reagents

• Table 5-2. Hazardous chemicals used in the Cellabs malaria antigen HRP2 ELISA

Product	Fire hazard	Health hazard	Toxicity	Storage requirements
Hydrogen peroxide (TMB)	Explosive under heat	Irritant to eyes/skin/nasal passage	Moderately toxic	Easily decomposes 2-8 ^{0}C
Hydrochloric acid (stop solution)	Flammable	Irritating to eyes/skin. Burns. Harmful by ingestion.	Toxic	Keep in a locked store
TMB	Flammable	Harmful swallowed/inhaled/ absorbed by skin	Toxic	Store solutions in light proof container at 4 -8 °C

2. Safety precautions

Recombinant *Plasmodium falciparum* HRP2 used as a standard has been shown to be non-infectious in a recombinant expression system.

Disposable latex or nitrile gloves must be worn while handling clinical specimens and reagents. All clinical material i.e. all components containing blood must be autoclaved before disposal. The assay stop solution contains hydrochloric acid a corrosive and hazardous substance. Avoid eye and skin contact by wearing protective clothing and eye protection.

Hands must be washed once work has been completed.

3. Technical precautions

- Components must not be used after their expiry date.
- Different batches/lots of reagents should never be interchanged.
- Storage of reagents must be at the recommended conditions.
- Contamination of reagents should be avoided by changing pipette tips where necessary.

PROCEDURE HISTORY

Date	Version	Comments	Initials
15 OCTOBER 2004	D	Draft Prepared	KB/PC
14 OCTOBER 2005	1	Version 1 introduced	DB
29 MAY 2008	5	Changed the ELISA method (replaced NBl hrp2 ELISA by SD Hrp2 ELISA)	DB/JL/PJ/SI/WO/CS
MAY 2014	7	Changed the ELISA method (replaced SD HRP2 ELISA by Cellabs Pty HRP2 ELISA) and updated the procedure, based on updates for the Product Testing Manual version 5	RRC, SI
FEBRUARY 2020	9	Formatting changes, renamed figures 1 and 2 to figure 5-1 and figure 5-2 respectively. Renamed tables 1 and 2 to table 5-1 and table 5-2 respectively	JL, CAL

SOP 5.02 SD pLDH ELISA Kit Procedure

PURPOSE

This SOP describes the materials, equipment, and procedures required to correctly and safely use the SD malaria antigen pLDH ELISA kit to diagnose malaria using blood samples. Protocol includes:

- Setting up dilutions of recombinant pLDH antigen for a standard calibration curve
- Preparation of blood samples for use in the assay
- Running of the ELISA in a 96 well plate format and interpretation of results

SCOPE

This procedure is part of the methods for malaria antigen ELISA described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". It has been developed for the training of laboratory personnel using the SD malaria antigen pLDH ELISA kit for malaria diagnosis in clinical and research settings. For the WHO malaria RDT evaluation programme, this SOP describes the use of this assay in the assessment of pLDH antigen content within patient blood samples that form part of the global specimen bank.

PROCEDURE

A. PRINCIPLE OF THE TEST

SD malaria antigen ELISA is suitable for the detection, in a blood sample, of the four species of malaria infecting humans. After whole blood is lysed, pLDH in the blood specimen is bound to antibodies conjugated with horseradish peroxidase enzyme. Subsequent transfer to the test plate allows this complex to bind well of the plate by means of monoclonal antibodies directed against pLDH. Unbound material is then removed with a wash step, a substrate solution of TMB is added to the wells and the reaction product is subsequently quenched using an acid stop solution. The colour intensity of the resulting product is directly proportional to the pLDH concentration and is measured as ΔOD 450/620 nm

B. ASSAY AND SPECIMEN REQUIREMENTS

NB: All reagents are allowed to equilibrate to room temperature (20-30°C) for 15 min before use.

Sarstedt tubes

Vortex

Preparation microtitre plate (uncoated)

Anti-pLDH coated test plate*

Recombinant pLDH

Lysis buffer*

Enzyme conjugate*

TMB substrate solution*

Stop solution*

1 X PBST

Plate lid

Micropipettes (50 – 200 µl and 100-1000 µl)

Multichannel micropipette (50 – 250 µl)

Automated plate washer

37 °C incubator

Spectrophotometer

*Contained within individual SD pLDH kit boxes.

C. INSTRUCTIONS FOR PERFORMING THE ASSAY

1. Preparation of standards and test samples

Eight standards are used as a reference positive and prepared in serial dilution for this assay. Human blood that has been frozen and thawed is used as a diluent for the standards and as a negative control. A purified recombinant form of pLDH is used to produce the standards that are diluted in human blood, see Table 5-3. Blood used to dilute the recombinant is pipetted into sarstedt tubes. Stock antigen is added to the first tube at an appropriate dilution to provide a starting concentration of 500 ng/ml. Doubling dilutions made thereafter form the 8 reference points that will generate the standard curve. Between transfers from one tube to another, blood should be pipetted up and down several times then a vortex used to mix each tube. A fresh pipette tip should be used between each transfer.

Depending on the concentration of pLDH in the specimen being tested, dilution may be appropriate in order for the test samples to fit within range of the calibration curve. If necessary, this should be done using human blood and a conversion factor applied during data analysis.

• Table 5-3. Recombinant pLDH (x ng/ml)* varies depending on aliquots being used

Conc required (ng/ml)	500	250	125	62.5	31.25	15.62	7.8	3.9
Working stock (ng/ml)	x	500	250	125	62.5	31.25	15.6	7.8
Volume stock (µl)	x	150	150	150	150	150	150	150
Volume diluent (µl)	x	150	150	150	150	150	150	150
Total volume (µl)	1000	300	300	300	300	300	300	300

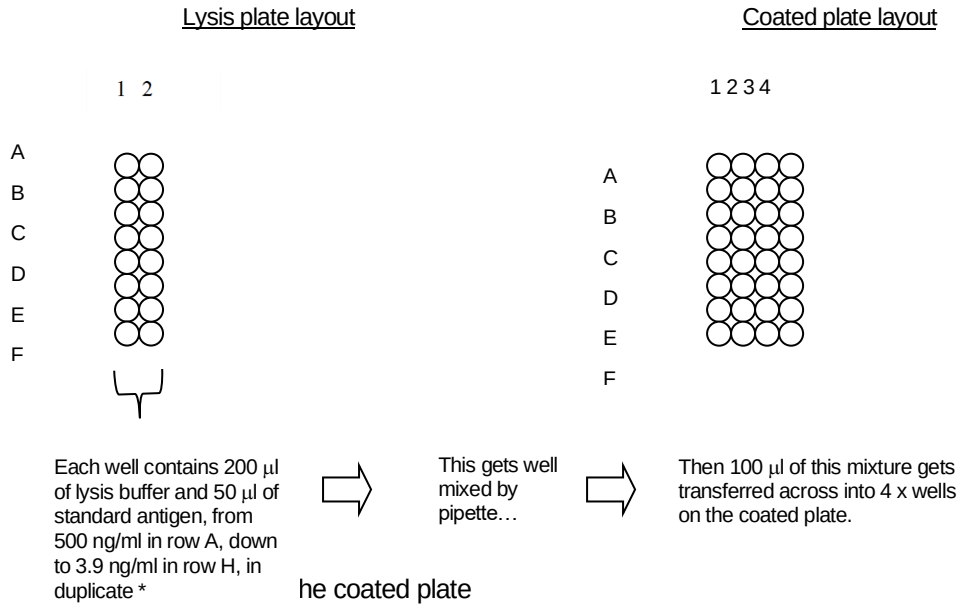
2. Preparation of the lysis plate

To prepare the working strength enzyme conjugate, the stock solution needs to be diluted to working strength i.e. 1 in 101. For a full 96 well plate, 11 ml should be prepared by adding 110 µl of the stock to 11 ml of lysis buffer.

Initially, 200 µl working strength enzyme conjugate should be dispensed into wells of the uncoated/lysis plate that will contain blood samples. 50 µl of each of the 8 pre-prepared standards (500-3.9 ng/ml) should then be added to the wells of column one (A-H) and two (A-H) in parallel.

The test samples should then be added to each of the wells consecutively from A3 as far as G6. H6 should contain 50 µl of human blood used as the negative control. For one plate, 31 specimens can be tested as all wells from the lysis plate will then be duplicated in the coated plate. When all samples have been added to the lysis plate, cover the plate with a plastic lid and leave the plate for 30 min at room temperature (20-30°C).

• Figure 5-3. Loading format for lysis and coated plates



3. he coated plate

With a multi-channel pipette, 100 µl should then be transferred from each well containing lysed blood to wells of an anti-pLDH coated test plate so that each column of wells is tested in duplicate filling the 96 well microtitre plate. The plate should then be covered with a lid and incubated at 37 °C for 90 min.

4. Wash steps and development of substrate

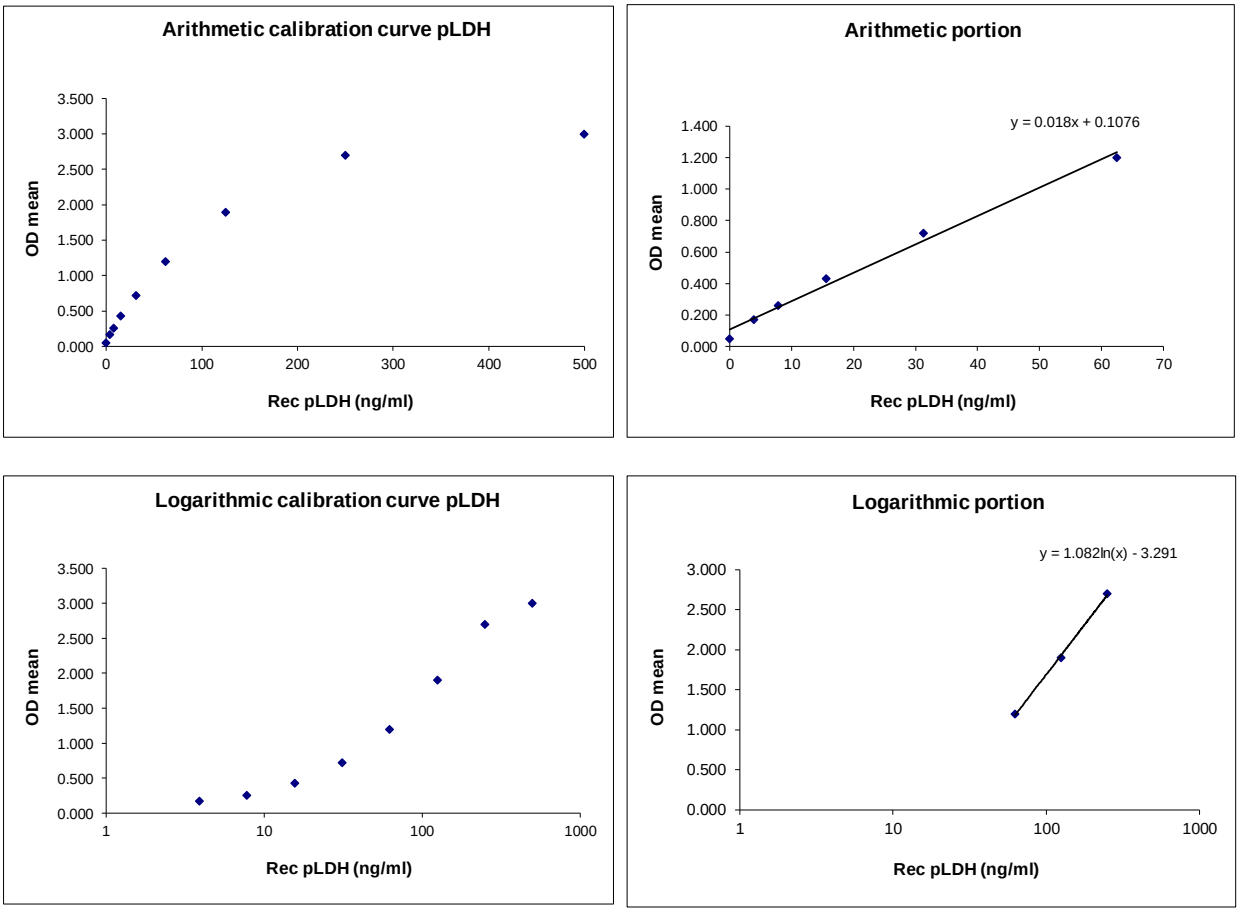
The wash solution contained within the assay kit is 10x PBST. A 1 Litre stock of 1 X PBST should be made up and used to wash the wells of the coated plate 3x with an automatic plate washer set to fill the wells with 350 µl solution. The TMB substrate contained within the kits is already at working strength. 100 µl of working substrate should be dispensed into each well using the multichannel pipette. The plate should then be covered with a plastic lid and incubated at room temperature (20-30°C) for 30 mins in the dark. The acid stop solution is contained within the assay kit and 100µl should be then dispensed in all wells. The endpoint absorbance of the wells should be read at 450 nm with a reference wavelength 620 nm.

5. Interpretation of results

The spectrophotometer will make a print out of ΔOD 450/620 nm results and these should then be transcribed to another PC. Each point comprising the standard curve has been replicated 4 times therefore a mean OD for each point should be calculated in EXCEL. The specimens are in duplicate and also need to be averaged. Mean ODs for the standards, specimens and negative control should all be entered into EXCEL to make a scatter graph (calibration curve). Both arithmetic and logarithmic curves are plotted. The arithmetic calibration curve will be parabolic in shape. The logarithmic calibration curve should be sigmoidal in shape. ODs that are > 500 ng/ml standard on the logarithmic curve will begin to plateau on the curve. Test samples with ODs higher than the 500 ng/ml standard should not be interpreted because the assay begins to saturate with pLDH antigen at this point. The negative specimen should have an OD < 0.100. Separate plots for selected points from the logarithmic and arithmetic curves are used for fitting trend-lines and obtaining equations which are used to calculate concentrations from OD for each test sample. The calculation uses a re-arrangement of the algebraic expression,

y = mx + c. ODs are converted to pLDH concentration in ng/ml. The result on a test specimen is adjusted for any pre-dilution of the specimen.

• Figure 5-4. Generation of logarithmic and arithmetic trend lines for data interpretation



1. Hazardous reagents

• Table 5-4. Hazardous chemicals used in the SD malaria antigen pLDH ELISA

Product	Fire hazard	Health hazard	Toxicity	Storage requirements
Hydrogen peroxide (TMB)	Explosive under heat	Irritant to eyes/skin/nasal passage	Moderately toxic	Easily decomposes 2-8 ^{0}C
Hydrochloric acid (stop solution)	Flammable	Irritating to eyes/skin. Burns. Harmful by ingestion.	Toxic	Keep in a locked store
TMB	Flammable	Harmful swallowed/inhaled/ absorbed by skin	Toxic	Store solutions in light proof container at 4 -8 °C

2. Safety precautions

Recombinant p LDH used as a standard has been shown to be non-infectious in a recombinant expression system.

Disposable latex or nitrile gloves must be worn while handling clinical specimens and reagents. All clinical material i.e. all components containing blood must be autoclaved before disposal. The assay stop solution contains hydrochloric acid a corrosive and hazardous substance. Avoid eye and skin contact by wearing protective clothing and eye protection.

Hands must be washed once work has been completed.

3. Technical precautions

- Components must not be used after their expiry date.
- Different batches/lots of reagents should never be interchanged.
- Storage of reagents must be at the recommended conditions.
- Contamination of reagents should be avoided by changing pipette tips where necessary.

PROCEDURE HISTORY

Date	Version	Comments	Initials
15 OCTOBER 2004	D	Draft Prepared	KB/PC
14 OCTOBER 2005	1	Version 1 introduced	DB
29 MAY 2008	5	Minor changes only	DB/JL/PJ/SI/WO
MAY 2014	7	Updated the procedure, based on updates for the Product Testing Manual version 5	RRC, SI
FEBRUARY 2020	9	Formatting changes, renamed figures 1 and 2 to figures 5-3 and 5-4 respectively. Renamed tables 1 and 2 to table 5-3 and table 5-4	JL, CAL

SOP 5.03 Biotinylation of Monoclonal Antibodies for Aldolase ELISA Procedure

PURPOSE

This Standard Operating Procedure (SOP) Describes procedures outlined in the EZ-Link® Sulfo-NHS-LC-Biotinylation Kit for biotinylation of monoclonal antibodies (MAb's) prior to Malaria Antigen Detection – Capture ELISA with anti-aldolase MAb.

BACKGROUND

Biotin is a small naturally occurring vitamin that binds with high affinity to avidin and streptavidin proteins. Because it is so small (244 Da), biotin can be conjugated easily to many proteins without altering their biological activities. The labeled protein or other molecule may then be detected easily in ELISA, dotblot or Western blot application using streptavidin or avidin probes. The following procedure usually yields incorporation of 8-12 biotins per molecule of IgG when labeling antibodies.

AIM

To improve sensitivity of Malaria Antigen Detection – Capture ELISA with anti-aldolase MAb

SCOPE

This procedure applies to the malaria RDT product testing programme of WHO and FIND with the US Centers for Disease Control and Prevention.

REAGENTS, SUPPLIES, AND EQUIPMENT

1. Reagents

a) Anti-aldolase Monoclonal Antibodies (National Bioproducts Institute)

Store at -20o C

- MAb M/B 7-20, 10 mg in PBS pH 7.2 [4.4 mg/ml]
- MAb C/D 11-4, 10 mg in PBS pH 7.2 [5.7 mg/ml]

b) Biotinylation Kit – Pierce catalog # 21430

- EZ-Link® Sulfo-NHS-LC-Biotin, 25 mg (Store at -20o C with desiccant.)
- HABA, 1 ml, 10 mM in 0.01 N NaOH (Store at 2-8o C)
- Avidin (Affinity Purified), 10 mg (Store at -20o C)

c) Ultrapure water (Mediatech Cellgro catalog # 25-055-CM)

d) PBS, 0.01 M, pH 7.2 (CDC BIOS catalog # CP0636)

2. Supplies

a) Screw-cap centrifuge tubes, 2 ml and15 ml polypropylene

b) Micropipet tips, 20 ul and 200 ul

c) Slide-A-Lyzer® Dialysis Cassette Kit, 10K MWCO, 0.5-3 ml – Pierce catalog #66382

d) Cuvettes for spectrophotometer

3. Equipment

a) Vortex Mixer

b) Spectrophotometer – Pharmacia Biotech Ultrospec 3000

c) Pipettors

d) Timer

PROCEDURE

1. General Safety

a) Wear gloves, lab coat, and safety glasses while handling all human or animal

b) blood products.

c) Dispose of all pipets, etc. into a covered pan; autoclave for 60 minutes.

d) Wipe work surfaces with disinfectant (e.g. 0.8% Vesphene).

2. Biotinylation Procedure

Calculations

By using the appropriate molar ratio of biotin to protein, the extent of labeling can be controlled. (With dilute protein solutions, a greater fold molar excess of biotin is necessary compared to more concentrated protein solutions.) Generally, use □12-fold molar excess of biotin for a 10 mg/ml protein solution or □ 20-fold molar excess of biotin for a 2 mg/ml protein solution.

Calculate amount of biotin to use

20 = molar fold excess of biotin for a 2 mg/ml protein sample.

$$\text{ml protein} \times \frac{\text{mg protein}}{\text{ml protein}} \times \frac{\text{mmol protein}}{\text{mg protein}} \times \frac{20 \text{ mmol biotin}}{\text{mmol protein}} = \text{mmol biotin}$$

Calulate the volume of 10 mM Sulfo-NHS-LC-Biotin (biotin) (prepared in step B.3.) to add to the reaction

557 = MW of Sulfo-NHS-LC-Biotin.

400 = # µl of water in which 2.2 mg of biotin is dissolved to make a 10 mM solution.

$$\text{mmol biotin} \times \frac{557\ \text{mg}}{\text{mmol biotin}} \times \frac{400\ \mu\text{l}}{2.2\ \text{mg}} = \mu\text{l biotin}$$

Example–for 1 ml of a [2.0 mg/ml] MAb (assume 150,000 MW) solution,

~27 µl of 10 mM biotin will be added.

$$1\ \text{ml MAb} \times \frac{2\ \text{mg MAb}}{1\ \text{ml MAb}} \times \frac{1\ \text{mmol MAb}}{150{,}000\ \text{mg MAb}} \times \frac{20\ \text{mmol biotin}}{1\ \text{mmol MAb}} = 0.000266\ \text{mmol biotin}$$

$$0.000266\ \text{mmol biotin} \times \frac{557\ \text{mg}}{\text{mmol biotin}} \times \frac{400\ \mu\text{l}}{2.2\ \text{mg}} = 26.9\ \mu\text{l biotin reagent}$$

Biotin labeling reaction

1. Remove vial of biotin from freezer and allow to come to room temperature (20-30°C) before opening in step 3.
2. Prepare [2.0 mg/ml] stock solutions of monoclonal antibodies.

- M/B 7-20, 10 mg in PBS pH 7.2 [4.4 mg/ml]

455 µl of [4.4 mg/ml] + 545 µl of PBS (0.1M, pH 7.2) = 1.0 ml of [2.0 mg/ml]

- C/D 11-4, 10 mg in PBS pH 7.2 [5.7 mg/ml]

351µl of [5.7 mg/ml] + 649 µl of PBS (0.1M, pH 7.2) = 1.0ml of [2.0 mg/ml]

3. Immediately before use, prepare a 10 mM biotin solution by adding 2.2 mg to 400 µl of ultra pure water.
4. Based on calculations (see section B), add the appropriate volume of the

biotin solution to the protein solution.

5. Incubate reaction for 30 minutes at room temperature (20-30°C) (or 2 hours on crushed ice).
6. Protein labeling is complete at this point.

Purification of the protein by removing excess (unbound) biotin for optimal stability and performance.

2. Dialysis method

1. For each protein sample to be purified, label a beaker and add 1,000 ml PBS.
2. For each 0.5-2.0 ml sample, remove one dialysis membrane cassette from pouch. Handle the cassette only on the frame. Do not touch the membrane.
3. Mark an "X" on the top corner port which will be used to inject sample.
4. Slip the top edge of the cassette into the groove of the appropriate size buoy.
5. Float this assembly in the beaker of PBS for 30 seconds to hydrate the membrane.
6. Remove the cassette from the buffer and tap bottom edge gently on paper towels to remove excess liquid. DO NOT BLOT MEMBRANE.
7. Fill a 5 ml syringe with sample, leaving a small amount of air in the syringe.
8. Taking care not to pierce the membrane, with the bevel sideways, insert the needle tip through the port marked with an "X".
9. Inject the sample slowly; inject the remaining air to flush any remaining sample.
10. With the needle still inserted in the cassette cavity, remove almost all of the air compressing the membrane windows so that the sample solution contacts the greatest window surface area. (Leave a small amount of air so that the needle does not pierce the membrane.)
11. Remove needle from cassette. The gasket will reseal so that the sample will not leak.
12. Slip top edge of cassette back into the groove of the buoy. Return the to the same, labeled beaker of PBS. Add a small magnetic stir bar.
13. Place on a magnetic stirrer (set to a slow speed). Allow to dialyze for 2 hours at room temperature (20-30°C).
14. Change the PBS. Allow to dialyze for 2 hours at room temperature (20-30°C), with slow stirring.
15. Again change the PBS. Allow to dialyze overnight at 2-8^0 C, with slow stirring.
16. To remove the sample after dialysis, fill a syringe with a volume of air at least equal to the sample volume.
17. With the needle bevel sideways, insert only the tip of the needle through the port. Using

the other (previously unused) top port, inject air into the cassette cavity. (Air is used to further separate the membrane so reduce risk of penetration by the needle.)

18. Rotate the cassette until the port with the syringe is on the bottom.
19. Slowly remove the dialyzed sample.
20. Remove the syringe needle from the cassette. Discard the membrane cassette.
21. Transfer contents to a 2 ml screw cap tube, labeled with the protein, biotin-labeled, dialyzed, concentration, and date. (Draw a ✱ on the cap.)
22. Store at 2-8^0 C.

4. HABA Assay for Measuring Level of Biotin Incorporation

Reagent preparation

1. Remove reagents from -20^{0}C or 2-8^o C and allow to come to room temperature (20-30°C)
2. HABA/avidin solution – 1 mg avidin
   60 µl 10 mM HABA in 1 N NaOH
   1.94 ml PBS
3. The A_{500} of this solution should be about 0.9 to 1.3
4. If a precipitate forms in the solution, it can be filtered and then used.
5. Stable if stored at 2-8^o C for up to 2 weeks.

Procedure

1. Set the spectrophotometer absorbance at 500 nm. Use PBS as a blank.
2. Pipette 90 µl of HABA / Avidin Solution into a 1 cm cuvette.
3. Measure the absorbance and record the value as **A_{500} HABA/avidin**.
4. Add 10 µl of biotinylated protein to this cuvette. Mix well.
5. Once the value remains constant for 15 seconds, measure the absorbance.
6. Record this value as A500 HABA/avidin/biotin sample.

- If this reading is ≤0.3, dilute the sample in PBS and repeat the assay (but remember to account for the dilution during calculations.)

Calculation of moles of biotin per mole of protein

These calculations are based on the Beer Lambert Law (Beer's Law):

$$A_\lambda = \varepsilon_\lambda bC$$

- A = the absorbance of a sample at a particular wavelength (λ).
- λ = 500 nm for the HABA assay.
- ε = absorptivity or extinction coefficient at the wavelength (λ). For HABA / Avidin
- samples at 500 nm, pH 7.0 extinction coefficient = 34,000 ml / (M^{-1} cm^{-1})
- b = cell path length expressed in centimeters (cm).
- A 10 mm square cuvette has a path length of 1 cm.
- C = the concentration of the sample expressed in mmoles/ml.

The following values are needed for calculating the number of moles of biotin per mole of protein or sample:

- Concentration of the protein or sample used expressed as mg/ml
- Molecular weight (MW) of the protein or sample used expressed as Daltons
- Absorbance at 500 nm for HABA/Avidin Solution (A500 HABA/avidin)
- Absorbance at 500 nm for HABA/Avidin Biotin Sample mixture
- (A500 HABA/avidin/biotin)
- Dilution factor (if the sample was diluted before addition to the HABA/avidin Solution)

Calculation #1 – biotinylated sample concentration (mmoles/ml)

$$\text{biotinylated sample (mmoles/ml)} = \frac{\text{protein concentration (mg/ml)}}{\text{MW of protein (Daltons)}} = \text{Calc \#1}$$

Calculation #2 – change in absorbance at 500 nm

$$\Delta \text{A500} = (0.9 \times \text{A500 HABA/avidin}) - (\text{A500 HABA/avidin/biotin}) = \text{Calc \#2}$$

Calculation #3 – concentration of biotin (mmoles /ml):

$$\frac{\text{mmoles biotin}}{\text{ml reaction mixture}} = \frac{\Delta A500}{(34{,}000 \times b)} = \frac{\text{Calc \#2}}{(34{,}000 \times b)} = \text{Calc \#3}$$

Calculation #4 – the mmoles of biotin per mmole of protein

$$\frac{\text{mmoles biotin}}{\text{mmoles protein}} = \frac{(\text{Calc \#3}) \times 10^{*} \times \text{dilution factor}^{**}}{\text{Calc \#1}}$$

* Since 90% of the HABA/avidin/biotin sample mixture is HABA/Avidin Solution and 10% is sample, a factor of 10 is used here.

**Use additional dilution factor only if sample was diluted before performing HABA assay.

MAb Samples:

Calculation #1 = mmoles biotinylated protein per ml =

$$\frac{\text{protein concentration (mg/ml)}}{\text{MW of protein (Daltons)}} = \frac{2.0\ \text{mg/ml}}{150{,}000} = 1.33 \times 10^{-5} \quad \textit{Calc \#1}$$

Calc #2 for MAb M/B 7-20 $\Delta A_{500} = (0.9 \times 0.946) - 0.64 = 0.2114$

Calc #2 for MAb C/D 11-4 $\Delta A_{500} = (0.9 \times 0.943) - 0.63 = 0.2187$

$$\frac{\text{mmoles biotin}}{\text{ml reaction mixture}} = \frac{\Delta A_{500}}{(\varepsilon \times b)} = \frac{\textit{Calc \#2}}{(34{,}000 \times b)} = \textit{Calc \#3}$$

Calc #3 for MAb M/B 7-20 $\frac{0.2114}{34{,}000} = 6.2 \times 10^{-6}$

Calc #3 for MAb C/D 11-4 $\frac{0.2187}{34{,}000} = 6.4 \times 10^{-6}$

$$\frac{\text{mmoles biotin}}{\text{mmoles protein}} = \frac{\textit{Calc \#3} \times 10 \times \text{dilution factor}}{\textit{Calc \#1}} = \textit{Calc \#4}$$

Calc #4 for MAb M/B 7-20 $\frac{6.2 \times 10^{-6} \times 10 \times 1}{1.33 \times 10^{-5}} = 4.66$ average # biotin molecules per MAb molecule

#4 for MAb C/D 11- 4 $\frac{6.4 \times 10^{-6} \times 10 \times 1}{1.33 \times 10^{-5}} = 4.81$ average # biotin molecules per MAb molecule

REFERENCES

Pierce – Instructions EZ-Link® Sulfo-NHS-LC-Biotinylation Kit

PROCEDURE HISTORY

Date	Version	Comments	Initials
15 OCTOBER 2004	D	Draft Prepared	KB/PC
14 OCTOBER 2005	1	Version 1 introduced	DB
29 MAY 2008	5	Minor changes only	DB/JL/PJ/SI/WO

SOP 5.04 CDC Aldolase ELISA for malaria antigen in blood

PURPOSE

This SOP describes the materials, equipment, and procedures required to correctly and safely use the CDC in-house aldolase ELISA to diagnose malaria using blood samples. Protocol includes:

- Setting up dilutions of recombinant Plasmodium vivax (Pv) aldolase antigen for a standard calibration curve
- Preparation of blood samples for use in the assay
- Running of the ELISA in a 96 well plate format and interpretation of results

SCOPE

This procedure is part of the methods for malaria antigen ELISA described in the Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests. This SOP has been developed for the training of laboratory personnel using the CDC in-house aldolase ELISA for malaria diagnosis in clinical and research settings. For the WHO malaria RDT evaluation programme, this SOP describes the use of this assay in the assessment of aldolase antigen content within patient blood samples that form part of the global specimen bank.

PROCEDURE

A. PRINCIPLE OF THE TEST

The CDC aldolase ELISA is suitable for the detection, in a blood sample, of the four species of malaria infecting humans. Uncoated plates with a high affinity to protein/peptide (Immulon HB2) are coated with monoclonal antibodies raised against Pv aldolase. Lysed blood test samples are then added to the plate such that Pv aldolase can bind to antibodies. A biotinylated detection monoclonal antibody is then added, which binds to a different epitope on the antigen. The addition of Avidin Peroxidase (Sigma) conjugated to substrate solution TMB will then cause a colour change in the substrate solution. After addition of an acid stop solution, the colour will become stabilised. The colour intensity of the resulting product is directly proportional to the aldolase concentration and is measured as ΔOD 450/620 nm.

B. ASSAY AND SPECIMEN REQUIREMENTS

NB: All reagents are allowed to equilibrate to room temperature (20-30°C) for 15 min before use.

Sarstedt tubes
Vortex
Plate rocker/orbital shaker that is able to rotate at 650rpm.
Preparation microtitre plate (uncoated)
High protein/peptide affinity binding plate (Immulon HB2)
Recombinant Pv aldolase
Capture monoclonal antibody: Unlabelled mAb M/B7-20
Biotinylated detection monoclonal antibody: C/D 11-4
Coating buffer: 0.1M Carbonate/bicarbonate buffer pH 9.6
Blocking buffer: 1% (w/v) BSA in 1 X PBST
Lysis buffer: 1% (w/v) BSA in 1 X PBST containing 0.5% (v/v) nonidet-P 40 (NP-40)
Wash buffer: 1 X PBST

Avidin Peroxidase (Sigma Aldrich)
TMB substrate solution (Millipore)
Stop solution: 1M H3PO4
Plate lid
Micropipettes (50 – 200 µl and 100-1000 µl)
Multichannel micropipette (50 – 250 µl)
Automated plate washer
Spectrophotometer

C. INSTRUCTIONS FOR PERFORMING THE ASSAY

1. Plate coating

Coating buffer should be made up fresh before each set of assays. Capture monoclonal mAb M/B 7-20 should be diluted in coating buffer to a final concentration of 2 µg/ml and 100 µl dispensed into all 96 wells of the uncoated Immulon HB2 plate. A plate lid should then be added and the plate left overnight at 4 ºC in preparation for performing the next steps of the assay the following day.

2. Wash steps and blocking

Freshly coated plates should be washed for 3 cycles with 250 µl 1 X PBST per well using an ELISA plate washer. To prevent non-specific binding of proteins to the antibodies, 250 µl blocking buffer should then be dispensed into all 96 wells of the plate. A plate lid should then be added and the plate incubated at room temperature (20-30°C) on a plate rocker/orbital shaker set to 650rpm for 1 hour. Prior to loading of the plate with test samples, the plate should then be washed again for 3 cycles with 250 µl 1 X PBST.

3. Preparation of standards and test samples

Eight standards are used as a reference positive and prepared in serial dilution for this assay. Human blood that has been frozen and thawed is used as a diluent for the standards and as a negative control. A purified recombinant form of Pv aldolase is used to produce the standards that are diluted in human blood, see Table 5-5. Blood used to dilute the recombinant is pipetted into sarstedt tubes. Stock antigen is added to the first tube at an appropriate dilution to provide a starting concentration of 250 ng/ml. Doubling dilutions made thereafter form the 8 reference points that will generate the standard curve. Between transfers from one tube to another, blood should be pipetted up and down several times then a vortex used to mix each tube. A fresh pipette tip should be used between each transfer.

Depending on the concentration of aldolase in the specimen being tested, dilution may be appropriate in order for the test samples to fit within range of the calibration curve. If necessary, this should be done using human blood and a conversion factor applied during data analysis.

- Table 5-5. Recombinant Pv aldolase (x ng/ml)* varies depending on aliquots being used

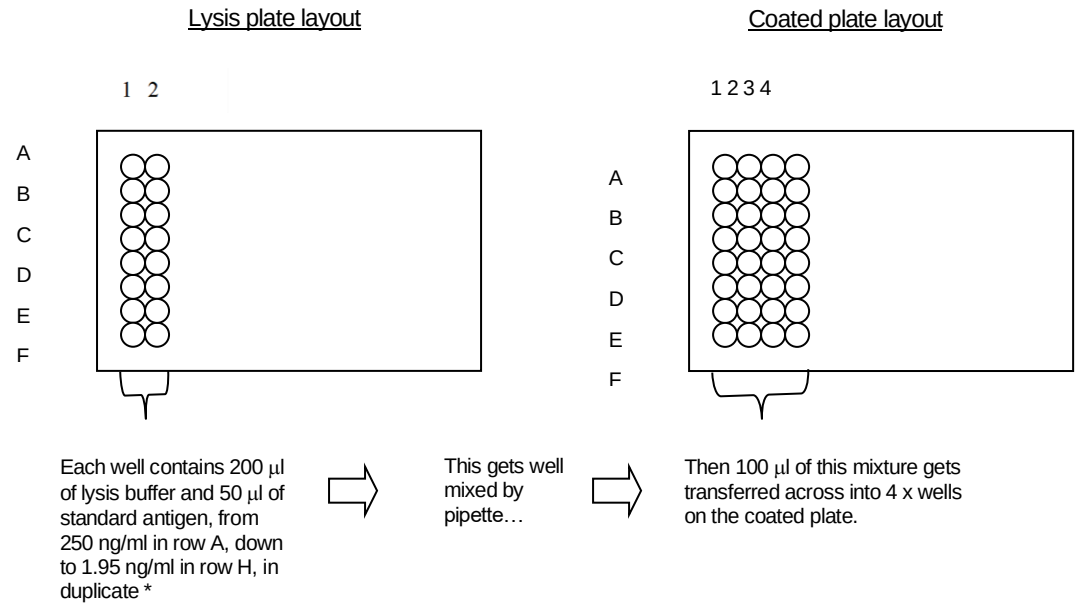
Conc required (ng/ml)	250	125	62.5	31.25	15.62	7.8	3.9	1.95
Working stock (ng/ml)	x	250	125	62.5	31.25	15.62	7.8	3.9
Volume stock (µl)	x	150	150	150	150	150	150	150
Volume diluent (µl)	x	150	150	150	150	150	150	150
Total volume (µl)	1000	300	300	300	300	300	300	300

4. Preparation of the lysis plate

To begin, 200 µl lysis buffer should be dispensed into wells of an uncoated/lysis plate that will contain blood samples. Subsequently, 50 µl of each of the 8 pre-prepared standards (250-1.95 ng/ml) should then be added to the wells of column one (A-H) and two (A-H) in parallel.

The test samples should then be added to each of the wells consecutively from A3 as far as F6. G6 should contain 50 µl of human blood used as the negative control. H6 should contain 50 µl blocking buffer as this will be used as a "blank" to control for any significant background buffer may give rise to. For one plate, 30 specimens can be tested as all wells from the lysis plate will then be duplicated in the coated plate.

- Figure 5-5. Loading format for lysis and coated plates



5. Transfer of blood to the coated plate

With a multi-channel pipette, 100 µl should then be transferred from each well containing lysed blood to wells of the anti-aldolase coated test plate so that each column of wells is tested in duplicate filling the 96 well microtitre plate. The plate should then be covered with a lid and incubated at room temperature (20-30°C) on a plate rocker/orbital shaker set to 650rpm for 1 hour.

6. Detection and development of substrate

Prior to detection steps, the coated plate should then be washed again for 3 cycles with 250 µl 1 X PBST. Biotinylated detection mAb C/D 11-4 should then be diluted in blocking buffer to a working concentration of 1 µg/ml and 100 µl of the solution dispensed into all 96 wells of the coated plate. A plate lid should be added and the plate incubated again at room temperature (20-30°C) on a plate rocker/orbital shaker set to 650rpm for 1 hour.

The plate should then be washed again for 3 cycles with 250 µl 1 X PBST. Working strength enzyme conjugate should then be prepared by diluting Avidin Peroxidase 1:4,000 in 1 X PBST and 100 µl of the solution dispensed into all but wells H11 and H12 (the "blank" wells). For the blank wells, 100 µl X PBST should be added. A plate lid should then be added and the plate left to stand on the bench at room temperature (20-30°C) for 30 mins.

The TMB substrate is already at working strength but should be equilibrated to room temperature (20-30°C) prior to use. The coated plate should be washed for another 3 cycles with 250 µl 1 X PBST after which, 100 µl of TMB should be dispensed into all 96 wells. The plate should then be covered with a plastic lid and incubated at room temperature (20-30°C) for 10 mins in the dark.

After 10 mins, 100µl acid stops solution should be then dispensed in all wells. The endpoint absorbance of the wells should be read at 450 nm with a reference wavelength 620 nm.

7. Interpretation of results

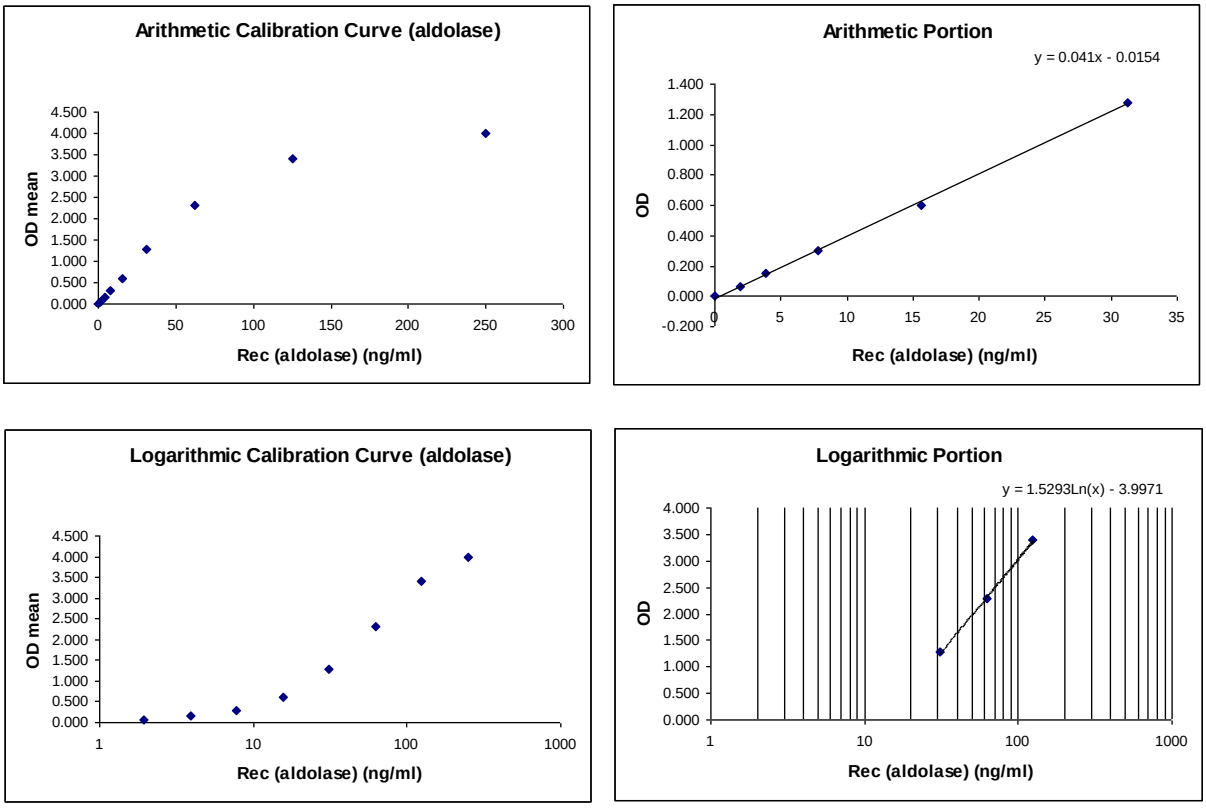
The spectrophotometer will make a print out of ΔOD 450/620 nm results and these should then be transcribed to another PC. Each point comprising the standard curve has been replicated 4 times therefore a mean OD for each point should be calculated in EXCEL. The specimens are in duplicate and also need to be averaged. Mean ODs for the standards, specimens and negative control should all be entered into EXCEL to make a scatter graph (calibration curve). Both arithmetic and logarithmic curves are plotted.

The arithmetic calibration curve will be parabolic in shape. The logarithmic calibration curve should be sigmoidal in shape. ODs that are > 250 ng/ml standard on the logarithmic curve will begin to plateau on the curve. Test samples with ODs higher than the 250 ng/ml standard should not be interpreted because the assay begins to saturate with aldolase antigen at this point. The negative specimen should have an OD < 0.100 but it is not uncommon that a higher than usual background may be observed in this assay owing to the use of a biotinylated detection antibody.

Separate plots for selected points from the logarithmic and arithmetic curves are used for fitting trend-lines and obtaining equations which are used to calculate concentrations from OD for each test sample. The calculation uses a re-arrangement of the algebraic expression,

$y = mx + c$. ODs are converted to aldolase concentration in ng/ml. The result on a test specimen is adjusted for any pre-dilution of the specimen.

• Figure 5-6. Generation of logarithmic and arithmetic trend lines for data interpretation



C. HEALTH AND SAFETY

1. Hazardous reagents

• Table 5-6. Hazardous chemicals used in the CDC aldolase ELISA

Product	Fire hazard	Health hazard	Toxicity	Storage requirements
Hydrogen peroxide (TMB)	Explosive under heat	Irritant to eyes/skin/nasal passage	Moderately toxic	Easily decomposes 2-8 ^{0}C
Orthophosphoric acid (stop solution)	Flammable	Irritating to eyes/skin. Burns. Harmful by ingestion.	Toxic	Keep in a locked store
TMB	Flammable	Harmful swallowed/inhaled/ absorbed by skin	Toxic	Store solutions in light proof container at 4 -8 °C

2. Safety precautions

Recombinant Plasmodium vivax aldolase used as a standard has been shown to be non-infectious in a recombinant expression system.

Disposable latex or nitile gloves must be worn while handling clinical specimens and reagents. All clinical material i.e. all components containing blood must be autoclaved before disposal. The assay stop solution contains orthophosphoric acid; a corrosive and hazardous substance. Avoid eye and skin contact by wearing protective clothing and eye protection.

Hands must be washed once work has been completed.

3. Technical precautions

- Components must not be used after their expiry date.
- Different batches/lots of reagents should never be interchanged.
- Storage of reagents must be at the recommended conditions.
- Contamination of reagents should be avoided by changing pipette tips where necessary.

PROCEDURE HISTORY

Date	Version	Comments	Initials
15 OCTOBER 2004	D	Draft Prepared	KB/PC
14 OCTOBER 2005	1	Version 1 introduced	DB
29 MAY 2008	5	Minor changes only	DB/JL/PJ/SI/WO
MAY 2014	7	Updated the procedure, based on updates for the Product Testing Manual version 5	RRC, SI
FEBRUARY 2020	9	Formatting changes, renamed figures 1 and 2 to figure 5-5 and 5-6. Renamed tables 1 and 2 to table 5-5 and 5-6. Renamed table 1 (checklist of information recorded in the handcover of the handbook) to table 5-7	JL, CAL

SOP 5.05 Dilution Protocol for Recombinant pLDH, HRP2, reagents and blood samples (ELISA)

PURPOSE

This Standard Operating Procedure (SOP) describes the procedure for calculating dilutions of reagents or samples used in the HRP2 or pLDH ELISA procedures.

SCOPE

This procedure is part of the methods for malaria antigen ELISA described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

PROCEDURE

1. Use the following calculation to perform any dilutions of reagents or samples.

$$\frac{\text{(Concentration required)}}{\text{Stock concentration}} \times \text{Total volume required} = \text{Volume of stock required}$$

2. Record the volumes used and relevant information in Form 5.01.

3. Keep all records associated with the Quality Assurance Scheme for at least five years (see SOP 6.12 for Documents storage)

4. Always use reverse pipetting when diluting blood or other viscous substances (see SOP 3.14).

5. Do not pipette a volume <20µL.

6. Use a separate disposable tip for each transfer to avoid cross-contamination.

PROCEDURE HISTORY

Date	Version	Comments	Initials
15 OCTOBER 2004	D	Draft Prepared	KB/PC
14 OCTOBER 2005	1	Version 1 introduced	DB
29 MAY 2008	5	Minor changes only	DB/JL/PJ/SI/WO
MAY 2014	7	Chamged title to clarify that procedures are for ELISA	SI

SOP 5.06 Protocol for Recording ELISA Results

PURPOSE

This Standard Operating Procedure (SOP) describes the procedure for recording results generated from ELISA work.

SCOPE

This procedure is part of the methods for malaria antigen ELISA described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

PROCEDURE

1. All laboratory work is to be recorded in a hardcover laboratory book. This will serve as a hard copy record for the results saved on computer and will also serve as a daily record and audit trail of the work carried out.
2. Record the date of the test, lot number of kit and reagents used, any deviations from the standard operating procedure and any problems encountered.
3. Record all dilutions on the dilution audit form as well (Form 5.01)
4. Record all results onto the computer.
5. Record all raw data onto the ELISA reporting form (Form 5.02).
6. The data may be entered manually or transferred onto the computer; it is preferable that two people check the results to avoid transcription errors.
7. All computer data must be backed regularly in case of a computer mishap (ideally weekly, at least monthly).
8. Laboratory notebooks must be photocopied on a regular basis and the photocopies stored in a folder away from the laboratory (ideally weekly, at least monthly). This is to prevent loss of data in case of a mishap.
9. See SOP 6.11 for Documents storage.

• Table 5-7: Checklist of information to be recorded in the hardcover laboratory book.

Date of test
Lot number of kit
Lot number of reagents (e.g. positive control, etc.)
Diluents used
How reagents were made up
Reason for deviation from SOP

Problems encountered
Record ambient temperature

PROCEDURE HISTORY

Date	Version	Comments	Initials
15 OCTOBER 2004	D	Draft Prepared	KB/PC
14 OCTOBER 2005	1	Version 1 introduced	DB
29 MAY 2008	5	Changed the frequencies of data back-up, minor changes only	DB/JL/PJ/SI/WO/CS

SOP 5.07 Extraction of Genomic DNA from Whole Blood Using QIAamp Protocol

PURPOSE

This SOP describes how to extract genomic DNA from whole blood samples, eventually to be used for *Plasmodium* species identification.

SCOPE

This procedure is part of the methods for sample characterization described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

REAGENTS

1. QIAGEN Protease (Proteinase K)
2. QIAGEN Buffer AL
3. QIAGEN Buffer AW1
4. QIAGEN Buffer AW2
5. ddH2O
6. Ethanol

PROCEDURE

NOTE: Heat a water bath or heating block to 56 °C for use in step 4

1. Pipette 20 µl QIAGEN protease (or Proteinase K) into the bottom of a 1.5 mL microcentrifuge tube
2. Add 200 µl whole blood sample to the microcentrifuge tube. If the volume is less than 200 µl add the appropriate amount of PBS
3. Add 200 µl Buffer AL to the sample. Mix by pulse-vortexing for 15 seconds.
4. Incubate at 56 °C for 10 minutes.
5. Briefly centrifuge the 1.5mL tube to remove drops from inside of the lid.
6. Add 200 µl ethanol (96-100%) to the sample, and mix again by pulse-vortexing for 15 seconds. After mixing, briefly centrifuge the tube to remove any residue from the lid
7. Carefully apply the mixture from step 6 to a QIAamp Spin Column (in a 2mL collection tube) without wetting the rim, close the cap, and centrifuge at 8000 rpm for 1 minute
8. Place the Spin Column in a clean 2 ml collection tube and discard the tube containing the filtrate
9. Carefully open the Spin Column and add 500 µl Buffer AW1 without wetting the rim, close the cap, and centrifuge at 8000 rpm for 1 minute
10. Place the Spin Column in a clean 2 ml collection tube and discard the tube containing the filtrate
11. Carefully open the Spin Column and add 500 µl Buffer AW2 without wetting the rim, close the cap, and centrifuge at 13000 rpm for 3 minutes

12. Place the Spin Column in a clean 1.5mL microcentrifuge tube and discard the collection tube containing the filtrate
13. Carefully open the Spin Column and add 200 µl ddH2O.
14. Incubate at room temperature (20-30°C) for 1 minute, and then centrifuge at 8000 rpm for 1 minute
15. Store isolated DNA at -20□C for future use

REFERENCES

Blood and Body Fluid Spin Protocol. QIAamp□ DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook. QIAGEN. February 2003.

PROCEDURE HISTORY

Date	Version	Comments	Initials
MAY 2014	7	Introduced the SOP, based on updates for the Product Testing Manual version 5	JB, JG, SI

SOP 5.08 Identification of Plasmodium Species by PCR Assay

PURPOSE

This SOP describes how to perform a nested Polymerase Chain Reaction (PCR)-based assay for the detection and identification of malaria parasites.

BACKGROUND

This assay will be performed on whole blood known or believed to be infected with Plasmodium spp. The results will be used to identify and differentiate between the four main human malaria species. This is a nested Polymerase Chain Reaction, amplifying a portion of the Plasmodium SSU rRNA gene, in which both genus and species specific primers are used.

SCOPE

This procedure is part of the methods for sample characterization described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

REAGENTS

1. Expand High Fidelity Enzyme Mix (Taq DNA polymerase and Tgo DNA polymerase)
2. Expand High Fidelity Buffer (10X) with 15 mM Mg Cl2
3. 2mM dNTP's
4. ddH2O
5. Genus and species-specific primers
6. Template DNA

PROCEDURE

A. General

1. Always record the date the assay was performed and note any changes to the SOP during the run in the hardcover laboratory book
2. Bring buffer solution, DNA template, and primers well to room temperature (20-30°C) (20-30º C) before use.
3. Keep Enzyme Mix at -20º C until needed
4. Optimal incubation times and temperatures for thermal cycling depend on the system used and are determined individually.
5. Positive controls for nest 1 will come from P. falciparum SSU rRNA gene
6. Prior to use, ensure species-specific primers are working properly by testing against positive and negative controls
7. Perform all mixing of reagents in a sterile environment
8. Use a separate disposable tip for each transfer to avoid cross contamination

B. Extraction of Genomic DNA from Whole Blood (Refer to SOP 5.07)

C. Preparation for Nest 1 PCR

1. Briefly vortex and centrifuge all reagents before starting
2. Prepare a master mix prior to addition of template DNA (Table 5-8)
3. Use a 1.5 mL microfuge tube when making master mix
4. Add 1.0 µL of forward and 1.0 µL reverse genus-specific primers (Table 5-12) for each reaction at a concentration of 100 ng/µL or 15 mM.
5. Upon completion, pipette up and down to mix reagents
6. A total of 3 PCR reactions will be performed; the sample in question, a positive, and a negative control
7. Add 18 µL of master mix to three 0.2 µL thin-walled PCR tube, and make note which tube will have sample DNA as well as positive and negative controls
8. Add 2 µL template DNA to sample tube and positive control, and 2 µL H2O to negative control to give 20 µL total volume per PCR tube

- Table 5-8: Nest 1 PCR Master Mix

REAGENTS	VOLUME NEEDED	NUMBER OF PCR REACTIONS	TOTAL VOLUME
dd H2O	11.8 µL	3	35.4 µL
10X Buffer	2.0 µL	3	6.0 µL
dNTP's	2.0 µL	3	6.0 µL
Primers	2.0 µL	3	6.0 µL
Polymerase	0.2 µL	3	0.6 µL
Total:	**18 µL**		**54 µL**

D. Thermal Cycling of Nest 1

1.Place samples in a thermal block cylinder, and start cycling using the thermal profile for nest 1 (Table 5-9)

2.Run for 30 cycles

3.Store PCR product at 4º C when not in use

• Table 5-9: Nest 1 Thermal Profile

	TEMPERATURE	TIME
Initial Denaturation	94º C	5 min
Denaturation	95º C	30 sec
Annealing	53º C	30 sec
Elongation	68º C	1 min 30 sec
Final Elongation	68º C	5 min
Cooling	4º C	Unlimited

Desired BP Size: 1.05 Kb

E. Preparation for Nest 2 PCR (Species Identification)

1. Briefly vortex and centrifuge all reagents before starting
2. Prepare a second master mix in a 1.5 µL microfuge tube prior to the addition of template DNA and primers (Table 5-10)
3. Upon completion, pipette up and down to mix reagents
4. A total of 6 PCR reactions will be performed; 4 using each species-specific set of primers, 1 negative control, and 1 positive control
5. Add 1.0 µL of forward and 1.0 µL reverse species-specific primers (Table 5-12) for each reaction at a concentration of 100 ng/µL or 15 mM.
6. Add 1.0 µL of template DNA (PCR product from nest 1 reaction) to each of the seven PCR tubes to give 20 µL total volume per tube

• Table 5-10: Nest 2 PCR Master Mix

REAGENTS	VOLUME NEEDED	NUMBER OF PCR REACTIONS	TOTAL VOLUME
dd H2O	12.8 µL	6	76.8 µL
10X Buffer	2.0 µL	6	12.0 µL
dNTP's	2.0 µL	6	12.0 µL
Forward Primer	1.0 µL	6	6.0 µL
Reverse Primer	1.0 µL	6	6.0 µL
Polymerase	0.2 µL	6	1.2 µL
Total:	**19 µL**		**114 µL**

F. Thermal Cycling of Nest 2

1. Place samples in a thermal block cylinder, and start cycling using the thermal profile for nest 2 (Table 5-11)

2. Run for 30 cycles
3. Store PCR product at 4º C when not in use

• Table 5-11: Nest 2 Thermal Profile

	TEMPERATURE	TIME
Initial Denaturation	94º C	5 min
Denaturation	95º C	30 sec
Annealing	55º C	30 sec
Elongation	68º C	1 min
Final Elongation	68º C	5 min
Cooling	4º C	Unlimited

G. Species Identification

1. Run PCR products from nest 2 on a 1.5 percent agarose gel
2. Only two bands should fluoresce; the positive control, and one species-specific PCR product
3. Match the band to proper species-specific primer, and identify, if any, which Plasmodium parasite the sample is infected with.

• Table 5-12: Genus and Species-Specific Primer Pairs for Nest 1 and Nest 2 PCR Reactions

Nest 1: Genus Specific	
rPLU6 (forward)	5′-TTA AAA TTG TTG CAG TTA AAA CG-3′
rPLU5 (reverse)	5′-CCT GTT GTT GCC TTA AAC TTC-3′
Nest 2: *P. falciparum* specific	
rFAL1 (forward)	5′-TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT-3′
rFAL2 (reverse)	5′-ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC-3′
Nest 2: *P. malariae* specific	
rMAL1 (forward)	5′-ATA ACA TAG TTG TAC GTT AAG AAT AAC CGC-3′
rMAL2 (reverse)	5′-AAA ATT CCC ATG CAT AAA AAA TTA TAC AAA-3′
Nest 2: *P. ovale* specific	
rOVA1 (forward)	5′-ATC TCT TTT GCT ATT TTT TAG TAT TGG AGA-3′
rOVA2 (reverse)	5′-GGA AAA GGA CAC ATT AAT TGT ATC CTA GTG-3′
Nest 2: *P. vivax* specific	
rVIV1 (forward)	5′-CGC TTC TAG CTT AAT CCA CAT AAC TGA TAC-3′
rVIV2 (reverse)	5′-ACT TCC AAG CCG AAG CAA AGA AAG TCC TTA-3′

Desired BP sizes:

P. falciparum	205 bp
P. Malariae	144 bp
P. Ovale	787 bp
P. Vivax	117 bp

PROCEDURE HISTORY

Date	Version	Comments	Initials
MAY 2014	7	Introduced the SOP, based on updates for the Product Testing Manual version 5	JB, JG, SI
FEBRUARY 2020	9	Formatting changes. Renamed tables 1-5 to table 5-8 to 5-12 respectively.	JL, CAL

Methods Manual for Laboratory Quality Control Testing of Malaria RDTs

Chapter 6: GENERAL LABORATORY QUALITY ASSURANCE

FORMS FOR CHAPTER 6:

6.01: Microscopy Competency Assessment Result Sheet

6.02: Microscopy Competency Assessment Collation Sheet

6.03: Microscopy Competency Assessment Reporting Form

6.04: Pipette Calibration Sheet

6.05: Incubator Calibration Sheet

6.06: Equipment Maintenance Sheet

6.07: Temperature Monitoring Form

6.08: Corrective Action Register

SOP 6.01 Laboratory Safety

PURPOSE

This Standard Operating Procedure (SOP) describes general safety procedures and must be understood and adhered to by all personnel.

SCOPE

This procedure is part of the methods for general laboratory quality assurance described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

PROCEDURE

Note: this SOP is only an outline of standard laboratory safety procedures. A more detailed biosafety guideline should be available in the laboratory and the staff be trained on, such as WHO Biosafety guidelines.

A. Laboratory Design

1. Adequate space should be provided for the safe conduct of laboratory work.
2. Bench tops should be stable, impervious to water and resistant to disinfectants, chemicals and moderate heat.
3. Facilities for storing outer garments and personal items should be provided outside the laboratory working area.
4. Facilities for eating and drinking should be provided outside the working areas.
5. Hand-washing basins, with running water and disinfectant soap, should be provided in each laboratory room.
6. Safety system should cover fire, electrical emergencies, emergency shower and eyewash facilities.
7. A dependable supply of good quality water is essential.
8. There should be a reliable and adequate electricity supply and a standby generator must be available for the support of essential equipment i.e. refrigerators, freezers, with an automated switching system in case of electricity shortages.

B. Laboratory Working Areas

1. All benches must be kept clean, tidy, and dry.
2. Work surfaces must be decontaminated at the end of the working day.
3. Packing and transportation must follow applicable national and/or international regulations.

4. All chemicals, solutions and specimens must be properly labeled. Labels must include name, date prepared, and expiry date, where applicable.
5. Glassware and other materials for reuse must be rinsed with de-ionized water after cleaning with detergent.
6. Supplies and materials must be kept in designated drawers and lockers that are labeled with respective contents on the outside.
7. Heavy equipment, glassware, and chemicals are not to be stored above eye level.
8. All equipment must be properly attached to electrical outlets in a way that prevents overloading and tripping hazards.

C. Personal protection

Human specimens are potential sources of communicable diseases such as HIV 1 and 2, and Hepatitis B and C, via direct contact with broken skin or mucous membrane. To minimize the biological and safety hazards inherent in handling human specimens, the following guidelines should be followed:

1. Laboratory gown and gloves must be worn at all times when doing work inside the laboratory, and especially when handling human body fluids.
2. Laboratory clothing should not be worn outside of the laboratory e.g. in canteens, offices, staff rooms or toilets.
3. Safety glasses face shield (visors) or other protective devices must be worn when it is necessary to protect the eyes and face from splashes.
4. Hands must always be washed using a skin disinfectant/antibacterial liquid (i.e. 4% chlorhexidine gluconate with added skin emollients) in case of contact with potentially infectious specimens, before and after work, and at any time before leaving the laboratory.
5. Open-toes footwear should not be worn in the laboratory.
6. Eating and drinking is prohibited in the laboratory working areas.
7. Storing human foods or drinks anywhere in the laboratory is prohibited.

D. Pipetting and manipulation of potentially infectious specimens

1. Work with potentially infectious specimens (e.g. human blood, serum, plasma) requires the use of disposable equipment and supplies, whenever possible. Otherwise, all reusable materials must be decontaminated before washing. See paragraph H for handling of biohazard material and waste.
2. Pipettes must be used properly, i.e. use pipetting aids with sterile disposable pipettes. Mouth pipetting is strictly forbidden.
3. All technical procedures must be performed in a way that minimizes the formation of aerosols and droplets. If available, whenever there is increased risk of aerosol production, work should be conducted in a biosafety cabinet.
4. Dilutions and aliquots of human blood should also be performed in a biosafety cabinet, whenever possible.
5. The use of hypodermic needles and syringes should be limited. They must not be used as substitutes for pipetting.

E. Essential Biosafety Equipment

1. Pipetting aids – to avoid mouth pipetting.
2. Screw-capped tubes and bottles.
3. Plastic disposable Pasteur pipettes, whenever available, to avoid glass.
4. Biological safety cabinets, to be used whenever
    a) Infectious materials are handled. Centrifuges may be placed in the open laboratory if sealed centrifuge safety cups are used and if they are loaded in a biological safety cabinet.
    b) There is an increased risk of aerosol production.
    c) Procedures with a high potential for producing aerosols are used; these may include centrifugation, grinding, blending, mixing, diluting and aliquoting.

F. Safety during blood collection

1. Universal precautions are to be adhered to, at all times for protection during blood collections.
2. Laboratory gown and gloves must be worn at all times when doing blood collection and handling blood samples.
3. Puncturing material such as hypodermic needles must be opened just prior to use and handled carefully. After use, needles should not be recapped, clipped or removed from disposable syringes. The complete assembly should be place in a dedicated sharps waste container.
4. Follow local institutional protocols and including PEP (post exposure prophylaxis) if an accidental needle sticks injury occurs.
5. See paragraph H for handling of biohazard material and disposal of infectious waste.

G. Injury and accidents with potentially infectious specimens

1. All spills or accidents involving potentially infectious specimens (e.g. blood, serum, plasma) must be reported to the designated infection control officer or to the laboratory supervisor. A written record of such accidents should be maintained.
2. In case of spill of potentially infectious fluids in the eye, and in case of injury with potentially infectious specimens (e.g. spill on broken skin, cutting with broken glass, puncture with needles/syringes), refer to standard safety procedures for immediate actions. Report the accident to the laboratory supervisor, and contact a physician as soon as possible.
3. Any pathological symptoms occurring after the accident should be reported to the laboratory supervisor, and a physician should be contacted if necessary.

H. Handling of Biohazard Material and disposal of infectious waste

1. “Sharps” (i.e. hypodermic needles, scalpels and broken glass) must be placed in specially labelled puncture-free (i.e. made of rigid plastic) “sharp containers” fitted with covers. When the container is three-quarters full, it should be closed and placed in an “infectious waste” container and incinerated, with prior autoclaving if laboratory practice requires it. Sharp containers must not be disposed of in landfills.

2. Apart from sharps, all bio-hazardous waste must be placed in specially designated "infectious waste containers" separately from non-infectious waste. Infectious waste containers should be made of rigid plastic, with covers and a colour-code or shape differentiating them from non-infectious waste containers.
3. All infectious material should be autoclaved or incinerated within the laboratory, and not be disposed of in landfills. Steam autoclaving is the preferred method for decontamination. For autoclaving, leak-proof containers e.g. autoclavable, colour-coded plastic bags should be used. If an incinerator is available on the laboratory site, autoclaving may be omitted. Reusable transfer containers should be leak proof and have tight-fitting covers. They should be disinfected and cleaned before they are returned to the laboratory. If both autoclaving and incineration are used for decontamination, use specific containers, e.g. autoclavable plastic bags that are colour coded to whether the contents are to be autoclaved or incinerated.
4. Any re-usable materials (e.g. glassware that is to be reused) should immediately be placed in containers (e.g. glass cans, one for re-usable material, one for disposable supplies) with a daily freshly prepared decontaminating solution (e.g. 0,05% hypochlorite solution) located at each work station. The material should remain in intimate contact with the disinfectant for the appropriate time required for the disinfectant. The disinfectant should then be poured into a container for autoclaving or incineration. The container should also be autoclaved and washed before use. Re-usable materials should be thoroughly washed with water and disinfectant, rinsed with de-ionized water, and autoclaved before use. The decontaminated disposable supplies should be placed in infectious waste containers for autoclaving or incineration.
5. Any spilled biological material must be covered with cloth soaked in 0.05% hypochlorite solution and left for 15 minutes before cleaning.

I. Biosafety Management

1. It is the responsibility of the laboratory supervisor (the person who has immediate responsibility for the laboratory) to ensure the development and adoption of a biosafety management plan and a safety operations manual.
2. The laboratory supervisor should ensure that regular training in laboratory safety is provided.
3. Personnel should be required to read the standard operation procedures manual on safety and a copy of this manual should be available in the laboratory.

REFERENCES

1. General Safety Standard Operating Procedure. Brisbane, Australian Army Malaria Institute, 2,000 (unpublished report).

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
27 MAY 2008	5	Added requirements of emergency generator, revised requirements for blood collection and laboratory injury/accidents,	DB/JL/PJ/SI/WO

SOP 6.02 Training

PURPOSE

This Standard Operating Procedure (SOP) describes the process for training new and existing staff members of the "WHO malaria rapid diagnostic test quality assurance initiative".

SCOPE

This procedure is part of the methods for general laboratory quality assurance described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

PROCEDURE

1. Every staff member is to be assessed for their competency to perform all relevant tasks within the department. The assessor will generally be the Supervisor or Head of Department, but may be any authorized person who has been assessed as being competent at that particular task. Assessment can be based on past experience or active assessment.
2. All relevant training completed is to be recorded on an appropriate form for each member of the department
3. The form is a record of competencies for each department member, including induction training for new staff. All tasks that they are expected to perform are to be included in the table. A member is not to perform departmental tasks until they are deemed competent. The assessor is to sign and date when the assessed member is competent.
4. Each member should have his or her competency re-assessed as required. This may be through within laboratory or inter-laboratory comparisons.

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
27 MAY 2008	5	Minor changes only	DB/JL/PJ/SO/WO
MAY 2014	7	Removed annual frequency for training	NC/SI

SOP 6.03 Microscope Maintenance

PURPOSE

This Standard Operating Procedure (SOP) describes the general maintenance of microscopes used for malaria slide reading.

SCOPE

This procedure is part of the methods for general laboratory quality assurance described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

PROCEDURE

A. Before each session

1. Check that the objectives and oculars are clean.
2. Check that the microscope is centered and focused.

B. After each session

1. Turn off the power switch.
2. Remove oil from the oil immersion objective lens with lens tissue or soft cotton cloth. Do not reuse with other objectives.
3. Wipe dirt or spilled specimens on the microscope stage using soft tissue.
4. Remove oil and grease from fingers and eyelashes that may be deposited on lenses and oculars using lens or soft tissue.
5. Keep the microscope protected using designated cover.
6. Make sure that the lamp is turned off and unplug the cord from the power switch.
7. If the microscope is not to be used for a long time, place inside its box with the door tightly closed.

C. Weekly

1. Clean dust from microscope outer surfaces using a soft cloth.
2. Using cotton bud dipped in window cleaning solution, clean the outer surfaces of the ocular, objective lens, condensers and filters. Wipe again using dry cotton bud afterwards.
3. Wind the sub-stage back and forth and sideways to the full length of its travel to spread the grease evenly on all surfaces.

D. Yearly

1. Microscopes are to be serviced at least annually by a qualified technician to ensure optimum condition.

E. Considerations when in the field

1. Transport microscopes in appropriate microscope boxes, making sure that they are labeled 'fragile' before check-in. Make sure that they are properly secured by means of a device that

screws into the base of the microscope. Where possible, designate microscopes especially for field use only.

2. Store equipment in areas where it can be observed.
3. Proper storage of microscopes in boxes requires the use of desiccants to avoid moisture and minimize the growth of fungi on the microscope oculars, lenses, and condensers. The desiccant should be dried each week.

REFERENCES

1. Basic Malaria Microscopy, Part 1. Learner's Guide. Geneva, World Health Organization, 1991 (unpublished document.LF.Q.AZ.1991 pt.1).
2. Microscope Maintenance Standard Operating Procedures. Brisbane, Australian Army Malaria Institute, 2,000 (unpublished report).

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
27 MAY 2008	5	Added a paragraph on maintenance before each session.	DB/JL/PJ/SI/WO

SOP 6.04 Malaria Microscopy Competency Assessment

PURPOSE

This Standard Operating Procedure (SOP) describes a process for assessing the competency of malaria microscopists and demonstrate the continuing improvement of microscopists in regards to malaria parasite speciation and parasite counts.

BACKGROUND

To prepare accurate dilutions of wild parasites, to be used in quality assurance of rapid diagnostic tests, it is vital to have good quality malaria microscopy to ensure accurate parasite counts (density). Methods to ensure accurate microscopy include multiple blinded readings and averaging of counts between readers (see SOP 4.01). However, it is also important to pre-qualify (check proficiency of) microscopists to ensure those performing the precise parasite counts used for calculation and preparation of quality control dilutions of wild parasites are of a high standard. Pre-qualifying should include both qualitative assessment (species identification) and quantitative assessment (parasite density).

SCOPE

This procedure is part of the methods for general laboratory quality assurance described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

This SOP should be seen as an example: different institutions may have modifications of this process.

PROCEDURE

MATERIALS AND SUPPLIES

1. Reference slides (may be obtained from the WHO/WPRO regional slide bank or other national or international slide repository)
2. Microscope(s)
3. Tally counters for counting parasites and WBCs
4. Oil immersion

PROCEDURE

1. About 4-5 months prior to each sample collection, the laboratory should gather for each microscopist, and send to FIND/WHO all or any of the following:

Document	Obtained from	Validity

Certificate as Level 1 or 2 microscopist	External Competency Assessments (ECA) provided by the WHO or other equivalent and recognized certification programme (international/national)	Obtained in the last 2-3 years
Evidence of regular participation in EQA or slide cross-checking programs with satisfactory performance (>90% score)	External Quality Assessment programme (EQA or proficiency testing) and/or slide cross-checking programme for malaria microscopy (international/national)	At least 1/year in the past 2 years
Training certificates and proof of satisfactory performance (>90%score)	Participation in training(s) (international/national)	Last 2 years

2. Only microscopists that are certified as level 1 or level 2 microscopists in WHO malaria microscopy workshops, should be considered to function as reference microscopists for the QC sample collection and preparation. Alternatively, if evidence can be provided for any of the two other performance criteria, WHO and FIND can jointly agree to exceptionally include microscopists that satisfy these other criteria.

3. In situations where the laboratory and its microscopists are not participating in any of the above quality assessments, or the validity of their certificates/evidences have exceeded the maximum, the performance of all the potential reference microscopists should be assessed through an EQA or proficiency testing.

4. The laboratory should communicate to FIND/WHO about its need to assess its microscopists, before the planned sample collection.

5. FIND/WHO coordinates and requests slides from an identified institution that can provide the required reference slides (e.g. WHO/WPRO slide bank).

6. The following slide sets are recommended:
   For assessment:
   - 20 slides, including at least 6 negatives and at least 10 positives
   - Positives should include different species and mixed infections, with densities >2,000 p/ul.
   - An optional set of 5 positive slides with densities <2,000 p/ul (down to <200 p/ul), for the lab's own interest (results will not be considered in the analysis)

   For refresher training (before the assessment, and for corrective action):
   - Another 25 slides, with the same composition as the above (but from different cases)

7. Upon receiving the slide set(s), the laboratory should implement proficiency testing among its microscopists.

- Each microscopist should examine the slides independently, and individually record the results (negative/positive diagnosis, *Plasmodium* species and parasite density for applicable cases) on printed paper record forms that are as part of the 'Malaria Microscopy Assessment form' (latest version to be obtained by the Project Manager).
- Results should only be shared with the laboratory head or another designated person that is not part of the microscopists to ensure blinded reading among all microscopists (no discussion, nor sharing of results).
- The microscopists should read the slides in a round Robin fashion, i.e., the first microscopist read all the slides before passing them on to the next reader, and so on, until all the microscopists have read the same slide set.
- Each microscopist should be given sufficient time (minimum 10-20 minutes/slide which will make up to about 1 day; or if the microscopist cannot read all of the slides in one sitting, 2-3 days should be allotted per microscopist).
- After all the microscopists have examined the slide set, the laboratory head (or designated staff) should collect the individual result forms and analyse them or send them to FIND for analysis.

8. Entry and analysis of the results will be performed using the latest version of the 'Malaria Microscopy Assessment form', to be obtained from the Project Manager. This form will automatically analyze the results and provide the final scores for each microscopist, according to the following principles:
    - Identification of positives/negatives/species
        - For each diagnosis and species identification, those that are in complete agreement with the "true" diagnosis/species will be considered correct.
    - Parasite density quantitation (to be analysed only for samples with >2,000p/ul)
        - For each parasite density, those within +/-20% difference from the "true" count will be considered correct (this aligns with requirements of the specimen collection procedures).
    - The "true" diagnosis/species and count will be obtained from the slide bank/repository that provided the slides.

9. Microscopists selected for the sample collection should comply with the following requirements, by prioritizing those who obtained the highest scores in both identification/speciation and parasite quantitation:
    - Minimum overall score of 80% in identification (i.e., 80% complete agreement of answers to the "true" diagnosis/species)
    - Minimum overall score of 50% in parasite counting (i.e. 50% of all slides counted are within the -/+20% difference from "true" count)
    - The above benchmarks were selected based on the WHO certification requirement for Level 1 malaria microscopists for parasite counting and level 2 microscopist for species identification.

10. Ideally, the laboratory should implement proficiency testing among its microscopists at least once a year. It should ideally be completed within 2-3 months before a planned sample collection to allow necessary corrective actions.

11. In case of non-compliance, or none of the microscopists achieving the requirements, corrective actions must be implemented. Internal discussion, first within the laboratory, and with FIND/WHO should be done to determine the next steps. Refresher or one-on-one training with one of the complying or qualified microscopists should be considered, using the recommended training slide set, or the EQA slide set if no training slide set is available. After an agreed period, the microscopist should re-read the examination slide set and pass.

12. If the microscopist does not pass this re-assessment, she/he should be considered as priority for refresher training at international level, and/or some training at national level with microscopist(s) having higher qualification level.

13. For the laboratory to proceed with QC sample collection, at least 2 of its microscopists should comply with the minimum requirements to be a reference microscopist for the sample collection activity.

14. The laboratory should keep all documents related to all or any of the above quality assessment activities on malaria microscopy in a designated folder, including certificates, score sheets, reports from EQA provider, etc.

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
14 OCTOBER 2005	2	Routine Revision: major changes made including the addition of details on the structure of the course	RG/KGL/DB
27 MAY 2008	5	Re-numbered from SOP 4.1 (version 4) to SOP 6.05 (version 5). Procedures removed, referenced only	DB/JL/PJ/SI/WO
MAY 2014	7	Introduced a more detailed procedure including requirements for prequalification and microscopy EQA	DB/JL/NC/SI

SOP 6.05 Operation of the Analytical Balance

PURPOSE

This Standard Operating Procedure (SOP) describes the process for weighing samples using the Analytical Balance.

SCOPE

This procedure is part of the methods for general laboratory quality assurance described in the Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests. The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

PROCEDURE

1. Switch on the balance by touching the ON/OFF key. The balance undergoes a brief test, and is then ready for weighing.
2. Open the balance door.
3. When using a weigh boat, reset the balance to zero by touching the TARE key.
4. Place the sample to be weighed on the weigh boat, and close the balance door.
5. Wait until the weight display becomes stable (in some balances, indicated by a stability detector symbol, such as a small ring to the left of the weight display), then the result can be recorded.

REFERENCES

1. Operation of the Mettler Toledo Analytical Balance Standard Operating Procedure. Brisbane, Australian Army Malaria Institute, 2,000 (unpublished report).

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
28 MAY 2008	5	Re-numbered from SOP 6.4 (version 4) to SOP 6.06 (version 5). More general formulation for all balance models.	DB/JL/PJ/SI/WO

SOP 6.06 Pipette Calibration

PURPOSE

This Standard Operating Procedure (SOP) describes the process for calibration of pipettes using the Gravimetric method.

SCOPE

This procedure is part of the methods for general laboratory quality assurance described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

PROCEDURE

A. Calibration of maximum and minimum volumes

1. If an adjustable pipette is to be calibrated, test both the maximum and minimum settings.
2. Place a small amount of distilled water in the analytical balance.
3. Tare the balance.
4. While waiting for the balance to stabilize, aspirate the sample using the forward mode.
5. Open the balance door, add the sample to the beaker, then close the balance door.
6. Record the value after the balance reading stabilizes (use Form 6.04 or equivalent).
7. Repeat steps 3. through 6. for both the maximum and minimum settings 20 times.
8. Calculations
   (a) Calculate the mean, standard deviation, and coefficient of variance for each pipette.
   (b) 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, by contacting/sending pipettes to the Suppliers technical service.

B. Leak test

1. Fit a pipette tip and set the volume at maximum.
2. Aspirate water and maintain liquid 20 seconds in the tip. Observe of a drop or leak appears at the orifice of the tip.
3. Re-immerse the tip in the test liquid. The fluid level in the tip should not descend.
4. If there is a leak, refer to the Suppliers recommendations (users manual or website) for appropriate actions.

REFERENCES

1. Farnell, H. Good Pipetting Practice. International Labmate XXV (V), 2002. (www.internationallabmate.com, date accessed: 17-10-02)
2. Pipette Calibration Standard Operating Procedure. Brisbane, Australian Army Malaria Institute, 2,000 (unpublished report).
3. http://www.gilson.com/ServiceTraining/pipeUsersGuides.asp (date accessed: 13-06-08)

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
28 MAY 2008	5	Added paragraph and reference on leak test.	DB/JL/PJ/SI/WO

SOP 6.07 Incubator Calibration and Maintenance

PURPOSE

This Standard Operating Procedure (SOP) describes the method for calibration and general maintenance of incubators.

BACKGROUND

Accuracy of incubators must be ensured as non-correspondence between dial and actual temperature readings have been noted. Hence, calibration of incubators must be carried out in such cases to ensure the integrity of the incubator settings.

SCOPE

This procedure is part of the methods for general laboratory quality assurance described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

PROCEDURE

A. Temperature Monitoring

1. Calibrated thermometers with appropriate temperature ranges are placed inside incubators for internal temperature monitoring.
2. Temperature monitoring is carried out, as outlined in SOP 6.09 (Equipment Temperature Monitoring).

B. Incubator Calibration

1. An incubator calibration sheet is posted in front of the incubator (Form 6.05 or equivalent)
2. Set the incubator to its lowest dial temperature. Record date, time, dial temperature and signature on the form.
2. Place a calibrated reference thermometer inside the incubator.
3. Allow the incubator to equilibrate for 24 hours.
4. Actual temperature readings are recorded the following day and the dial temperature increased at appropriate intervals.
5. Repeat steps 3. to 4., until prescribed temperature ranges are reached.
6. Designated lab personnel are to update the dial and actual temperature readings at approximately the same time each day (e.g. 08:00 am).
7. Calibration Sheets for each incubator must be kept in a folder for future reference.

C. Incubator Maintenance

1. Incubators must be checked regularly (e.g. at least every 6 months) and documented using Form 6.06.

2. In cases of incubator malfunctioning, lab personnel must report the malfunction to the head of the department, and if indicated, the equipment manufacturer/supplier.

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
28 MAY 2008	5	Added need of calibrated thermometers.	DB/JL/PJ/SI/WO

SOP 6.08 Equipment Temperature Monitoring

PURPOSE

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.

Hence, this SOP describes the procedure for temperature checks, as well as maintenance on all appropriate equipment.

SCOPE

This procedure is part of the methods for general laboratory quality assurance described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

PROCEDURE

1. Calibrated thermometers with appropriate temperature ranges are used for each incubator, refrigerator and freezer in the laboratory. Refer to the thermometer suppliers' recommendations and/or standard protocols for regular calibration and appropriate use of the thermometers.
2. Daily temperature readings are recorded in daily temperature monitoring sheets posted in front of the equipment (Form 6.07 or equivalent).
3. Temperature checks are done at a set time every day by designated lab personnel.
4. Personnel should make arrangements with other staff to perform the temperature monitoring if they are way on annual or sick leave.
5. At the end of each month, daily temperature monitoring sheets are placed in a folder, and arranged in convenient order.
6. Relevant personnel must be immediately (within the day) notified in case of temperature deviations outside acceptable ranges.

REFERENCES

1. Unit Temperature and Maintenance Records Standard Operating Procedure. Brisbane: Australian Army Malaria Institute, 2,000 (unpublished report).

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
28 MAY 2008	5	Added need of calibration of thermometers and of immediate notification	DB/JL/PJ/SI/WO

SOP 6.09 Operation of pH meter

PURPOSE

This Standard Operating Procedure (SOP) describes the method for using a pH meter.

SCOPE

This procedure is part of the methods for general laboratory quality assurance described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

REAGENTS/EQUIPMENT

pH meter

pH 4.0 or pH 10.0 buffer

pH 7.0 buffer

Distilled H2O

Beaker

PROCEDURE

This procedure is specific for pH meter ORION Model 410A. For other pH meter models, refer to the supplier's/manufacturer's recommendations and/or User Manual.

A. Principle

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

B. Measurement and Auto calibration with Two Buffers

1. Select two buffers that cover the range of expected pH. One of the buffers should be near the iso-potential point (pH 7.0) and the other, near the expected sample pH (e.g. pH 4.0 or pH 10).
2. Rinse electrode with distilled water and blot dry.
3. Place electrode on pH 7.0 buffer, then press MODE key. Calibration will be displayed on screen.
4. Press YES. P1 will show on the lower field of the screen.
5. When the electrode is stable, Ready will appear on screen, and the temperature-corrected pH of the buffer is displayed.
6. 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.

7. Rinse the electrode with distilled water, blot dry, then place on the second buffer.
8. When Ready appears, press YES.
9. The pH meter automatically advances to the Measure Mode. Measure is displayed above the main field. Rinse electrode with distilled H2O, blot dry, then place on sample.
10. Once stable, record pH reading from meter display.
11. Rinse electrode with distilled water. Store the electrode in an appropriate and regularly changed storage buffer, as recommended by the pH meter's supplier/manufacturer.

REFERENCES

1. pH Meter Standard Operating Procedure. Brisbane, Australian Army Malaria Institute, 2,000 (unpublished report).
2. Instruction Manual for pH meter ORION Model 410A.

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
28 MAY 2008	5	Added mentions for other pH meter models and for storage of electrode.	DB/JL/PJ/SI/WO

SOP 6.10 Document Control

PURPOSE

To detail the control of Quality Documents for WHO-coordinated malaria rapid diagnostic test quality assurance.

SCOPE

This procedure is part of the methods for general laboratory quality assurance described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests". The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

PROCEDURE

A. General

1. WHO controls the issue, approval and updating of all quality-related documents and data. The Quality Manager's role 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 internal audits adequately address the issue of document control in their Internal Audit Checklists.

2. This SOP applies to:

   Quality Policy

   Methods Manual (Standard operating procedures)

   Forms

   Standards, Acts, Regulations and Codes

   Electronic Data

B. Registers

1. This Methods Manual (SOP) acts as a register for quality documentation such as Forms, SOPs and Work Instructions. Templates are maintained in the Methods Manual as registered forms.
2. A Distribution List is maintained by WHO showing where the copies of the Methods Manual are located.

C. Issue Status

All Quality documentation is to have an issue status in order that obsolete documents can be identified.

D. Amendments/ Raising

Amendments are made only by the authorized WHO officer. Suggested amendments should be communicated to the officer.

E. Obsolete Documents

Obsolete documents are to be removed from the active electronic documentation system and placed in archive folders. Hard copies are to be removed from the department and destroyed or placed in archive folders where their retention is important.

F. Authorisation

Some Quality Documents require Authorisation, for example, Duty Statements and SOPs.

PROCEDURE HISTORY

Date	Version	Comments	Initials
1 FEBRUARY 2004	1	Modified from template	KGL
11 FEBRUARY 2004	1	Routine review, minor typo changes	KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
28 MAY 2008	5	Minor changes only	DB/JL/PJ/SI/WO

SOP 6.11 Document Storage

PURPOSE

This SOP describes the process for storage of documents produced as part of WHO-coordinated malaria rapid diagnostic test quality assurance.

SCOPE

This procedure is part of the methods for general laboratory quality assurance described in the Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests. The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

PROCEDURE

A. Documents

1. All documents generated as part of the RDT-QA are to be archived for 5 years.
2. Records must be legible.
3. If paper-based records are kept, they are to be filed in an organised manner.

B. Computer

1. The computer must be password protected.
2. Records stored electronically are to be well organised.
3. Data stored on the computer must be backed-up regularly (ideally weekly, at least monthly) and the back-up ideally stored in a separate building.
4. An electronic copy should be sent to the Project Manager for archiving.

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	1	Routine Revision, minor changes only	RG
28 MAY 2008	5	Minor changes only	DB/JL/PJ/SI/WO

SOP 6.12 Corrective Action

PURPOSE

This Standard Operating Procedure (SOP) describes the system for recording problems and creating solutions, as part of the WHO-coordinated malaria rapid diagnostic test quality assurance.

SCOPE

This procedure is part of the methods for general laboratory quality assurance described in the Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests. The SOP may be adapted by the head of department to be compatible with pre-existing SOPs and local conditions, retaining the elements of this SOP.

PROCEDURE

1. The Corrective Actions Register (CAR) (Form 6.08 or equivalent) is to be used to record all incidents that impact on the normal operation of the RDT QA laboratory, both administrative and technical. The Register is to be used to record suggestions as to how systems may be improved.
2. When an incident occurs (e.g. test fails to work, results reported incorrectly, incubator tenperature out of range, freezer alarms) the incident is to be noted in the Register and the head of department or Scientist responsible is to be notified.
3. All staff are authorised to record incidents in the Register. Entries are to include a brief description of the incident, action taken to address the issue and staff initials and date.
4. The head of the department is responsible to review the register in their department to familiarise themselves with what has been occurring and then initial the register to indicate that the entries has been sighted and they are familiar with the action taken (this should occur weekly).
5. Where action taken is incorrect or inadequate, the head of the department should provide feedback to the staff member/departmental staff on further action taken.
6. Review of the Register should be an agenda item for all staff meetings as it promotes the culture of continuous improvement and is a useful training tool.
7. The assumption with CARs is that any action initially taken to address the incident is in most cases of a temporary nature and the problem required an investigation into all aspects of the problem, consultation with external parties and the identification of the Cause of the Problem. Once identified, preventative action is put in place.
8. Preventative action often requires significant effort such as changing procedure/forms, raising additional administrative paperwork, development and delivery of training, etc.

PROCEDURE HISTORY

Date	Version	Comments	Initials
14 OCTOBER 2005	1	Draft Introduced	RG/KGL
28 MAY 2008	5	Minor changes only	DB/JL/PJ/SI/WO

SOP 6.13 On-site External Quality Assessment (Supervisory Visits)

PURPOSE

This Standard Operating Procedure (SOP) describes the process of assessment of the performance of laboratories conducting quality assurance (QA) for rapid diagnostic tests (RDTs).

BACKGROUND

A major component of any quality assurance scheme is external quality assessment (EQA). EQA is a process to assess laboratory performance and can be achieved using the following mechanisms:

a. On-site assessments (supervisory visits),

b. Comparison of results of panel testing with another laboratory, and confirmatory testing of routine work by another laboratory,

c. Proficiency assessments (e.g. for malaria microscopy).

This SOP will cover on-site assessments.

A major advantage of on-site evaluations is that assessment of the laboratory occurs under actual working conditions and necessary corrective actions are implemented immediately. It also provides an environment where there is direct contact between staff and the assessor. The major disadvantage of these assessments is that they are quite resource intensive, i.e. travel costs and assessor salary. WHO has developed a Laboratory Assessment Tool (LAT) through the WHO/CSR Lyon office, specifically for RDT QC Laboratory EQA.

SCOPE

This procedure is part of the methods for general laboratory quality assurance described in the "Methods Manual for laboratory quality control testing of malaria rapid diagnostic tests".

PROCEDURE

A. Assessment coordination

1. On-site assessments should ideally be performed annually, and/or before each sample collection, if possible.
2. At present this is coordinated by the Project Manager, using an external evaluation by means of a Laboratory Assessment Tool designed for this purpose.
3. The person selected to perform the assessment must be appropriately trained in using the LAT and have considerable expertise, either in malariology/parasitology (ideally in malaria diagnosis), or in laboratory quality assurance, or ideally in both fields.
4. The assessment should be coordinated with the laboratory regarding proposed dates. This ensures the relevant staff will be present at the time of the evaluation.

B. Assessment process

1. The evaluation is carried out by using a Laboratory Assessment Tool in MS Excel format, and by following the guidelines of the associated LAT User Manual (up-to-date versions of these documents are provided by the Project Manager). 2. The assessor should first explain the purpose and content of the assessment to the laboratory staff, then make a general visit of the laboratory.
3. The checklists of the LAT are covering the following areas: lab information, general conditions of infrastructure, workplace conditions, staff and supervision, general laboratory quality assurance, internal and external quality control, safety, QC samples preparation, quality control of RDTs and general RDT quality assurance.
4. For laboratories doing lot testing, all the questionnaire sheets (areas) should be covered, while for laboratories doing sample collection only, the assessment can be restricted to the following areas: lab information, general conditions of infrastructure, workplace conditions, internal and external quality control, QC samples preparation. For laboratories doing lot testing, the assessment should be done on an annual basis, while for laboratories doing sample collection only, it should be carried out before or right at the start of the collection campaign, as much as possible, and feedback should be given immediately on-site, especially for all critical points (flags).
5. The same checklists are used each year to assess improvement in performance over time.
6. At the end of the evaluation, the checklists are discussed in a constructive manner with the staff of the laboratory. The assessor should highlight major strengths and major weaknesses to the laboratory staff. Immediately recommended corrective actions should be discussed where needed.
7. The assessor then completes the report and summary sections of the LAT.
8. The final version of the completed LAT can eventually be sent to laboratory staff to cross-check again for avoiding any misunderstandings or major disagreements between the assessor and the laboratory staff.
9. A copy of the completed LAT is forwarded to the supervisor of the laboratory at the end of the assessment and the Project Manager.
10. Required improvements and corrective actions should be discussed between the assessor, the Project Manager and the laboratory supervisor. It should be agreed on eventually required assistance by WHO or FIND or other organizations, in terms of subventions, equipment/material supply, training of laboratory staff, etc.
11. The interpretation of the general EQA indicator (GEI) and appropriate decisions is described in the external quality assurance program procedure in chapter 2.

REFERENCES

1. World Health Organization. Quality Assurance of Sputum Microscopy in DOTS Programmes. Regional Guidelines for Countries in the Western Pacific. Geneva, World Health Organization, 2003.
2. User's Manual, EQA Laboratory Assessment tool, WHO Malaria RDT Evaluation Programme, July 2013 (unpublished document).

PROCEDURE HISTORY

Date	Version	Comments	Initials
13 NOVEMBER 2002	D	Draft Introduced	RG/DB
27 NOVEMBER 2002	1	Version 1 introduced	DB
22 DECEMBER 2003	1	Routine review, minor format and typo changes	RG/KGL/DB
15 OCTOBER 2004	1	External on-site assessment, minor changes only	KGL
14 OCTOBER 2005	2	Routine review: Form 001 expanded	RG
AUGUST 2006	4	Revised to LAT	DB
27 MAY 2008	5	Re-numbered from SOP 2.4 (version 4) to SOP 6.13 (version 5), Added more detail on the assessment process, mentioned discussion of corrective actions and eventual assistance. Added reference to the LAT User Manual.	DB/JL/PJ/SI/WO
MAY 2014	7	Reference to the EQA Programme procedure in chapter 2, specified EQA in lot testing versus sample collection laboratories, updated reference to the user's manual	NC/SI

Methods Manual for Laboratory Quality Control Testing of Malaria RDTs

Chapter 7: FORMS

Institute: _____

2.01: Responsibilities of RDT-QC staff

This form is optional and is to be used only if the institute organization requires such tracking

Task	Responsible staff (names)	Contact details (phone number, email)	Documents to be provided and explained
Front desk receipt (contact responsible lab staff, store at ≤25°C)			**RDT register (SOP 2.01, 2.02, Form 2.03)**
Receipt in lab (inspection, register, labelling, appropriate storage)			**RDT register and storage (SOP 2.01, 2.02, 2.03)**
QC of RDTs (QC planning, performing QC, reporting results) and entering results in the database			**Chapter 2**
QC of RDTs (replacement staff)			**Chapter 2**
Supervision of RDT QC activity			**Chapter 2**

Institute: _____

(signing QC reports)			

Institute: _____

2.02: Lot-testing Request Form

Form 2.02: Malaria RDT Lot Testing Request Form

REQUESTING DETAILS

	WHO IS SENDING THE RDTs?	WHO IS REQUESTING THE TESTING? (final recipient of the report)
MANUFACTURER		
PROCUREMENT AGENCY		
INSTITUTION		
NGO		
COUNTRY		
OTHER		

Minimum number of RDTs required per lot: **Pf-only RDTs :** 100 tests
Combination RDTs : 150 tests

RDT DETAILS

RDT PRODUCT NAME (As in product insert)	MANUFACTURER	CAT. NUMBER	LOT NO.	MANU. DATE dd/mm/yyyy	EXP. DATE dd/mm/yyyy	NO. OF BOXES	NO. OF TESTS/ BOX	LOT SIZE (n° of RDTs manfactured per lot)	LOT SIZE (N°. of RDTs sent per country)	Country where the RDTs will be sent to (If known)

(delete/extend rows as needed)

CONTACT DETAILS FOR RECEIPT OF RESULTS: (Delete/extend columns as necessary)

CONTACT NAME			
POSITION			
INSTITUTION/ADDRESS			
TEL. /FAX NO.			
EMAIL ADDRESS			
COMMENTS			

NOTE : This form should be sent by email prior to sending the RDTs to Malaria_rdt@who.int. Include also a hard copy with the RDTs. A summary of results report will be published regularly and this will include the product name but the procurer agency name will be excluded.

Institute: _____

2.03: RDT Front Desk Register (Optional)

For boxes addressed to RDT QC laboratory

Received by (sign)	Date received (dd/mm/yy)	RDT product name	Catalog number	Storage temperature	Label on box, comments

Institute: _____

2.04: RDT Register

RECEIPT										DESCRIPTION	
Received by:	Date received	Received from	Name of RDT	Source Manufacturer	Catalog number	Lot/ Batch	Expiry	Quantity received	Storage T°	Condition and type of packaging	Other Comments
Sign	dd/mm/yy	Sender	Product name	Manufacture, country			dd/mm/yy	Boxes/ tests per box		Cooler box or not ? Damage or not?	e.g. temperature monitor included?

Institute: _____

2.05: Storage and Internal Movements of Malaria RDTs

Initial storage area

(e.g. incubator n°1): _____

Storage temperature: _____

RDT product name e.g. ICT Diagnostics Malaria Combo	Catalog number e.g. ML02	RDT Lot / batch e.g. 50124	Reception Date dd/mm/yy	Stored by: Sign	Date stored dd/mm/yy	Quantity stored Boxes/ tests per box	Moved by: Sign	Date moved dd/mm/yy	Storage area / T° e.g. incubator n°2 / 28°C	Quantity moved Boxes/ test per box	Comment e.g. insufficient space

Institute: _____

2.06: RDT Dispatch

RDT product name e.g. ICT Diagnostics Malaria Combo	Catalog number e.g. ML02	RDT Lot / batch e.g. 50124	Reception Date dd/mm/yy	Dispatched by: Sign	Date dispatched dd/mm/yy	Destination e.g. IPC, Cambodia	Quantity dispatched Boxes/ test per box	Transport condition e.g. cold box with temp. monitor

Institute: _____

2.07: RDT QC Results Sheet

Month 0 testing (initial testing):

☐ Primary testing laboratory
☐ First testing (with initial QC samples)
☐ Confirmatory testing laboratory
☐ Repeat testing (with different QC samples)
☐ Testing with stock RDT (validation of QC samples)

RDT product name (lot RDT or stock RDT)	Manufacturer	Catalog n°	Lot Number	Expiry date	Reception date

Date of Testing ___/___/____(dd/mm/yy) Technician Signature_____

Sample ID	Dilution (parasites per µl)	Pf eg.2+	Pan eg.1+	Pv eg.NA	Control e.g. 3+	Result e.g. Pf pos	Comments e.g. blood flow problem
Neg. control:	0						
Neg. control:	0						
Neg. control:	0						
Neg. control:	0						
Neg. control:	0						
Neg. control:	0						
Neg. control:	0						
Neg. control:	0						
Neg. control:	0						
Neg. control:	0						
Pf:	200						
	200						
	200						
	200						
	200						
	200						
Pf:	200						
	200						
	200						
	200						
	200						
	200						
Pf:	200						
	200						
	200						

Institute: _____

	200						
	200						
	200						
Pf:	200						
	200						
	200						
	200						
	200						
	200						

Institute: ____

Pv:	200						
	200						
	200						
	200						
	200						
	200						
Pv:	200						
	200						
	200						
	200						
	200						
	200						
Pv:	200						
	200						
	200						
	200						
	200						
	200						
Pv:	200						
	200						
	200						
	200						
	200						
	200						

2.08: RDT Quality Control Report

In word format as follows, or created through the lot-testing database (same information/content)

Malaria RAPID DIAGNOSTIC TEST

Quality Control Report

(Lot testing)

Place Institutional Logo Here

Report prepared by: name(s)	
QA-RDT network laboratory (Institute):	
Institution that requested the RDT-QC: name	
Place from where tests were sent: Institution, town, country	
Contact: email(s)	
For the attention of: name(s)	
Date of report: dd/mm/yyyy	

Interpretation of results: see last page

RDT product name: as per product insert	
Catalog No:	
Manufacturer: name	
Date received: dd/mm/yyyy	
Place received:	
Transport method: Sending Institution to Testing Institution	
Storage condition during transport: Sending Institution to Testing Institution	
Condition of RDT's on receipt:	

Kit content (Please cross out)

☐ Buffer	☐ Lancet	☐ Alcohol swab	☐ Blood transfer device
Other:			

Lot No:	Expiry: dd/mm/yyyy or mm/yyyy	N° of Boxes received	N° of tests / box	Testing interval	Result

Insert additional rows if necessary

Summary of results:

Testing Interval	Temp. of storage (°C)	Date tested dd/mm/yyyy	Product (lot N°)			
			Result	% positive results		Observations
				Pf *200*	*Pv* *200*	
6 months before expiry						
0 month						

Notes: expand with new rows as necessary at time of report, Result = Pass', 'Deferred', 'Fail',

% positive result: total % of RDTs testing positive for a given Plasmodium species (Pf = P. falciparum, Pv = P. vivax) at a given parasite density (200 parasites/µL).

Method:

1. Definition of routine and non-routine testing

Non-routine : testing against quality control samples (malaria positive and/or negative) that is prompted by quality concerns post deployment of the product in the field. Standard protocols may be modified as per the availability of recovered RDTs.

Routine: testing against quality control samples according to standard procedures either pre or post shipment or post deployment (in absence of quality concerns).

2. QC testing Method:

RDTs were tested with frozen QC samples based on the algorithm described in SOP 2.06 of the WHO Quality Control Methods Manual for Malaria RDTs. An accessory assessment is performed and released with the report and photos of the testing carried out. For a lot of RDTs to pass the QC assessment, all quality control dilutions must be positive (100%) and essential accessory to perfom the testing, such as buffer, must be available in sufficent quantity. RDTs which do not meet these criteria will be forwarded to a second laboratory for confirmation or will immediately fail in case of insufficient buffer to perform the testing. Testing results with false positive rate of ≥10% agains negative QC samples is considered as failure - no retesting will be done at confirmatory laboratory. Additionally, false positive results for the wrong Plasmodium spp will be reported in the 'Observations' section.

3. Samples used for QC testing:

Quality control (QC) samples of dilutions from wild-parasites prepared according to SOP 3.08 of the WHO Quality Assurance Methods Manual for Malaria RDTs. Samples are stored at -70°C.

Samples used include:

a) Negative control: 0 parasites/µl of *Plasmodium spp.*
b) Low Positive Control: 200 parasites/µl of *Plasmodium falciparum*
c) Low Positive Control: 200 parasites/µl of *Plasmodium vivax**

4. RDT preparation method:

RDTs were tested as per manufacturer instructions, using micropipette for blood transfer.

Details of RDT QC testing results: ☐ month testing

EXAMPLE RESULT TABLES. USE ONLY ONE TABLE. TABLE CAN BE EXTENDED TO INCLUDE MULTIPLE LOTS OF THE SAME PRODUCT. (ALWAYS USE DIFFERENT REPORTS FOR DIFFERENT PRODUCTS)

Table 1: Initial Testing

Quality control dilutions		***Product (lot):***			***Comments***
Sample ID	(parasites/µl)	RDTs Tested	RDTs Positive	% Positive	
Pf	200	6			
Pf	200	6			
Pf	200	6			
Pf	200	6			
Pv	200	6			
Pv	200	6			
Pv	200	6			
Pv	200	6			
		RDTs Tested	RDTs negative	% negative	
Negative control *List sample IDs*	0	10			

Table 2: Repeat Testing with different QC panels

Quality control dilutions		***Product (lot):***			***Comments***
Sample ID	(parasites/µl)	RDTs Tested	RDTs Positive	% Positive	
Pf	200	6			
Pf	200	6			
Pv	200	6			
Pv	200	6			
		RDTs Tested	RDTs negative	% negative	
Negative control *List sample IDs*	0	1			

Table 3: Repeat Testing of QC samples against Stock RDTs to ensure QC sample integrity

Quality control dilutions		***Product (lot):***			***Comments***
Sample ID	(parasites/µl)	RDTs Tested	RDTs Positive	% Positive	
Pf	200	2			
Pf	200	2			
Pv	200	2			

Pv	200	2			
		RDTs Tested	RDTs negative	% negative	
Negative control *List sample IDs*	0	1			

Note: Delete Pv sections if not applicable.

5. Interpretation of results:

For a lot of RDTs to pass the QC assessment, all positive quality control dilutions must be positive (100%), false positives against negative samples must be <10%, and essential accessories, such as buffer must be available in sufficient quantity to perform the testing. False positive results for the wrong Plasmodium spp should be taken into account when interpreting results.

Interpretation of results:

- PASS: This RDT lot passed the quality control test and the RDT sample assessed detects antigen at a threshold SUFFICIENT FOR USE in the field.
- DEFERRED: This RDT lot failed this assessment on quality control dilutions, 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 QC assessment and also failed confirmatory testing at another institution. It is recommended that this lot should **NOT BE USED** in the field as it has been assessed as lacking sufficient sensitivity. This RDT lot could not be tested due to buffer evaporation thus declared as a failure. It is recommended that the manufacturer be contacted and advised of the results. Further, testing results with ≥ 10% false positive rate against negative samples and >5% invalid observations are both considered as failure.

NB: Non-routine testing often does not follow standard protocols for a variety of reasons and therefore results are not assigned PASS/FAIL, rather the results and all observations are presented and can be used to corroborate or refute findings in the field which triggered the initial testing.

Note:

This assessment is performed in collaboration with the World Health Organization (WHO) and the Research Institute for Tropical Medicine (RITM). The report is prepared for the confidential information of the institution that submitted these Rapid Diagnostic Tests (RDTs) for assessment. The results are for use of the institution that submitted the RDTs for assessment as evidence that the stored samples of the particular lot of RDTs tested performed with sufficient sensitivity for use. They must not be used for purposes of advertising or otherwise promoting a product, or as evidence of formal approval or recommendation of a product, without the written permission of the testing institution and World Health Organization. Other than confirmation of sufficient sensitivity of the sample of the tested lot, the results listed here do not indicate endorsement of the RDT product by the World Health Organization or the testing institution. While the results indicate that the RDTs tested detect antigen to an acceptable threshold in the QC parasite samples used for testing, they do not necessarily reflect actual sensitivity in the field where local storage conditions, variation in parasite antigen, and host factors may affect operation. Lot tesing is performed using well characterized cryopreserved parasites samples prepared from malaria cases in endemic countries (see "Method Manual for Laboratory Quality Control Testing of Malaria Rapid Diagnostics Tests") on the WHO website. Recommendations on use and storage of RDTs in the field can be obtained from the WHO website https://www.who.int/teams/global-malaria-programme/case-management/diagnosis/rapid-diagnostic-tests, or by email from Malaria_rdt@who.int.

Signed:

Technician **Laboratory head**

Copies of report:

Include email copy to: Requesting Institution.

WHO Lot-testing coordinator

Hard copy to be retained by testing institute

2.09: Accessory Assessment form

Date: LTR #: _____

RDT KIT

RDT (name/brand)	Manufacturer	Product Code

RESULTS (check the box of the relevant answers)

If more than one lot of the same product is sent as part of the same lot testing request, only one lot is to be tested (selected randomly)

	Results	Comments
Instructions for Use (Initial testing only)		
In English and other common language?	YES ☐ No ☐ NA ☐	
Target malaria species specified? *	YES ☐ No ☐ NA ☐	To be specified here
Target antigens specified for each test line?	YES ☐ No ☐ NA ☐	To be specified here
Picture showing target antigen and/or species for each test line?	YES ☐ No ☐ NA ☐	
Volume of blood specified?	YES ☐ No ☐ NA ☐	To be specified here
Use of blood transfer device explained?	YES ☐ No ☐ NA ☐	
Type of blood transfer device	☐ Inverted cup ☐ Loop ☐ Pipette	
Picture/text explaining appropriate well for blood?	YES ☐ No ☐ NA ☐	
Volume of buffer specified (number of drops or volume)?	YES ☐ No ☐ NA ☐	To be specified here
Use of individual buffer ampoules explained?	YES ☐ No ☐ NA ☐	
Picture/text explaining appropriate well for buffer?	YES ☐ No ☐ NA ☐	
Reading time specified?	YES ☐ No ☐ NA ☐	To be specified here
Text AND picture explaining test results interpretation for:		
- Each of the detected species (positive)	YES ☐ No ☐ NA ☐	
- Negative result	YES ☐ No ☐ NA ☐	
- Invalid test result	YES ☐ No ☐ NA ☐	

Accessories **To be assessed for each lot***		
Alcohol swabs in intact envelopes, and humid	YES ☐ No ☐ NA ☐	
Dessicant confirms there is no exposure to humidity	YES ☐ No ☐ NA ☐	
Sufficient buffer volume for testing	YES ☐ No ☐ NA ☐	
Same color for all buffer vials	YES ☐ No ☐ NA ☐	

*in case of anomaly (ies), the lot number concerned is to be specified in the comment field related to the anomaly

FINAL APPRECIATION:

All assessment items are successfully fulfilled: ( )

Some assessment items are not fulfilled: ( )

Number of unfulfilled assessment items: ___ out of ___

Comments: _____

_____

_____

Signed:

Technician Laboratory Head

Institute: _____

3.01: Preparatory activities for RDT QC Sample Preparation

Note: the below timelines are indicative and may need to be adapted based on local context.

Activities	Date Checked/ Accomplished	Signature of Responsible Staff	Remarks
Month 1			
1. Obtain ethical clearance.			
2. Purchase or ensure availability of materials, reagents and equipments. Complete **Form 3.02**.			
3. Perform QC testing of malaria screening RDTs.			
4. Pre-qualify two microscopists, provide certificates.			
5. Review potential field collection sites and choose.			
6. Determine availability of field and lab staff.			
Month 2			
7. Coordinate with local authorities and clinic heads of the recruitment site(s).			
8. If field collection site is far: - arrange for nearby laboratory with adequate equipment,			
- book travel tickets and accomodation,			
- arrange for QC sample transport at -70°C (dry ice).			
9. Identify potential donor(s) or blood bank(s) for *Plasmodium*-negative blood and AB+ fresh frozen plasma.			
10. Arrange for nearby hematology services.			
11. Arrange for hep. B/C and HIV testing by ELISA.			
12. If needed, arrange for hep. B/C and HIV RDTs.			
13. Arrange for HIV counseling/results management.			
14. Check supplies/equipment purchases (Form 3.02)			
15. Arrange for sufficient storage space at -70°C.			
Month 3			
Week 1 & 2			
16. Final check of arrangements with all partners.			
17. Final check of supplies/equipments (Form 3.02).			
18. Check quality of: - Giemsa stain,			
- micropipettes (calibrate if needed),			
- equipments (proper functioning).			

Institute: _____

19. Brief staff involved on all procedures and forms, delegate responsibilities and complete **Form 3.03**, distribute copies of necessary procedures and forms.			
Week 3 & 4			
20. Prepare supplies/equipments in the working area.			
21. Secure donor blood and plasma; store properly; perform hepatitis B/C and HIV screening.			
22. Print required number of all forms			

Institute: _____

3.02: Supplies and Equipments Checklist

Note: the below quantities are indicative and may need to be adapted based on local context and particular collection needs, as discussed with the project manager.

1. Please check supplies every three months.
2. Three months before the QC sample preparation campaign, check if all ordered items have arrived, if not, chase up the supplies.
3. The day prior to the field trip, pack up all items and check off in final column.

Supply / Equipment	Stock required (Example only)	STOCK AVAILABLE	Amount ordered	Date ordered	Company ordered from	Date received	Final check (tick)
Field collection site							
A. Blood collection							
5 or 10 mL EDTA tubes	100 - 200						
5 mL plain tubes (no additives)	50						
Lancets	200						
Vacutainers and adaptors	30						
Vacutainer needles	30						
21g Needles	30						
23g Needles	30						
10 mL syringes	30						
5 mL syringes	30						
Cotton balls	100						
Tourniquet	1						
Alcohol Swabs	100						
B. Slides and blood spots preparation							
Microscopy slides	100						
If blood spots are prepared in the field:							
Filter Paper (Whatmann 3M) for blood spots	50 pieces for 2 spots						
Small individual plastic bags for filter paper.	50						
Dessicant (if not taken from RDT pouchs)	For 50 plastic bags						

Institute: _____

Supply / Equipment	Stock required (Example only)	STOCK AVAILABLE	Amount ordered	Date ordered	Company ordered from	Date received	Final check (tick)
C. RDTs and/or Microscopy							
Malaria pLDH RDTs	e.g. 150						
Malaria HRP2 or pfLDH RDTs +/- aldolase	e.g. 150						
20 µL micropipette	1						
20 µL pipette tips	e.g. 150						
HIV, HepatitisB and C RDTs (optional)	*e.g. 50*						
Micropipette and tips as needed for virus RDTs	*1 pipette, e.g. 150 tips*						
Microscopy slides	200						
Staining container/jar/tray	2						
Giemsa Stain (stock), buffered water	500 mL						
Drying rack	2						
Slide trays	2						
Slide boxes	2						
D. Other supplies for field site							
Sharps containers	2						
Gloves - small, medium, large	≥1 box each						
Pens – variety of pens, pencils, marker pens							
Paper towels							
Coolers with ice packs for storage/transport							
E. Forms for field site							
Forms 3.04 -Consent form							
–Form 3.05 - Patient Screening							
–Form 3.06 - Patient Record							
–Form 3-07 - Venepuncture							

Institute: _____

Supply / Equipment	Stock required (Example only)	STOCK AVAILABLE	Amount ordered	Date ordered	Company ordered from	Date received	Final check (tick)
Laboratory where QC samples are processed							
A. Pipetting equipments							
Multi-dispense pipette (50 –100 µL capacity)	2						
Tips for multi-dispense pipette (dispense of 50µL)	50 - 100						
20 µL micropipette	2						
200 µL micropipette	2						
1000 µL micropipette	2						
Pipette tips	500 per pipette						
Wide-bore pipettes or disposable sterile pipettes (5, 10 and 20 mL)	100 each						
Pipette aid/pump/ gadget (allowing slow dispensing)	2						
B. For dilution/ mixing of blood							
Sample rotator or rocker	1						
Sterile 2 mL tubes (round bottom)	100						
Sterile 15 mL tubes (round bottom)	100						
Sterile 50 mL tubes (conical bottom)	100						
Group AB Fresh Frozen Plasma	Equiv. 1 L						
Group O+ or O- donor blood	Equiv. 1 L						
C. For preparing QC sample aliquots							
Sarstedt cryotubes 0,5 ml with external screw cap and O-ring	15,000 – 20,000 pcs.						

Institute: _____

Supply / Equipment	Stock required (Example only)	STOCK AVAILABLE	Amount ordered	Date ordered	Company ordered from	Date received	Final check (tick)
Foam racks	10 (for ≥1200 cryotubes)						
Freezer boxes (10x10)	As required						
Dry Ice *(if QC aliquots transport from distant lab to QA-RDT lab)*	As required						
D. For testing/ characterization of QC samples							
Microscopy slides	100						
Cover slips	100						
Saline solution (0,9% w/v NaCl)	100 mL						
Malaria pLDH RDTs	e.g. 100						
Malaria HRP2 RDTs +/- aldolase	e.g. 100						
Earl Perez slides	e.g. 100						
If blood spots are prepared in the lab :							
Filter Paper (Whatmann 3M)	50 pieces for 2 spots						
Small individual plastic bags for filter paper.	50						
Dessicant (if not taken from RDT pouchs)	For 50 plastic bags						
E. Other supplies for laboratory							
Gloves - small, medium, large	≥3 boxes each						
Refrigerator thermometers	3						
Freezer thermometers	2						
Extension cord with appropriate connections	1						
Sharps containers	2						
Ethanol 70%	250 mL						
Pens – variety of pens, pencils, marker pens							

Institute: _____

Supply / Equipment	Stock required (example only)	STOCK AVAILABLE	Amount ordered	Date ordered	Company ordered from	Date received	Final check (tick)
F. Forms							
Form 3.08 – Parasite-free blood preparation	10						
Form 3.09 – Microscopist 1, read 1	30						
Form 3.10 - Microscopist 2, read 1	30						
Form 3.11 - Microscopist 1, read 2	30						
Form 3.12 - Microscopist 2, read 2	30						
Form 3.13– Density & Dilution Calculation	30						
Form 3.14 - Dilution Preparation	30						
Form 3.15 – RDT results sheet	20						
Form 3.16 - Checklists	30						
G. Equipments							
Microscope with 100x oil immersion objective	2-3						
Manual counter	2-3						
Water bath (37°C)	1						
Refrigerator 4ºC	1						
Freezer –20ºC	1						
Freezer -70°C (sufficient space for all QC sample aliquots)	1						
Centrifuge (for 1-2 mL tubes)	1						
Centrifuge (for 2-50 mL tubes)	1						
HAEMATOLOGY ANALYSER FOR BLOOD CELL COUNTS (OR MANUAL COUNT)	1 (evtl. easy access in other lab)						

3.03: Staff Responsibilities

Date: ___/___/_____ (dd/mm/yyyy)

Tasks	Responsible staff (name)	Documents to provide and explain
Coordination of field work, communication with lab staff	**Phone:**	Chapter 3
Interview of patient (selection for malaria screening)		Chapter 3, Part 1 and Part 2
Malaria screening (RDT / microscopy)		Chapter 3, Part 1 and Part 2
Patient information, counseling, obtain signed consents		Chapter 3, Part 1 and Part 2
Virus screening with RDT (hepatitis B/C, HIV)		Chapter 3, Part 1 and Part 2
Venepuncture, preparation of thick/thin films and blood spots		Chapter 3, Part 1 and Part 2
Coordination of lab work, communication with field staff	**Phone:**	Chapter 3
Procurement and preparation of "parasite-free" blood		Chapter 3, Part 1 and Part 3
White blood cell counts and eventual blood group tests		Chapter 3, Part 1 and Part 3
Preparation of serum for virus serology (hep.B/C, HIV)		Chapter 3, Part 1 and Part 3
Preparation of blood spots (if not prepared in the field)		Chapter 3, Part 1 and Part 3
Microscopist 1 (pre-qualified)		Chapter 3, Part 1 and Part 3, Chapter 4
Microscopist 2 (pre-qualified)		Chapter 3, Part 1 and Part 3
Serologies of hep.B/C, HIV (eventual partner lab)		Chapter 3, Part 1 and Part 3
Dilution Calculations with MS Excel calculator		Chapter 3, Part 1 and Part 3
Dilution of blood (pipeting, mixing)		Chapter 3, Part 1 and Part 3

Institution: _____

Clumping test mixture and clumping tests (microscopy)		Chapter 3, Part 1 and Part 3
RDT of QC sample dilutions		Chapter 3, Part 1 and Part 3
Labeling of aliquots		Chapter 3, Part 1 and Part 3
Aliquoting and freezing		Chapter 3, Part 1 and Part 3
Check-up / signature of forms (supervisor)		Chapter 3

3.04a: Information Sheet and Consent Forms for study participation

Draft Minimum Standard Consent Form

- *To be modified to fulfill local requirements and national regulations, retaining each element below.*
- *Further consent or counseling is required for HIV testing, according to national regulations (Form 3.04b).*
- *Initial finger-prick blood screening may be included if this is not part of normal clinical practice for the presenting symptom.*

Patient Information and Consent- # 1

….insert: Institution, city, country…..

Collection of Wild type Plasmodium falciparum from Clinical Samples for the Development of Panels for Quality Assurance of Malaria Rapid Diagnostic Tests

Patient Information

Introduction: Fever is a common way for malaria to present but not all fevers are caused by malaria. The World Health Organization (WHO) recommends that all people suspected to have malaria have a test to confirm it, prior to being given treatment. Thus, it is very important that malaria diagnostic tests work well because health workers rely on them to make decisions about appropriate treatment.

Purpose:

I am …… and I work with the ….. (insert: Institution, city, country)….

The WHO and the Foundation for Innovative New Diagnostics (FIND) is working with the **….***(insert: Institution, city, country)* …. to make sure that the malaria tests we are using are working well. We do this by collecting and storing samples of blood from people who have malaria and then intermittently using these blood samples to see if the malaria tests we buy and use are working (give a positive result) or not working (give a negative result when they shouldn't). Today you have presented with a fever and could have malaria. I am going to give you information and invite you to be part of this sample collection, because we think that a sample of your blood could help us in the future to check if malaria tests are working.

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change. If you choose not to participate in this research project, you will receive the treatment that is routinely offered in this clinic/hosptial for fever including … (insert malaria tests, treatments and any other item according to national guidelines for fever management)…. You may change your mind later and stop participating even if you agreed earlier.

Procedures:

If you agree to take part in the research, there will be two steps:

First, we need to find out if you have malaria, this will be done using a 'rapid diagnostic test'. The blood is taken from a finger prick.

If the malaria test is negative, you will be referred for care for non-malaria illness and your participation in this project is ended. However if the malaria test is strongly positive, we will ask you if you agree to contribute to a project to assure that malaria tests are working well worldwide, by agreeing to the following:

i) Answer some questions regarding recent treatment/medicines that you've received

ii) Provide a blood sample (15 ml or three teaspoons) taken from your vein that will be tested for other types of infection (see below) and stored in a freezer for use in the future to tell if malaria rapid diagnostic tests are working properly and, also, if you agree, for future research to improve diagnosis of malaria.

ii) Using the same blood sample, allow for additional tests for infection that include hepatitis and HIV infection and will be done only after you have been counselled and have had a chance to ask questions. If you refuse counselling related to HIV testing or to have HIV testing done, then you cannot participate in this project. If you agree to HIV testing and the other required tests, then should one of those extra tests be positive, you will receive counselling and be referred for appropriate care.

After you have donated the 15 ml of blood, you will receive treatment with *…..(insert treatment according to national guidelines)*…

Benefits:

There will be no direct benefit from your taking part in this initial part of the project but it will also not incur any costs and if you agree eventually to donate your blood then your participation may result in public health programmes being sure that the malaria tests used are of good quality and give accurate results. Furthermore, **by permitting us to use your blood for future malaria diagnostic research, you will be making available samples that can be used to develop new and improved methods to diagnose malaria.**

Risks and discomfort:

The risks involved in this study are minimal. They include the discomfort of a slight delay in the treatment, drawing a sample of blood, rare bruising and infection at the site of lancet stick. New lancet and needles will be used for each patient so there is no risk for transmitting diseases.

Compensation:

There will be no compensation to you if you decide to take part in this study.

Confidentiality:

All information that you provide will be considered confidential, and no mention of your name or any other identifying information will appear on the stored samples or in any publication in connection with this study. The information will NOT be stored together with the samples. Only the research staff and the health care workers overseeing your immediate care will have access to any information that identifies you individually. Only these persons will have the key to link the samples and the information attached to your name for the purposes of returning your test results.

Right to Refuse or Withdraw:

You may also choose that you do not participate in this study and you may refuse to participate at any time without penalty or loss of benefits to which he/she would otherwise be entitled. You do not have to explain why you do not wish to participate or withdraw.

Contact information:

If you have any questions, or if any problems arise, you may contact:

….(insert contact information for collecting institution)….

If you have additional questions you may also contact:

….(insert contact information for local ethics committee)….

Certificate of consent # 1

I have been invited to participate in a research project to help ensure malaria tests are working well. I agree to be screened for malaria by a rapid diagnostic test (RDT) using a blood test and if it is strongly positive (or if negative in the first 15 cases) then I will be asked later to agree to be asked some questions about my health complaints and recent treatments and to give a blood sample and have additional testing for other infections. I have read the foregoing information (or it has been read to me). I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate in this **first** part of the study.

Print Name of Participant_____

Signature of Participant_____

Date _____

Day/month/year

If illiterate (Statement of witness)

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness _____***AND*** ***Thumb print of parent***

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the person understands that the following will be done:

1. That a malaria test will be performed using finger prick blood.

2. That he/she will be asked later to consent to other procedures including answering questions about his/her health, collecting a blood sample and having other testing including HIV and hepatitis done.

3. That the appropriate treatment for the condition will be provided after the blood sampling.

I confirm that the potential participant was given an opportunity to ask questions about the study, and all the questions asked have been answered correctly to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent _____

Signature of Researcher /person taking the consent _____

Date _____

Day/month/year

Patient Information and consent #1

for parents/guardians of children 5-18 years

….insert: Institution, city, country…..

Collection of Wild type Plasmodium falciparum from Clinical Samples for the Development of Panels for Quality Assurance of Malaria Rapid Diagnostic Tests

Parent Information

Introduction:

Fever is a common way for malaria to present but not all fevers are caused by malaria. The World Health Organization (WHO) recommends that all people suspected to have malaria have a test to confirm it, prior to being given treatment. Thus, it is very important that malaria diagnostic tests work well because health workers rely on them to make decisions about appropriate treatment.

Purpose:

I am …….. and I work with the ….(insert Institution, city, country)….

The WHO and the Foundation for Innovative New Diagnostics (FIND) is working with ….(insert Institution, city, country)….. to make sure that the malaria tests we are using are working well. We do this by collecting and storing samples of blood from people who have malaria and then intermittently using these blood samples to see if the malaria tests we buy and use are working (give a positive result) or not working (give a negative result when they shouldn't). Today your child has presented with a fever and could have malaria, I am going to give you information and invite you to allow your child to be part of this sample collection, because we think that a sample of his/her blood could help us in the future to check if malaria tests are working.

Your child's participation in this research is entirely voluntary. It is your choice whether he/she participates or not. Whether you choose for him/her to participate or not, all the services he/she receives at this clinic will continue and nothing will change. If you choose for your child not to participate in this research project, he/she will receive the treatment that is routinely offered in this clinic/hosptial for fever including … (insert malaria tests, treatment and any other item according to national guidelines for fever management)…. He/she may change his/her mind later and stop participating even if he/she agreed earlier.

Procedures:

If your child agrees to take part in the research, there will be two steps:

First, we need to find out if your child has malaria, this will be done using a 'rapid diagnostic test'. The blood is taken from a finger prick.

If the malaria test is negative, you will be referred for care for non-malaria illness and your participation in this project is ended. However if the malaria test is strongly positive (or if it is negative and your child is one of the first 15 patients with fever that we test), we will ask you if you agree for him/her to contribute to a project to assure that malaria tests are working well worldwide, by agreeing to the following:

i) Answer some questions regarding recent treatment/medicines that your child has received

ii) Provide a blood sample (15ml or three teaspoons) taken from your child's vein that will be tested for other types of infection (see below) and stored in a freezer for use in the future to tell if malaria rapid diagnostic tests are working properly and, also, if you agree, for future research to improve diagnosis of malaria.

ii) Using the same blood sample, allow for additional tests for infection that include hepatitis and HIV infection and will be done only after you and your child have been counselled and have both had a chance to ask questions. If you/your child refuse counselling related to HIV testing or to have HIV testing done, then your child cannot participate in this project. If you agree to allow your child to have HIV testing and the other required tests, then should one of those extra tests be positive, you and your child will receive counselling and he/she will be referred for appropriate care.

After your child has donated the 15 ml of blood, he/she will receive treatment with …(insert treatment according to national guidelines)….

Benefits:

There will be no direct benefit from your child taking part in this initial part of the project but it will also not incur any costs and if you agree to allow your child to participate and then eventually to donate blood then his/her participation may result in public health programmes being sure that the malaria tests used are of good quality and give accurate results. Furthermore, by permitting us to use your child's blood for future malaria diagnostic research, your child will be making available samples that can be used to develop new and improved methods to diagnose malaria.

Risks and discomfort:

The risks involved in this study are minimal. They include the discomfort of a slight delay in the treatment, drawing a sample of blood, rare bruising and infection at the site of lancet stick. New lancet and needles will be used for each patient so there is no risk for transmitting diseases.

Compensation:

There will be no compensation to you or your child if he/she decides to take part in this study.

Confidentiality

All information that you provide regarding your child will be considered confidential, and no mention of his/her name or any other identifying information will appear on the stored samples or in any publication in connection with this study. The information will NOT be stored together with the samples. Only the research staff and the health care workers overseeing your child's immediate care will have access to any information that identifies him/her individually. Only these persons will have the key to link the samples and the information attached to your child's name for the purposes of returning his/her test results.

Right to Refuse or Withdraw:

You/your child may also choose not to participate in this study and he/she may refuse to participate at any time without penalty or loss of benefits to which he/she would otherwise be entitled. You do not have to explain why your child does not wish to participate or withdraw.

Contact information:

If you/your child have any questions, or if any problems arise, you/he/she may contact:

….(insert contact information for collecting institution)….

If you have additional questions you may also contact:

….(insert contact information for local ethics committee)….

Certificate of consent # 1

(Parents/guardians of children 5-18 years)

My child has been invited to participate in a research project to help ensure malaria tests are working well. I agree for him/her to be screened for malaria by a rapid diagnostic test (RDT) using finger prick blood and if it is strongly positive (or if negative in the first 15 cases) then I agree to be asked later if I agree to be asked some questions about my child's health complaints and recent treatments and to give a sample of my child's blood and have additional testing for other infections. I have read the foregoing information (or it has been read to me). I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily for my child to participate in this **first** part of the study.

Print Name of Participant_____

PrintName of Parent or Guardian_____

Signature of Parent or Guardian _____

Date _____

Day/month/year

If illiterate (Statement of witness)

I have witnessed the accurate reading of the consent form to the potential participant's parent/guardian, and this individual has had the opportunity to ask questions. I confirm that the individual has given consent for their child to participate, freely.

Print name of witness_____ ***AND*** ***Thumb print of parent***

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the parent of the potential participant, and to the best of my ability made sure that the person understands that the following will be done to his/her child:

1. That a malaria test will be performed using finger prick blood.

2. That on behalf of his/her child they will be asked later to consent to other procedures including answering questions about their child's health, collecting a blood sample and having other testing including HIV and hepatitis done.

3. That the appropriate treatment for the condition will be provided after the blood sampling.

I confirm that the parent of the potential participant was given an opportunity to ask questions about the study, and all the questions asked have been answered correctly to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

Patient Information and Assent Form

(For children between the ages of 5-18 years of age)

….insert: Institution, city, country…..

Collection of Wild type Plasmodium falciparum from Clinical Samples for the Development of Panels for Quality Assurance of Malaria Rapid Diagnostic Tests

Patient Information

Introduction: Fever is a common way for malaria to present but not all fevers are caused by malaria. The World Health Organization (WHO) recommends that all people suspected to have malaria have a test to confirm it, prior to being given treatment. Thus, it is very important that malaria diagnostic tests work well because health workers rely on them to make decisions about appropriate treatment.

Purpose:

I am …….. and I work with … (insert Institution, city, country)….

The WHO and the Foundation for Innovative New Diagnostics (FIND) is working with … (insert Institution, city, country)…. to make sure that the malaria tests we are using are working well. We do this by collecting and storing samples of blood from people who have malaria and then intermittently using these blood samples to see if the malaria tests we buy and use are working (give a positive result) or not working (give a negative result when they shouldn't). Today you have presented with a fever and could have malaria. I am going to give you information and invite you to be part of a research study.

You can choose whether or not you want to participate. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change. If you choose not to participate in this research project, you will receive the treatment that is routinely offered in this clinic/hosptial for fever including … (insert malaria test, treatment and any other item according to national guidelines for fever management)….. You may change your mind later and stop participating even if you agreed earlier.

We have discussed this research with your parent(s)/guardian and they know that we are also asking you for your agreement. If you are going to participate in the research, your parent(s)/guardian also have to agree. But if you do not wish to take part in the research, you do not have to, even if your parents have agreed.

You may discuss anything in this form with your parents or friends or anyone else you feel comfortable talking to. You can decide whether to participate or not after you have talked it over. You do not have to decide immediately.

There may be some words you don't understand or things that you want me to explain more about because you are interested or concerned. Please ask me to stop at anytime and I will take time to explain.

I have checked with the child and he/she understand that participation is voluntary ____(initial)

Procedures:

If you agree to take part in the research, there will be two steps:

First, we need to find out if you have malaria, this will be done using a 'rapid diagnostic test'. The blood is taken from a finger prick.

If the malaria test is negative you will be referred for care for non-malaria illness and your participation in this project is ended. However if the malaria test is strongly positive, we will ask you if you agree to contribute to a project to assure that malaria tests are working well worldwide, by agreeing to the following:

i) Answer some questions regarding recent treatment/medicines that you've received

ii) Provide a blood sample (15ml or three teaspoons) taken from your vein that will be tested for other types of infection (see below) and stored in a freezer for use in the future to tell if malaria rapid diagnostic tests are working properly and, also, if you agree, for future research to improve diagnosis of malaria.

ii) Using the same blood sample, allow for additional tests for infection that include hepatitis and HIV infection and will be done only after you have been counselled and have had a chance to ask questions. If you refuse counselling related to HIV testing or to have HIV testing done, then you cannot participate in this project. If you agree to HIV testing and the other required tests, then should one of those extra tests be positive, you will receive counselling and be referred for appropriate care.

After you have donated the 15 ml of blood, you will receive treatment with …(insert treatment according to national guidelines)….

I have checked with the child and he/she understands the procedures _____(initial)

Benefits:

There will be no direct benefit from your taking part in this initial part of the project but it will also not incur any costs and if you agree eventually to donate your blood then your participation may result in public health programmes being sure that the

malaria tests used are of good quality and give accurate results. Furthermore, by permitting us to use your blood for future malaria diagnostic research, you will be making available samples that can be used to develop new and improved methods to diagnose malaria.

I have checked with the child and he/she understands the benefits_____ (initial)

Risks and discomfort:

The risks involved in this study are minimal. They include the discomfort of a slight delay in the treatment, drawing a sample of blood, rare bruising and infection at the site of lancet stick. New lancet and needles will be used for each patient so there is no risk for transmitting diseases.

I have checked with the child and he/she understands the risks and discomforts ____ (initial)

Compensation:

There will be no compensation to you if you decide to take part in this study.

Confidentiality:

We will not tell other people that you are in this research and we won't share information about you to anyone who does not work in the research study.

Sharing the Findings :

Immediately after performing the test, I will tell you and your parent the result of the malaria test. I will also give you a paper with the results written down. With this result, the health worker will handle your case properly.

Right to Refuse or Withdraw: Can I choose not to be in the research? Can I change my mind?

You may also choose that you do not participate in this study and you may refuse to participate. No one will be mad or disappointed with you if you say no. It’s your choice. You can think about it and tell us later if you want. You can say "yes" now and change your mind later and it will still be okay.

Contact information:

You can ask me questions now or later. You can ask the nurse questions. I have written a number and address where you can reach us or, if you are nearby, you

can come and see us. If you want to talk to someone else that you know like your teacher or doctor or auntie, that's okay too.)

Also if you have any questions, or if any problems arise, you may contact:

….(insert contact information for collecting institution)….

If you have additional questions you may also contact:

….(insert contact information for local ethics committee)….

Certificate of assent # 1

(For children between the ages of 5-18 years of age)

I have been invited to participate in a research project to help ensure malaria tests are working well. I agree to be screened for malaria by a rapid diagnostic test (RDT) using a blood test and if it is strongly positive (or if negative in the first 15 cases) then I will be asked later to agree to be asked some questions about my health complaints and recent treatments and to give a blood sample and have additional testing for other infections. I have read the foregoing information (or it has been read to me). I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I agree to take part in this **first** part of the study.

OR

I do not wish to take part in the research and I have not signed the assent below._____(initialled by child/minor)

Only if child assents:

Print name of child _____

Signature/thumb print of child: _____

Date:_____

day/month/year

If illiterate:

A literate witness must sign (if possible, this person should be selected by the participant, not be a parent, and should have no connection to the research team). Participants who are illiterate should include their thumb print as well.

I have witnessed the accurate reading of the assent form to the child, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness (not a parent)_____ AND Thumb print of participant

Signature of witness _____

Date _____

Day/month/year

I have accurately read or witnessed the accurate reading of the assent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given assent freely.

Print name of researcher_____

Signature of researcher_____

Date_____

Day/month/year

Statement by the researcher/person taking consent

Print Name of Participant_____

Signature of Participant_____

Date _____

Day/month/year

Statement by the researcher/person taking assent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the person understands that the following will be done:

1. That a malaria test will be performed using finger prick blood.

2. That he/she will be asked later to consent to other procedures including answering questions about his/her health, collecting a blood sample and having other testing including HIV and hepatitis done.

3. That the appropriate treatment for the condition will be provided after the blood sampling.

I confirm that the potential participant was given an opportunity to ask questions about the study, and all the questions asked have been answered correctly to the best of my ability. I confirm that the individual has not been coerced into giving assent, and the assent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the assent_____

Signature of Researcher /person taking the assent_____

Date _____

Day/month/year

3.04b: Information Sheet and Consent Forms for virus testing and blood storage

Draft Minimum Standard Consent Form

To be modified to fulfill local requirements and national regulations, retaining each element below.

Patient Information and Consent - # 2

(Collection and storage of blood, HIV/hepatitis testing)

….insert: Institution, city, country…..

Collection of Wild type Plasmodium falciparum from Clinical Samples for the Development of Panels for Quality Assurance of Malaria Rapid Diagnostic Tests

Introduction

You agreed to take part in the research that is collecting and storing samples of blood from people who have malaria (and from a small number of people who have fever but not malaria) and then intermittently using these blood samples to see if the malaria tests we buy and use are working before they are used on patients. These same samples may be useful to scientists developing new tests for malaria or improving the current ones. Therefore, we are also seeking permission to store your unused samples for further malaria diagnostic research at *…(insert Institution, city, country)…*and also with our partners, WHO and FIND.

Procedures

i) The test that we did on your sample has come out to be strongly positive, which means that you have malaria. You will receive the treatment for malaria very shortly.

I would now like to ask you if you agree that we continue and ask you some questions about your health. Specifically, we need to know if you have taken any medicines for malaria in the past two weeks. If you have then we cannot use your blood sample, but if not, then as explained earlier we would like to take 15ml blood (equal to 3 teaspoons) from your vein to test for HIV, hepatitis viruses and if these are all found negative, then we will store (approximately 10ml) of it in freezer specimen bank. Furthermore, if any part of the blood sample you have provided for this project is unused or leftover then we will give it, free of charge, to scientists only for research that supports development of new or improved diagnostic tests for malaria.

Regarding HIV testing, HIV is a type of germ that can cause AIDS (acquired immunodeficiency syndrome), which is usually a serious health problem and can

be deadly. Someone can look and feel perfectly healthy and still be infected. The only way to know is by doing a blood test. Over time HIV infection decreases the body's fighting power and increases a person's risk of catching other diseases, including tuberculosis. In order to help you decide whether or not you wish to be tested for HIV infection, the services of a counsellor are available. You will be counselled before testing for HIV infection. The reason why we would like to test if you have HIV/Hepatitis B or C or not, is that the tests we normally use to diagnose malaria may not work as well in patients who also have HIV/Hepatitis B or C and the results of a wrong diagnosis may be serious. Therefore it is important for us to have these results on the blood samples.

The benefit of HIV testing is that, if you agree, you will be given the results of these tests and if positive, you will be referred for counselling and care to …*(insert reference center for HIV counselling and care according to national guidelines)*… which offers free HIV treatment for those who require it. This research project will not pay for any form of HIV treatment. In addition, if you are positive for Hepatitis B or C, you will receive standard routine care provided by this facility immediately when the result is communicated to you.

There may be emotional discomfort or stress associated with knowledge of the results of this test.

If you accept, then we will first collect your blood and then give you the treatment for malaria (…*insert treatment according to national guidelines*…), then you will have counselling for the HIV test and the hepatitis tests. If you decide now that you do not wish to donate your blood samples we will not proceed further and your care will not be affected. Alternatively, if after the counselling you refuse to have the HIV or hepatitis testing processed, then we will destroy your blood samples and not proceed further.

If you agree, and after testing is completed and the blood is stored, all information linking the blood samples to you will be removed and further information on any future tests may not be available to you. Your stored blood sample will not be sold for profit and any research which uses your sample will have been approved by the WHO Ethics Review Committee (ERC) and the *…(insert local/national ethics committee)….*

CONSENT CERTIFICATE #2

I consent to answering questions about my health and specifically concerning any medicines I have taken for malaria recently. I also agree to provide a blood sample for HIV and hepatitis viruses testing and agree to the storage of the remaining blood sample to be stored and used in the future to determine if malaria diagnostic tests are working or not before they are used on people. I have read the foregoing information (or it has been read to me). I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate in this **second** part of the study.

Print Name of Participant_____

Signature of Participant_____

Date _____

Day/month/year

If illiterate (Statement of witness)

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ ***AND Thumb print of parent***

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the person understands that the following will be done:

1. That he/she will be asked questions about recent health and taking of malaria medicines

2. That he/she agrees to a blood draw for HIV and hepatitis testing, which includes pre-test and post-test counseling for HIV.

3. That he/she agrees that additional blood will be stored and used in the future to determine if malaria tests work properly.

I confirm that the potential participant was given an opportunity to ask questions about the study, and all the questions asked have been answered correctly to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

CONSENT CERTIFICATE #3

Long term storage of blood samples for malaria diagnostic research

I understand that my blood samples may additionally be useful to scientists developing new tests for malaria or improving the current ones. I consent that, if any of the blood sample I provided for this project is unused or leftover when the project is completed, then:

(Tick **one** choice from each of the following boxes)

- ☐ I wish my blood sample to be destroyed immediately.
- ☐ I want my blood sample to be destroyed after ____ years.
- ☐ I give permission for my blood sample to be stored indefinitely

AND (if the sample is to be stored)

- ☐ I give permission for my blood sample to be stored and used in future malaria diagnostics research.

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I consent voluntarily to have my samples stored in the manner and for the purpose indicated above.

Print Name of Participant_____

Signature of Participant _____

Date _____

Day/month/year

If illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ ***AND Thumb print of parent***

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. Samples will be stored for a long time in the freezer for testing of the quality of malaria rapid diagnostic tests.
2. That unused samples will be used for further research on malaria diagnosis.

I confirm that the participant was given an opportunity to ask questions about the nature and manner of storage of the samples, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

Patient Information - # 2

For parents/guardians of children 5-18 years

(Collection and storage of blood, HIV/hepatitis testing)

…..insert: Institution, city, country…..

Collection of Wild type Plasmodium falciparum from Clinical Samples for the Development of Panels for Quality Assurance of Malaria Rapid Diagnostic Tests

Introduction

You agreed for your child to take part in the research that is collecting and storing samples of blood from people who have malaria (and from a small number of people who have fever but not malaria) and then intermittently using these blood samples to see if the malaria tests we buy and use are working before they are used on patients. These same samples may be useful to scientists developing new tests for malaria or improving the current ones. Therefore, we are also seeking permission to store your unused samples for further malaria diagnostic research at *…(insert Institution, city, country)…*and also with our partners, WHO and FIND.

Procedures

The test that we did on your child's blood sample has come out to be strongly positive, which means that he/she has malaria. Your child will receive the treatment for malaria very shortly.

I would now like to ask you if you agree that we continue and ask you some questions about your child's health. Specifically, we need to know if he/she has taken any medicines for malaria in the past two weeks. If he/she has then we cannot use your child's blood sample, but if not, then as explained earlier we would like to take 15ml of your child's blood (equal to 3 teaspoons) from his/her vein to test for HIV, hepatitis viruses and if these are all found negative, then we will, store (approximately 10ml) of it in freezer specimen bank. Furthermore, if any part of your child's blood sample is unused or leftover then we will give it, free of charge, to scientists only for research that supports development of new or improved diagnostic tests for malaria.

Regarding HIV testing, HIV is a type of germ that can cause AIDS (acquired immunodeficiency syndrome), which is usually a serious health problem and can be deadly. Someone can look and feel perfectly healthy and still be infected. The only way to know is by doing a blood test. Over time HIV infection decreases the body's fighting power and increases a person's risk of catching other diseases, including tuberculosis. In order to help you decide whether or not you wish for

your child to be tested for HIV infection, the services of a counsellor are available. You and your child will be counselled before testing for HIV infection. The reason why we would like to test if you have HIV/Hepatitis B or C or not, is that the tests we normally use to diagnose malaria may not work as well in patients who also have HIV/Hepatitis B or C and the results of a wrong diagnosis may be serious. Therefore it is important for us to have these results on the blood samples.

The benefit of HIV testing is that, if you agree, you will be given the results of your child's tests and if positive, you and your child will be referred for counselling and care to …(*insert reference center for HIV counselling and care according to national guidelines)…* which offers free HIV treatment for those who require it. This research project will not pay for any form of HIV treatment. In addition, if your child is positive for Hepatitis B or C, he/she will receive standard routine care provided by this facility immediately after the result is communicated to you.

There may be emotional discomfort or stress associated with knowledge of the results of this test.

If you accept, then we will first collect your child's blood and give him/her the treatment for malaria (*…insert treatment according to national guidelines…*), then you and your child will have counselling for the HIV test and the hepatitis tests. If you decide now that you do not wish to donate your child's blood samples we will not proceed further and your child's care will not be affected. Alternatively, if after the counselling you refuse to have the HIV or hepatitis testing processed, then we will destroy your child's blood samples and not proceed further.

If you agree, and after testing is completed and the blood is stored, all information linking the blood samples to your child will be removed and further information on any future tests may not be available to you or your child. Your child's stored blood sample will not be sold for profit and any research which uses your child's sample will have been approved by the WHO Ethics Review Committee (ERC) and *…(insert local/national ethics committee)…..*.

CONSENT CERTIFICATE #2

(Parents/guardians of children 5-18 years)

I consent to answering questions about my child's health and specifically concerning any medicines he/she has taken for malaria recently. I also agree to allow collection of a blood sample from my child for HIV and hepatitis viruses testing and agree that the remaining blood sample can be stored and used in the future to determine if malaria diagnostic tests are working or not before they are used on people. I have read the foregoing information,(or it has been read to me). I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate in this **second** part of the study.

Print Name of Participant_____

Print Name of Parent or Guardian_____

Signature of Parent or Guardian _____

Date _____

Day/month/year

If illiterate (Statement of witness)

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ ***AND Thumb print of parent***

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the person understands that the following will be done:

1. That he/she will be asked questions about his/her child's recent health and taking of malaria medicines

2. That he/she agrees to allow a blood draw for his/her child for HIV and hepatitis testing, which includes pre-test and post-test counseling for HIV.

3. That he/she agrees that additional blood will be stored and used in the future to determine if malaria tests work properly.

I confirm that the potential participant was given an opportunity to ask questions about the study, and all the questions asked have been answered correctly to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

CONSENT CERTIFICATE #3

(Parents/guardians of children 5-18 years)

Long term storage of blood samples for malaria diagnostic research

I understand that my child's blood samples may additionally be useful to scientists developing new tests for malaria or improving the current ones. I consent that, if any of the blood sample my child provided for this project is unused or leftover when the project is completed, then:

(Tick **one** choice from each of the following boxes)

- ☐ I wish my child's blood sample to be destroyed immediately.
- ☐ I want my child's blood sample to be destroyed after ____ years.
- ☐ I give permission for my child's blood sample to be stored indefinitely

AND (if the sample is to be stored)

- ☐ I give permission for my blood sample to be stored and used in future malaria diagnostics research.

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I consent voluntarily to have my child's samples stored in the manner and for the purpose indicated above.

Print Name of Participant_____

Print Name of Parent or Guardian_____

Signature of Parent or Guardian _____

Date _____

Day/month/year

If illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness*_____ *AND Thumb print of parent

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

1. I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

2. Samples will be stored for a long time in the freezer for testing of the quality of malaria rapid diagnostic tests.

3. That unused samples will be used for further research on malaria diagnosis.

I confirm that the participant was given an opportunity to ask questions about the nature and manner of storage of the samples, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent _____

Date _____

Day/month/year

Patient Information and <u>Assent Form</u> - # 2

(For children between the ages of 5-18 years of age)

(Collection and storage of blood, HIV/hepatitis testing)

….insert: Institution, city, country…..

Collection of Wild type Plasmodium falciparum from Clinical Samples for the Development of Panels for Quality Assurance of Malaria Rapid Diagnostic Tests

Introduction

You agreed to take part in the research that is collecting and storing samples of blood from people who have malaria (and from a small number of people who have fever but not malaria) and then intermittently using these blood samples to see if the malaria tests we buy and use are working before they are used on patients. These same samples may be useful to scientists developing new tests for malaria or improving the current ones. Therefore, we are also seeking permission to store your unused samples for further malaria diagnostic research at *…(insert Institution, city, country)…*and also with our partners, WHO and FIND.

Procedures

The test that we did on your sample has come out to be strongly positive, which means that you have malaria. You will receive the treatment for malaria very shortly.

I would now like to ask you if you agree that we continue and ask you some questions about your health. Specifically, we need to know if you have taken any medicines for malaria in the past two weeks. If you have then we cannot use your blood sample, but if not, then as explained earlier we would like to take 15ml blood (equal to 3 teaspoons) from your vein to test for HIV, hepatitis viruses and if these are all found negative, then we will store (approximately 10ml) of it in freezer specimen bank. Furthermore, if any part of the blood sample you have provided for this project is unused or leftover then we will give it, free of charge, to scientists only for research that supports development of new or improved diagnostic tests for malaria.

Regarding HIV testing, HIV is a type of germ that can cause AIDS (acquired immunodeficiency syndrome), which is usually a serious health problem and can be deadly. Someone can look and feel perfectly healthy and still be infected. The only way to know is by doing a blood test. Over time HIV infection decreases the body's fighting power and increases a person's risk of catching other diseases, including tuberculosis. In order to help you decide whether or not you wish to be tested for HIV infection, the services of a counsellor are available. <u>You will be counselled before testing for HIV infection.</u> The reason why we would like to test if you have HIV/Hepatitis B or C or not, is that the tests we normally use to diagnose malaria may not work as well in patients who also have HIV/Hepatitis B

or C and the results of a wrong diagnosis may be serious. Therefore it is important for us to have these results on the blood samples.

The benefit of HIV testing is that, if you agree, you will be given the results of these tests and if positive, you will be referred for counselling and care to the standard HIV medical care services at *…(insert reference center for HIV counselling and care according to national guidelines)…* which offers free HIV treatment, for those who require it. This research project will not pay for any form of HIV treatment. In addition, if you are positive for Hepatitis B or C, you will be receive standard routine care provided by this facility immediately the result is communicated to you.

There may be emotional discomfort or stress associated with knowledge of the results of this test.

If you accept, then we will first collect your blood and give you the treatment for malaria (…*insert treatment according to national guidelines*…), then you will have counselling for the HIV test and the hepatitis tests. If you decide now that you do not wish to donate your blood samples we will not proceed further and your care will not be affected. Alternatively, if after the counselling you refuse to have the HIV or hepatitis testing processed, then we will destroy your blood samples and not proceed further.

If you agree, and after testing is completed and the blood is stored, all information linking the blood samples to you will be removed and further information on any future tests may not be available to you. Your stored blood sample will not be sold for profit and that any research which uses your sample will have been approved by the WHO Ethics Review Committee (ERC) and *…(insert local/national ethics committee)….*

ASSENT CERTIFICATE #2

(For children between the ages of 5-18 years of age)

I assent to answering questions about my health and specifically concerning any medicines I have taken for malaria recently. I also assent to provide a blood sample for HIV and hepatitis viruses testing and agree to the storage of the remaining blood sample to be stored and used in the future to determine if malaria diagnostic tests are working or not before they are used on people. I have read the foregoing information (or it has been read to me). I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I assent to participate in this **second** part of the study.

OR

I do not wish to take part in the research and I have not signed the assent below._____ (initialled by child/minor)

Only if child assents:

Print name of child _____

Signature/thumb print of child: _____

Date: _____

Day/month/year

If illiterate (Statement of witness)

I have witnessed the accurate reading of the assent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given assent freely.

Print name of witness_____ ***AND Thumb print of parent***

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking assent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the person understands that the following will be done:

1. That he/she will be asked questions about recent health and taking of malaria medicines

2. That he/she agrees to a blood draw for HIV and hepatitis testing, which includes pre-test and post-test counseling for HIV.

3. That he/she agrees that additional blood will be stored and used in the future to determine if malaria tests work properly.

I confirm that the potential participant was given an opportunity to ask questions about the study, and all the questions asked have been answered correctly to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this assent form has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

ASSENT CERTIFICATE #3

(For children between the ages of 5-18 years of age)

Long term storage of blood samples for malaria diagnostic research

I understand that my blood samples may additionally be useful to scientists developing new tests for malaria or improving the current ones. I assent that, if any of the blood sample I provided for this project is unused or leftover when the project is completed, then:

(Tick **one** choice from each of the following boxes)

☐ I wish my blood sample to be destroyed immediately.

☐ I want my blood sample to be destroyed after ____ years.

☐ I give permission for my blood sample to be stored indefinitely

AND (if the sample is to be stored)

☐ I give permission for my blood sample to be stored and used in future malaria diagnostics research

OR

I do not wish to take part in the research and I have <u>not</u> signed the assent below._____ (initialled by child/minor)

<u>Only if child assents:</u>

Print name of child _____

Signature/thumb print of child: _____

Date: _____

Day/month/year

If illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the assent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given assent freely.

Print name of witness*_____*AND Thumb print of parent

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. Samples will be stored for a long time in the freezer for testing of the quality of malaria rapid diagnostic tests.

2. That unused samples will be used for further research on malaria diagnosis.

I confirm that the participant was given an opportunity to ask questions about the nature and manner of storage of the samples, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving assent, and the assent has been given freely and voluntarily.

A copy of this assent form has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

Institute: _____ Collection round (n°): _____

3.05: Malaria Patient Screening Form

Recruitment site:_____ Date:___/___/_____ (dd/mm/yyyy)

Malaria RDTs	RDT (name)	Manufacturer	Catalog No.	Lot No. /Expiry
RDT No.1 (e.g. pfHRP2)				
RDT No. 2 (e.g. pLDH)				

Virus RDTs	RDT (name)	Manufacturer	Catalog No.	Lot No. /Expiry
Hepatitis B				
Hepatitis C				
HIV				

Patient / sample		**Malaria RDT No. 1 (e.g. pfHRP2-based) ***						**Malaria RDT No. 2 (e.g. pLDH-based) ***						**Microscopy**	**Virus RDTs †**			**Exclusions**
Patient number	QC sample ID	Control	Pf	Pan	Pv	Result	Time	Control	Pf	Pan	Pv	Result	Time	Species, Parasitaemia	Hep. B	Hep. C	HIV	Reasons for exclusion
PH 001	e.g. PH01 F01	e.g. 3+	e.g. 2+	e.g. 1+	e.g. NA	e.g. Pf pos	e.g. 9:45h	e.g. 3+	e.g. 3+	e.g. 2+	e.g. NA	e.g. Pf pos	e.g. 9:50h	e.g. Pf, 8000 p/µL	e.g. neg	e.g. neg	e.g. neg	e.g. anaemia

* Rating of RDT band intensity according to the standard color chart. † Screening of viral infections (hepatitis B, hepatitis C, HIV 1&2) with RDTs in the field is optional.

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Institute: _____ Collection round (n°): _____

3.06: Patient Record

Patient Number _____ Date: ___/___/_____ (dd/mm/yyyy) Time:_____ (hh:mm)

QC sample ID _____ (after assignment)

PATIENT DETAILS

NAME _____ AGE _____ (in yrs) SEX M ☐ F ☐

Province/District _____ Recruitment site: _____

CLINICAL HISTORY

Symptoms

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

Fever **yes** ☐ **no** ☐

Chills **yes** ☐ **no** ☐

Sweating **yes** ☐ **no** ☐

Headache **yes** ☐ **no** ☐

Others **yes** ☐ **no** ☐

(If others, specify_____)

Access to medication

(Do you keep any malaria medicine at home?)

yes ☐ **no** ☐

Treatment

(In the past two weeks, what medicines have you taken?)

Chloroquine **yes** ☐ **no** ☐

Sulfadoxine/Pyrimethamine **yes** ☐ **no** ☐

Primaquine **yes** ☐ **no** ☐

ACT **yes** ☐ **no** ☐

Others **yes** ☐ **no** ☐

(If others, specify_____)

History of treatment (if treatment taken):

(How long time ago have you taken the medicine?)

_____**(days)**_____**(weeks)**

MALARIA RDT RESULTS (finger-prick)

RDT (name/brand)	Manufacturer	Catalog No.	Lot No. /Expiry	Pf eg.2+	Pan eg.1+	Pv eg.NA	Control e.g. 3+	Result e.g. Pf pos

Rating of RDT band intensity according to the standard color chart

MALARIA MICROSCOPY (thin/thick film)

Plasmodium species: _____ Parasite density (p/µL): _____

VIRUS RDT RESULTS (hepatitis B, hepatitis C, HIV) - optional

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Institute: _____ Collection round (n°): _____

RDT (name/brand)	Manufacturer	Catalog No.	Lot No. /Expiry	Virus +/- e.g. neg	Control +/- e.g. pos	Result e.g. HIV neg

---

VENEPUNCTURE (If the patient meets the following criteria, then perform a venepuncture)

1) Strong malaria RDT signal (2+ OR 3+) OR parasite density ≥ 2,000 p/µl (rapid count on thick film),

2) Age (as approved in the study protocol),

3) NO malaria medicine in the past two weeks (preferably) or at least in the last week,

4) Informed and signed consent provided.

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Institute: _____ Collection round (n°): _____

3.07: Venepuncture

VENEPUNCTURE (If the patient meets the following criteria, then perform a venepuncture)

1) Strong malaria RDT signal (2+ OR 3+) OR parasite density ≥ 2,000 p/µl (rapid count on thick film),

2) Age (as approved in the study protocol),

3) NO malaria medicine in the past two weeks (preferably) or at least in the last week,

4) Informed and signed consent provided.

Patient Number _____ Date: ___/___/_____ (dd/mm/yyyy) Time:_____ (hh:mm)

QC sample ID _____ (after assignment)

Patient Consent Read yes ☐ no ☐

Volume of venous blood required: ≥ 10 mL in EDTA tubes, 5 mL in plain tube

Volume of blood collected:

Tube	No. of Tubes	Volume per tube	Total volume
EDTA			
Plain			

Time of blood collection: _____ (hh:mm)

Time of blood refrigeration: _____ (hh:mm)

Temperature of refrigerator / cooler box: _____ (°C)

Other samples required (to be prepared with fresh venous blood):

2 thick and thin films

2 filter paper blood spots (if not prepared in the lab)

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Institute: _____ Collection round (n°): _____

2 thick and thin films prepared: yes ☐ no ☐

2 filter paper blood spots prepared: yes ☐ no ☐

Please ensure samples are labeled with the date and the patient number

Initials: _____ Signature: _____(of technician doing venepuncture)

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Institute: _____ Collection round (n°): _____

3.08: Parasite-free blood preparation

"Universal blood mixture": fill in parts 1, 2 and 3 "Matched blood group": fill in part 1 only

---

1) WHOLE BLOOD (from donor / blood bank)

Source: Volunteer donor ☐ Name _____ No assigned to blood _____

Blood bank ☐ Name _____ No of blood bag _____

Blood Group _____ Volume (mL):_____

Date collected: ___/___/_____ (dd/mm/yyyy) Refrigeration temp. (°C): _____

Date of expiry: ___/___/_____ (dd/mm/yyyy) Refrigeration delay (min): _____

Virus screening (serology):

Lab performing test _____ Date of test ___/____/_____ (dd/mm/yyyy)

Hepatitis B: pos ☐ neg ☐ Hepatitis C: pos ☐ neg ☐ HIV 1&2: pos ☐ neg ☐

Malaria screening (microscopy and RDT):

Date of testing ___/____/_____ (dd/mm/yyyy) Microscopy (thick film): pos ☐ neg ☐

RDT (name/brand)	Manufacturer	Catalog No.	Lot No. /Expiry	Pf	Pan	Pv	Control	Result

---

2) AB+ PLASMA (from donor / blood bank / supplier)

Source: Volunteer donor ☐ Name _____ No assigned to blood _____

Blood bank ☐ Name _____ No of blood/plasma bag _____

Supplier ☐ Name _____ Lot No _____

Volume (mL):_____ Date blood collected / plasma received: ___/___/_____ (dd/mm/yyyy)

Date plasma prepared from blood: ___/___/_____ (dd/mm/yyyy)

Date of expiry: ___/___/_____ (dd/mm/yyyy)

Virus screening (serology): (if plasma prepared from donor / blood bank blood)

Lab performing test _____ Date of test ___/____/_____ (dd/mm/yyyy)

Hepatitis B: pos ☐ neg ☐ Hepatitis C: pos ☐ neg ☐ HIV 1&2: pos ☐ neg ☐

Malaria screening (microscopy and RDT): (if plasma prepared from donor / blood bank blood)

Date of testing ___/____/_____ (dd/mm/yyyy) Microscopy (thick film): pos ☐ neg ☐

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

RDT (name/brand)	Manufacturer	Catalog No.	Lot No. /Expiry	Pf	Pan	Pv	Control	Result

---

3) UNIVERSAL BLOOD MIXTURE PREPARATION (plasma replacement)

Plasma thawing: Date: ___/___/_____ (dd/mm/yyyy) Time: _____ (hh:mm)

Plasma replacement: Date: ___/___/_____ (dd/mm/yyyy) Time: _____ (hh:mm)

Refrigeration: Temperature (°C): _____ Time: _____ (hh:mm)

Number assigned: _____

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

3.09: Malaria Microscopy Record (microscopist 1, first read)

Patient Number _____ Date: ___/___/_____ (dd/mm/yyyy) Time:_____ (hh:mm)

QC sample ID _____ (after assignment)

White blood cell count (number of white cells per microlitre of blood): _____ (WBC/µL)

MICROSCOPY

Count number of parasites relative to 500 white blood cells.

If number of parasites is > 150 for the first 200 white blood cells, counting can be stopped.

Count all species present and record separately gametocytes and asexual parasites.

If a mixed species infection is detected, DO NOT use the blood sample.

Species	Pf		Pv		Pm		Po	
	Asex	Gam	Asex	Gam	Asex	Gam.	Asex	Gam.
Number of parasites counted								

Number of white cells counted	

Comments:

_____

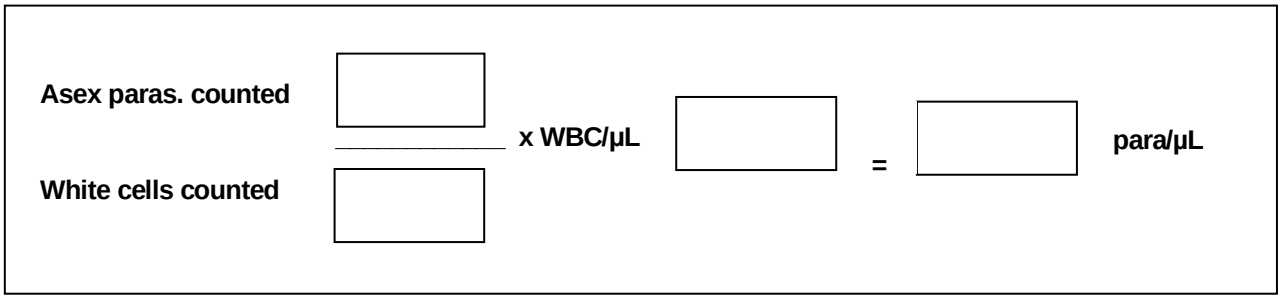
Initials: _____ Signature: _____ (of microscopist)

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

PARASITE DENSITY

Calculate parasite density in number of parasites per microlitre of blood (para/µL).

Use number of asexual parasites only.



Institute: _____ Collection round (n°): _____

3.10: Malaria Microscopy Record (microscopist 2, first read)

Patient Number _____ Date: ___/___/_____ (dd/mm/yyyy) Time:_____ (hh:mm)

QC sample ID _____ (after assignment)

White blood cell count (number of white cells per microlitre of blood): _____ (WBC/µL)

MICROSCOPY

Count number of parasites relative to 500 white blood cells.

If number of parasites is > 150 for the first 200 white blood cells, counting can be stopped.

Count all species present and record separately gametocytes and asexual parasites.

If a mixed species infection is detected, DO NOT use the blood sample.

Species	Pf		Pv		Pm		Po	
	Asex	Gam	Asex	Gam	Asex	Gam.	Asex	Gam.
Number of parasites counted								

Number of white cells counted	

Comments:

_____

Initials: _____ Signature: _____ (of microscopist)

PARASITE DENSITY

Calculate parasite density in number of parasites per microlitre of blood (para/µL).

Use number of asexual parasites only.

Asex paras. counted					
		x WBC/µL		**=**	**para/µL**
White cells counted					

Institute: _____ Collection round (n°): _____

3.11: Malaria Microscopy Record (microscopist 1, second read)

Patient Number _____ Date: ___/___/_____ (dd/mm/yyyy) Time:_____ (hh:mm)

QC sample ID _____ (after assignment)

White blood cell count (number of white cells per microlitre of blood): _____ (WBC/µL)

MICROSCOPY

Count number of parasites relative to 500 white blood cells.

If number of parasites is > 150 for the first 200 white blood cells, counting can be stopped.

Count all species present and record separately gametocytes and asexual parasites.

If a mixed species infection is detected, DO NOT use the blood sample.

Species	Pf		Pv		Pm		Po	
	Asex	Gam	Asex	Gam	Asex	Gam.	Asex	Gam.
Number of parasites counted								

Number of white cells counted	

Comments:

_____

Initials: _____ Signature: _____ (of microscopist)

PARASITE DENSITY

Calculate parasite density in number of parasites per microlitre of blood (para/µL).

Use number of asexual parasites only.

Asex paras. counted					
		x WBC/µL		**=**	**para/µL**
White cells counted					

Institute: _____ Collection round (n°): _____

3.12: Malaria Microscopy Record (microscopist 2, second read)

Patient Number _____ Date: ___/___/_____ (dd/mm/yyyy) Time:_____ (hh:mm)

QC sample ID _____ (after assignment)

White blood cell count (number of white cells per microlitre of blood): _____ (WBC/µL)

MICROSCOPY

Count number of parasites relative to ≥ 500 white blood cells.

If number of parasites is > 150 for the first ≥ 200 white blood cells, counting can be stopped.

Count all species present and record separately gametocytes and asexual parasites.

If a mixed species infection is detected, DO NOT use the blood sample.

Species	Pf		Pv		Pm		Po	
	Asex	Gam	Asex	Gam	Asex	Gam.	Asex	Gam
Number of parasites counted								

Number of white cells counted	

Comments:

_____

Initials: _____ Signature: _____ (of microscopist)

PARASITE DENSITY

Calculate parasite density in number of parasites per microlitre of blood (para/µL).

Use number of asexual parasites only.

Asex paras. counted ____ **x WBC/µL** ____ **=** ____ **para/µL**

White cells counted ____

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Institute: _____ Collection round (n°): _____

3.13: Parasite density & Dilution Calculations

Patient Number _____ Date: ___/___/_____ (dd/mm/yyyy)

QC sample ID _____ (after assignment)

PARASITE DENSITY CALCULATION

PARASITE DENSITY (NUMBER OF PARASITES / µL OF BLOOD)	1) Microscopist 1, first read	2) Microscopist 2, first read
Calculate Mean Density: (Density 1 + Density 2) / 2		
Calculate Discrepancy (%): (Diff/mean) x 100		

If discrepancy is ≤ 20%- Use mean parasite density for the dilution calculations below.

If discrepancy is >20% - Repeat Microscopy (use 0 ***and*** 0***)***

PARASITE DENSITY (NUMBER OF PARASITES / µL OF BLOOD)	3) Microscopist 1, second read	4) Microscopist 2, second read

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

Calculate Mean Density: (Density X + Density Y) / 2	
Calculate Discrepancy (%): (Diff/mean) x 100	

If discrepancy is ≤ 20% - Use mean parasite count for the dilution calculations below.

If discrepancy is >20% - do not use this sample for dilutions

---

DILUTION CALCULATION

Use the MS Excel "Dilution_Calculator"

Mean Parasite density used for calculations: _____ parasites / µL

Total Volume prepared (mL) (*V*)	Dilution Factor (*D*)	Parasite density prepared (p/µL) (*P*)	Parasite density of "parasitized" blood (p/µL) *(n) or (P)*	Volume of "parasitised" blood (mL) ($V_p = V / D$)	Volume of "parasite-free" blood (mL) (V-V_p).	Volume used for QC aliquots (mL) (*VA*)	Volume remaining for other aliquots/margin (mL) (*VR*)

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Institute: _____ Collection round (n°): _____

3.14: Dilution Preparation

Patient Number _____ Date: ___/___/_____ (dd/mm/yyyy)

QC sample ID _____ (after assignment)

DILUTION PREPARATION

Place used for mixing (identify cold room / refrigerator): _____

	Mixing start: Time, temp. (hh:mm, °C)	Mixing end: Time, temp. (hh:mm, °C)	1 EP prepared for counts ?	1 EP or 1 TT prepared for archive ?	RDT No. 1 result in Form 3.15?	RDT No. 2 result in Form 3.15?
2,000 p/µL			yes ☐ no ☐	yes ☐ no ☐	yes ☐ no ☐	yes ☐ no ☐
200 p/µL			yes ☐ no ☐	yes ☐ no ☐	yes ☐ no ☐	yes ☐ no ☐

EP = Earle Perez thick film, TT = thick and thin film

CLUMPING TEST: positive (clumping) ☐ negative (no clumping) ☐

If clumping is positive, do not use the blood sample.

If clumping is negative, the blood sample can be used for preparing QC sample aliquots.

"PARASITE-FREE" BLOOD used for dilution (record number assigned to donor / from blood bank)

"universal mixture": O+/- blood: _____

AB+ plasma: _____

"matched blood group": A/B/O blood: _____

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Institute: _____ Collection round (n°): _____

ALIQUOTS

RDT QC aliquots (50 µL volume)	**No of aliquots**	**Freezing time (hh:mm)**	**Freezing temp. (°C)**
Dilution at 2,000 p/µL			
Dilution at 200 p/µL			

"High volume" aliquots	**Prepared ?**	**Freezing time (hh:mm)**	**Freezing temp. (°C)**
6 x 100 µL for ELISA (patient blood)	yes ☐ no ☐		
1 x 1 mL whole blood (patient blood)	yes ☐ no ☐		
Pellet of 1 x 1 mL (patient blood)	yes ☐ no ☐		
Plasma of 1 x 1 mL (patient blood)	yes ☐ no ☐		
8 x 250 µL for ELISA (dilution 200 p/µL)	yes ☐ no ☐		
4 x 250 µL for ELISA (dilution 2,000 p/µL)	yes ☐ no ☐		

VIRAL INFECTIONS (serology)

Lab performing test _____ Date sent for testing ___/____/_____ (dd/mm/yyyy)

Hepatitis B: pos ☐ neg ☐ Hepatitis C: pos ☐ neg ☐ HIV 1&2: pos ☐ neg ☐

If any of these tests is positive, the QC aliquots can not be used for QC of RDTs (discard).

COMMENTS (e.g. reasons for exclusion, particular observations, etc.):

_____

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Institute: _____ Collection round (n°): _____

3.15: RDT Results Sheet

Date: __/__/_____ (dd/mm/yyyy)

RDT KITS

RDT (name/brand)	Manufacturer	Catalog No.	Lot No.	Expiry date

RESULTS

Rating of RDT band intensity according to the standard color chart

ID	Dilution (p/µL)	Time of testing (hh:mm)	Name of RDT:					Name of RDT:				
			Pf eg.3+	Pan eg.2+	Pv eg.NA	Control e.g. 3+	Result Pf pos	Pf eg.2+	Pan eg.1+	Pv eg.NA	Control e.g. 3+	Result Pf pos
	2,000											
	200											
	2,000											
	200											
	2,000											
	200											
	2,000											
	200											
	2,000											
	200											
	2,000											
	200											

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Institute: _____ Collection round (n°): _____

3.16: QC Sample Preparation Checklists

Patient Number _____ Date: ___/___/_____ (dd/mm/yyyy)

QC sample ID _____ (after assignment)

ALIQUOTS / TESTS checklist

	Aliquots / tests	Prepared / Recorded
Patient blood (plain tube)	Serum for virus screening	yes ☐ no ☐
Patient blood (EDTA tubes)	2 thick/thin films	yes ☐ no ☐
	2 filter paper blood spots	yes ☐ no ☐
	1 aliquot 1 mL whole blood	yes ☐ no ☐
	1 aliquot 1 mL centrifuged:	
	- pellet	yes ☐ no ☐
	- plasma	yes ☐ no ☐
	6 aliquots 100 µL for ELISA	yes ☐ no ☐
Dilution (2,000 p/µL	_____ aliquots 50 µL	yes ☐ no ☐
	1 Earle Perez (EP) for counts	yes ☐ no ☐
	1 EP or thick/thin film for archive	yes ☐ no ☐
	malaria RDT No 1 (Form 3.15)	yes ☐ no ☐
	malaria RDT No 1 (Form 3.15)	yes ☐ no ☐
	4 aliquots of 250 µL for ELISA	
Dilution 200 p/µL	_____ aliquots 50 µL	yes ☐ no ☐
	1 Earle Perez (EP) for counts	yes ☐ no ☐
	1 EP or thick/thin film for archive	yes ☐ no ☐
	malaria RDT No 1 (Form 3.15)	yes ☐ no ☐
	malaria RDT No 1 (Form 3.15)	yes ☐ no ☐
	8 aliquots 250 µL for ELISA	yes ☐ no ☐

FORMS checklist

Title	Number	Completed
Consent forms	3.04	yes ☐ no ☐
Patient screening	3.05	yes ☐ no ☐

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Institute: _____ Collection round (n°): _____

Patient record	3.06	yes ☐ no ☐
Venepuncture	3.07	yes ☐ no ☐
"Parasite-free blood" preparation	3.08	yes ☐ no ☐
Microscopy (microscopist 1, read 1)	3.09	yes ☐ no ☐
Microscopy (microscopist 2, read 1)	3.10	yes ☐ no ☐
Microscopy (microscopist 1, read 2)	3.11	yes ☐ no ☐
Microscopy (microscopist 2, read 2)	3.12	yes ☐ no ☐
Parasite Density & Dilution Calculation	3.13	yes ☐ no ☐
Dilution Preparation	3.14	yes ☐ no ☐
RDT Results Sheet	3.15	yes ☐ no ☐

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Institute: _____ Collection round (n°): _____

3.17: Negative Control Samples

WHOLE BLOOD (from donor / blood bank)

Source: Volunteer donor ☐ Name _____ No assigned to blood _____

Blood bank ☐ Name _____ No of blood bag _____

Volume collected (mL):_____

Date collected: ___/___/_____ (dd/mm/yyyy) Refrigeration temp. (°C): _____

Date of expiry: ___/___/_____ (dd/mm/yyyy) Refrigeration delay (min): _____

Virus screening (serology):

Lab performing test _____ Date of test ___/____/_____ (dd/mm/yyyy)

Hepatitis B: pos ☐ neg ☐ Hepatitis C: pos ☐ neg ☐ HIV 1&2: pos ☐ neg ☐

Malaria screening (microscopy and RDT):

Date of testing ___/____/_____ (dd/mm/yyyy) Microscopy (thick film): pos ☐ neg ☐

RDT (name/brand)	Manufacturer	Catalog No.	Lot No. /Expiry	Pf	Pan	Pv	Control	Result

Other screening tests:

Lab performing test _____ Date of test ___/____/_____ (dd/mm/yyyy)

Specify test_____ Test result _____

NEGATIVE CONTROL SAMPLE ALIQUOTS

Type of negative control sample (“clean”, others):_____

Date of preparation: ___/___/_____ (dd/mm/yyyy)

Freezing time: _____ (hh:mm)

Freezing temperature (°C): _____

Number of aliquots: _____

Negative Control Sample ID assigned: _____

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Institute: _____

3.18: Internal Movements of RDT QC Samples

QC sample ID	Description (type of sample, number of tubes, volume per tube)	Reason for internal movement	Previous storage area (temperature, place)	New storage area (temperature, place)	Date of movement (dd/mm/yy)	Delay of transfer (min)	Remarks	Signature
e.g. PH01 F04 2,000	e.g. QC aliquots, 400 tubes, 50 µL/tube	e.g. Defreezing of freezer	e.g. -70°C freezer in malaria lab	e.g. -70°C freezer in storage room		e.g. 30 min		

Institute: _____

3.19: QC Sample Referral Log

QC SAMPLE ID	DESCRIPTION (type of sample,number of tubes,volume per tube)	REASON FOR REFERRAL	DESTINATION	MODE OF TRANSPORT	DATE SENT	DATE RECEIVED	REMARKS	SIGNATURE
e.g. PH01 F04 2,000	e.g. QC aliquots, 400 tubes, 50 µL/tube	e.g. for product testing at CDC	e.g. CDC, Atlanta, USA	e.g. airway, with dry ice	dd/mm/yy	dd/mm/yy		

Institute: _____

5.01: ELISA Dilution Form

Date of Testing _ _ / _ _ / _ _ _ _ (dd/mm/yyyy) Technician ----------

Name of stock to be diluted e.g. Recombinant HRP2 ----------

Conc required	Stock Conc	Total volume required	Size of automatic pipette used	Volume of Stock in total volume	Size of automatic pipette used	Volume of diluent in total volume

5.02: ELISA Reporting Form

ELISA Kit	Manufacturer	Diluent Used (for recombinant Ag)	Lot Number	Expiry date

Date of Testing _ _ / _ _ / _ _ _ _ (dd/mm/yyyy) Operator _ _ _ _ _ _ _ _

96 well template (enter sample number)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

96 well OD readings result template (enter OD reading)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Result table (may need to include RDT results)

Sample number	Sample ID (e.g. blank, standards, controls, tests, PCW)	OD reading	Extrapolated Concentration ng/ml	RDT result
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
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44				
45				
46				
47				

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Sample number	Sample ID (e.g. blank, standards, controls, tests, PCW)	OD reading	Extrapolated Concentration ng/ml	RDT result
48				
49				
50				
51				
52				
53				
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57				
58				
59				
60				
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62				
63				
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96				

SIGNATURE OF SUPERVISOR : _____ DATE :_____ (DD/MM/YYYY)

Institute: _____

6.01: Pipette Calibration Sheet

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	
11		11		11	
12		12		12	
13		13		13	
14		14		14	
15		15		15	
16		16		16	
17		17		17	
18		18		18	
19		19		19	
20		20		20	
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	

INSTITUTE: _____

6.02: Incubator Calibration Sheet

Name, Location and Temperature Range of Incubator: _____
Name and Serial Number of Reference Thermometer: _____

DATE	TIME	DIAL TEMPERATURE (^{0}C)	SIGNATURE	DATE	TIME	ACTUAL TEMPERATURE (^{0}C)*	SIGNATURE

* Measure actual temperature using reference thermometer

INSTITUTE: _____

6.03: Equipment Maintenance Sheet

Equipment: _____ Location: _____

Date Maintenance Due	Date Maintenance Performed	Comments/Corrective Action

INSTITUTE: _____

6.04: Temperature Monitoring Form

Equipment: _____ Location: _____ Month: _____

T(^{0}C)																															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Sign:																															

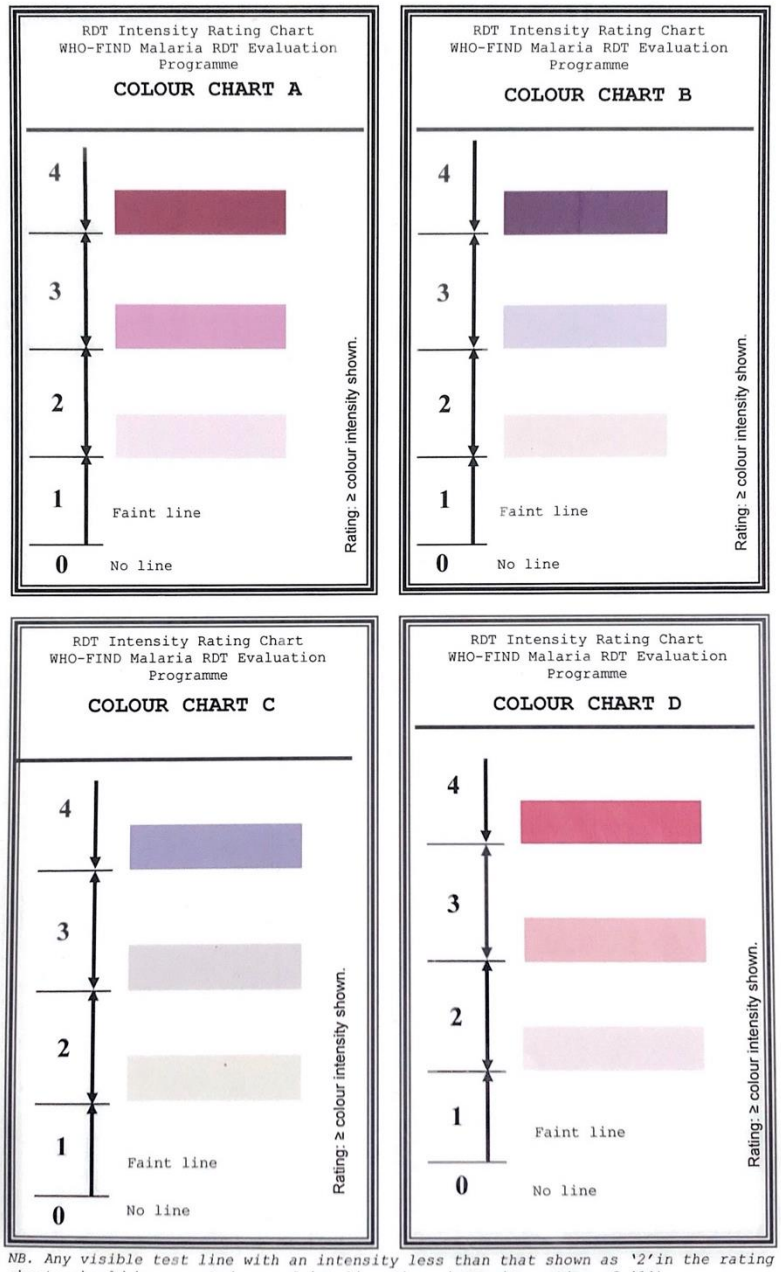
Date: Comments:

_____ _____
_____ _____
_____ _____
_____ _____
_____ _____

6.05: Corrective Action Register

Description of Problem/Incident	
Signature	Date _ _ / _ _ / _ _ _ _ (dd/mm/yyyy)
Action Taken to Resolve Problem	
Signature	Date _ _ / _ _ / _ _ _ _ (dd/mm/yyyy)
Cause of Problem	
Preventative Action Taken	
Signature	Date _ _ / _ _ / _ _ _ _ (dd/mm/yyyy)
Verification of Effectiveness	
Signature	Date _ _ / _ _ / _ _ _ _ (dd/mm/yyyy)

Annex 1: RDT Intensity Rating Charts for Stability Assessment



NB. Any visible test line with an intensity less than that shown as '2' in the rating charts should be reported as a faint line (i.e. intensity rating of '1')
All other ratings (2-4) are read as intensity consistent with or greater than the line shown in the chart.

Annex 2: Guide For the Interpretation of Observations Noted During Lot Testing of Malaria

What kind of comments can be found in a report?

This document is intended to guide the requester/user in the interpretation of observations noted in the lot-testing reports. The comments on anomalies noted on malaria RDTs that undergo lot-testing at laboratories of the WHO-FIND malaria RDT evaluation programme are reported according to a standard format, designed to be consistent between technicians and laboratories.

The lot-testing process

The evaluation carried out by the lot-testing reference laboratories is conducted according to the testing protocol provided by the manufacturer (testing procedure), and the Standard Operating Procedures (SOPs) of the Methods Manual[1].

See: https://cdn.who.int/media/docs/default-source/malaria/methods-manual-laboratory-quality-control-testing-malaria-rdt.pdf?sfvrsn=96ad896c_8&download=true

Interpretation of results during lot-testing

RDTs must detect parasite-positive panels at 200 parasites per microliter of blood in order to pass the quality control evaluation. This is considered close to the minimum density likely in a clinically- significant malaria infection.[2] Any visible line is considered a positive result. Very faint lines observed at such low density does not mean that the same result will be noted during testing in the field since parasite density is likely to be higher, and the test band correspondingly more intense.

The following comments are observations that are intended to bring to the attention of the procurer issues that may sometimes affect field use and make interpretation more difficult, or require emphasis in training. The importance of these observations needs to be considered in the light of their frequency, and the intended use of the RDTs.

The absence of comments in the report means that the result is good and the anomalies were not detected (i.e. clear test bands, no red background, no incomplete clearing etc).

When possible, photos of the testing are attached to the report so that the requesters can see the results of the RDTs tested.

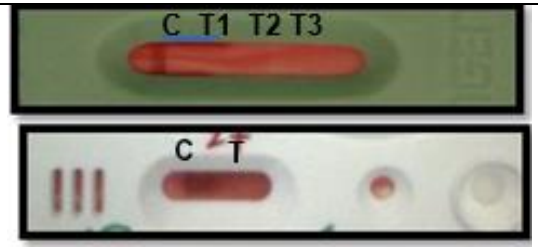
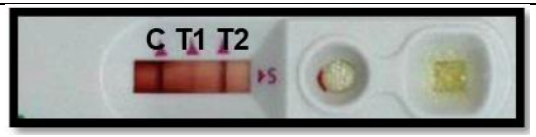
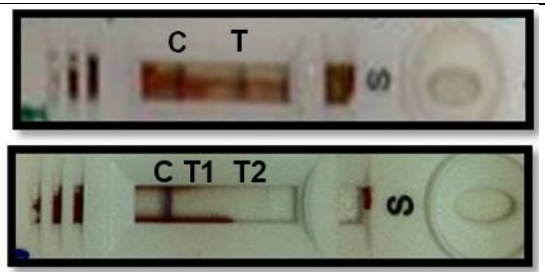
A test result is positive as long as the test line is visible, irrespective of the intensity of

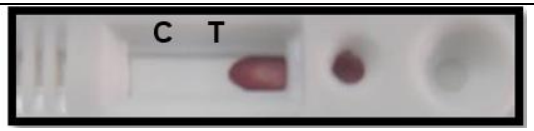
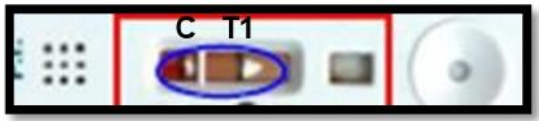
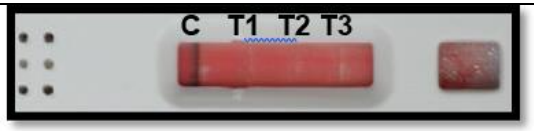
the line. A test result is negative when test line is not visible.

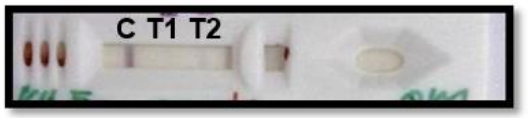
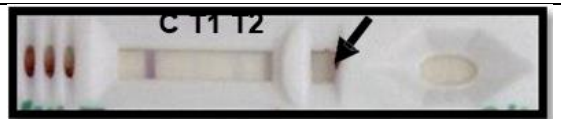
[1] WHO (2023). Methods Manual for Laboratory Quality Control Testing of Malaria Rapid Diagnostic Tests, Version Ten. Geneva, World Health Organization.

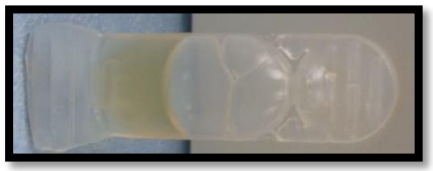
[2] WHO (2010). Parasitological confirmation of malaria diagnosis - Report of a WHO technical consultation GENEVA, 6–8 October 2009. Geneva, World Health Organization.

REPORTED COMMENTS (in lot testing reports)	ILLUSTRATED EXAMPLES	NOTE/EXPLANATION	REPORTED TEST RESULTS (in lot testing reports)
A) Typical test results (positive, negative, invalid)			
No comment (Positive test result)		Clear control line and clear test line, clean background	*Positive test result*
No comment (Negative test result)		Clear control line, but no test line, with clean background.	*Negative test result*
False Positive		Positive when **tested against a negative sample.**	*False positive*
Invalid	 	Absence of the control line. *(The test is repeated using the same sample)*	*Invalid test result*

B) Observations on the test strip (see section K for more examples)			
Red background that obscures test line(s)		A red background, if intense, may obscure weak positive test lines, causing false negative results. In this example, the result is 'negative' since test line is not visible. If the test line is not seen (obscured) with a parasite positive sample.	*Red background that obscures test line(s). This is noted as a negative result.*
Red background		Faint background staining is relatively common. In this example, the result is positive since test lines are positive.	*Red background*
Incomplete clearing with streaking blood		Poor clearing of blood with a clear blood streaking line. Poor clearing of blood may obscure weak positive test lines, causing false negative results. In this example, the result is positive since test line is visible.	*If the test line is not seen (obscured) with a parasite positive sample, this is noted as a negative RDT result.* *If the test line is seen, this is noted as a positive RDT result.*
Incomplete clearing		Poor clearing of blood may obscure weak positive test lines, causing false negative results. In this example, the result is positive since test line is visible.	*If the test line is not seen (obscured) with a parasite positive sample, this is noted as a negative RDT result.* *If the test line is seen, this is noted as a positive RDT result.*

C) Observations of flow problems (see section K for more examples)			
Failure to flow		Blood and buffer did not run the length of the strip	*This is noted as 'invalid' (no control line), and the RDT is repeated as per the standard procedures.*
Irregular migration that obscures test line(s)		One portion of the nitrocellulose near the test band was non absorptive and remained dry during wicking creating irregular migration of blood/buffer with red background that may obscure test line.	*Irregular migration. This is noted as a negative since test line is not visible.*
Irregular migration		One portion of the nitrocellulose near the test band was non absorptive and remained dry during wicking creating irregular migration of blood/buffer with red background. In this example the result is positive since test line is clearly visible.	*Irregular migration. This is noted as a positive result since test line is visible.*
D) Observations on test lines (see section K for more examples)			
Ghost test lines		White test lines on a stained (red) or clear (gray/off-white) background. The staining is on either side of the test line but not on the test line itself. In this example, the result is negative since test line is not dark thus not visible.	*This is noted as a negative RDT result.*
Patchy broken test line(s)		The test line is visible but interrupted (broken).	*Visible test lines are noted as positive, even if incomplete.*

Faint test line(s)		Faint test line results will be noted as a comment and are considered as a positive test result.	*Visible test lines are noted as positive, even if faint.*
Diffuse test line(s)		Test line wider than control, without clearly-defined edge.	*Visible test lines are noted as positive, even if diffuse.*
Indistinct shadowing		Vague, indistinct gray shadow over the region of the test line. Observed only with direct bright light source and not easily captured by photography.	*This is noted as a negative RDT result.*
E) RDT structural problems			
Strip misplaced in the cassette		Strip can only partially be seen in the results window.	*NA (RDT cannot be used for testing).*
Specimen pad not seen in sample window		Normally, the colour of the conjugated antibody can be seen in the sample window (commonly purple, pink or blue.	*NA (RDT cannot be used for testing).*
Container does not puncture		The puncture system to open the buffer bottle is not working well. The use of a scissor is necessary to open the bottle.	*The difficulty to puncture the bottle or ampoule is reported.*
Evaporated bottle / ampoule of buffer		This can be a manufacturing problem (filling of the bottle or ampoule), an evaporation issue or a leakage problem due to the porosity of the ampoule/ bottle. This evaporation issue is more and more frequently	*Buffer bottles/ampoules from other RDT lots should not be used, as their quality is controlled for that other lot only (and there will be not enough buffer for testing RDTs that were shipped with this buffer).*

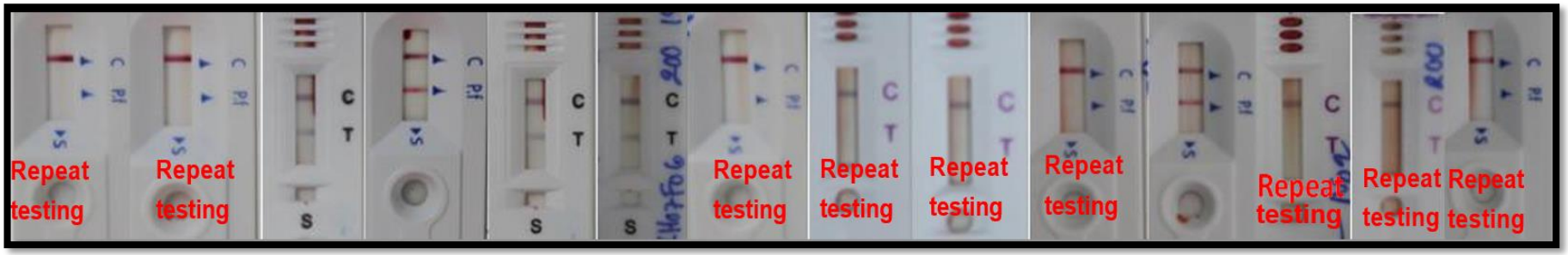
		noted during long term testing (after storage at 37°C during 18 months) and in ampoules recovered from field settings.	*The requester is informed that no testing can be carried out.*
Insufficient volume		This can occur either because of a problem at manufacturing (filling of the bottle), or during transport and storage (leakage or evaporation).	*Complete testing cannot be carried out. Requester is informed that no testing can be carried out because of the lack of buffer.*
Discolored buffer		This can occur either because of a problem at manufacturing (quality of buffer), or during transport and storage (e.g. exposure to high temperatures). It was frequently noted that the buffer becomes yellowish. This can alter the flow of buffer in the strip, thus alter results. Often coupled with evaporation issue.	*Buffer should not be used for testing and testing cannot be carried out. The requester is informed.* *Buffer bottles/ampoules from other RDT lots should not be used, as their quality is controlled for that other lot only.*
G) Desiccant			
Desiccant color indicates humidity		The colour indicating humidity depends on each desiccant (most of them turn from blue to red/pink; others turn from white to blue). Some RDT insert sheets clarify which colour indicates humidity, others do not. Comparison with desiccant of other RDT pouches and/or exposing a desiccant sachet to water can help clarifying.	*If the desiccant color clearly indicates humidity, the RDT should not be used. All the boxes meant for the long term testing are checked as well. Testing cannot be carried out and the requester is informed*
Damaged sachet of desiccant		The sachet of desiccant could be damaged, but desiccant colour could still be normal (i.e. not indicating	*If the desiccant colour does not indicate humidity, and the RDT does not show any damage, the RDT can*

		humidity). Check if the RDT nitrocellulose seems damaged by the desiccant granules.	*be used.*
H) RDT pouch			
Damaged RDT pouch		If the RDT pouch is damaged, humidity can enter and degrade the RDT. The desiccant sachet is to be checked and if it shows trace of humidity, the requester is to be informed.	*The requester is informed and testing is not carried out if the desiccant shows some trace of humidity, if not, testing is carried out and problem observed noted in the report.*
Wrong labelling		Labelling on the RDT pouch is inconsistent with labelling on the RDT kit (box), e.g. different name, catalog number, lot number etc.	*RDTs can be used for testing, but the inconsistencies should be reported immediately to the point of contact.*
I) RDT package (box)			
Damaged RDT package (box)		If the RDT package (box) is damaged, but not the RDT pouches, RDTs are not necessarily affected.	*RDTs can be used for testing, and issue noted in the report.*
Wrong labelling		Labelling on the RDT package (box) is inconsistent with labelling on the individual RDT pouches, e.g. different name, catalog number, lot number etc.	*The labelling issue is noted in the report.*
Missing labelling		No catalog number, no CE marking or no manufacturing dates have y been noted on RDT boxes.	*The missing information is noted in the report*
Missing essential test accessories		RDT kits can lack essential items, e.g. buffer bottles/ampoules, blood transfer devices, desiccants, etc.	*Missing items/accessories are noted in the report. If buffer is missing, testing cannot be carried out and requester is informed.*
J) IFU (Instruction For Use)			
Lacking information		e.g. no information on blood volume, reading time, target antigen, etc.	*The missing information is noted in the report and If without the information,*

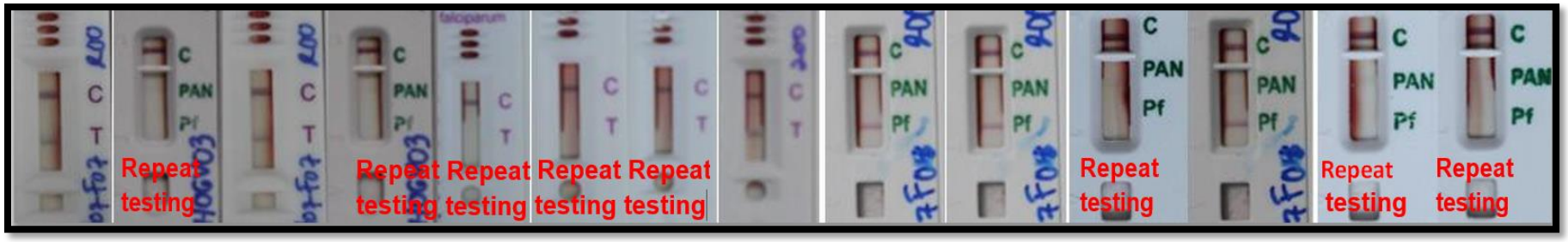
			testing cannot be carried out, the requester is informed.
Discrepant or wrong information		Different information on RDT box and IFU (i.e. on the outer box, it is mentioned that thy are to be stored at maximum 30°c and IFU mentions that they are to be stored at max. 40°C (already noted) or i.e. inconsistencies between the pictograms and the text (wrong order of test lines, etc.)	*The discrepancy is noted in the comment field of the report.*

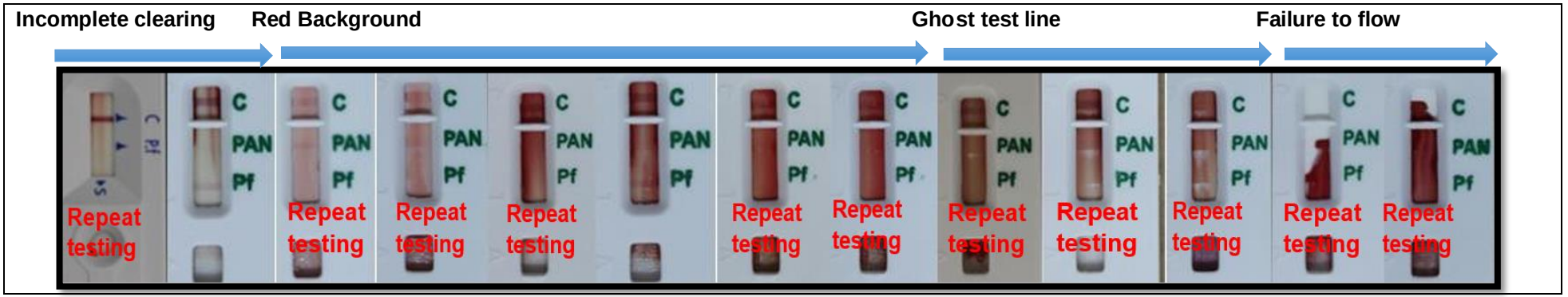
K) Examples of incomplete clearings – red backgrounds and failures to flow – of RDTs tested against Pf samples

No comments → **Incomplete clearing**



Incomplete clearing




Incomplete clearing
Red Background
Ghost test line
Failure to flow
C
PAN
Pf
Repeat testing