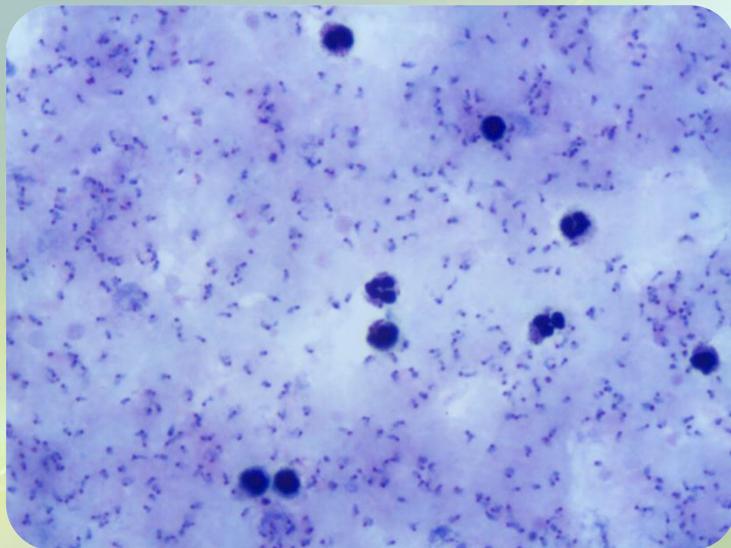


# National Malaria Diagnosis Quality Assurance Guidelines

National Department of Health

**South Africa**



health

Department:  
Health  
REPUBLIC OF SOUTH AFRICA

National Malaria Diagnosis Quality Assurance Guidelines

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Department of Health

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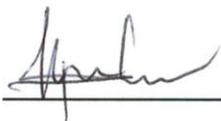
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Ms MP Matsoso

Director-General: Department of Health

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## ABBREVIATIONS

DoH	Department of Health
HCW	Healthcare workers
IQC	Internal quality control
MRC	Medical Research Council
NDoH	National Department of Health
NHLS	National Health Laboratory Service
NICD	National Institute for Communicable Diseases
NMCP	National Malaria Control Programme
PCR	Polymerase chain reaction
PMCP	Provincial Malaria Control Programme
PTS	Proficiency testing scheme
QA	Quality assurance
QBC	Quantitative buffy coat
QC	Quality control
RDT	Rapid diagnostic test
RBC	Red blood cell
SOP	Standard operating procedure
WHO	World Health Organization

## EXECUTIVE SUMMARY

Case management, through accurate and timely diagnosis and treatment, is one of the key strategies for reducing malaria related morbidity and mortality in South Africa. Additionally, it is a critical part in supporting, achieving and ultimately maintaining the National Malaria Elimination Strategy (2011-2018). Since quality assurance is vital for ensuring reliable malaria diagnosis, it has become imperative that standardised guidelines be readily available. The National Department of Health, together with key stakeholders, has therefore drafted these guidelines to support quality diagnosis of malaria in South Africa.

The purpose of these Quality Assurance Guidelines is to standardise both malaria detection methods (microscopy and rapid diagnostic tests) and to ensure timely reporting of accurate malaria diagnostic results, within private laboratories, the National Health Laboratory Service and the Provincial Malaria Control Programme laboratory services.

These guidelines detail competency requirements of healthcare workers who perform malaria diagnosis by rapid diagnostic tests and microscopy. The guidelines also outline elements for an external quality assurance system. Detailed standard operating procedures are also provided to help standardise malaria diagnostic procedures, irrespective of the setting.

The guidelines presented here are intended for use by healthcare workers in both the public and private sectors and should be used to strengthen laboratory and field-based diagnosis of malaria in South Africa.

## 1. INTRODUCTION

In South Africa all clinically suspected malaria cases must be confirmed either by microscopy or by a rapid diagnostic test (RDT) prior to the initiation of therapy. Since the management of malaria is highly dependent upon accurate and timely diagnostic analyses, this comprehensive quality assurance (QA) guideline has been compiled to facilitate the standardisation of both the malaria detection methods and the timely reporting of accurate malaria diagnostic results in South Africa. Improvement in the quality of diagnosis as well as diagnostic standardisation is critical as with the improvement of public health malaria control methods, the detection of low parasite counts becomes even more important.

For the purpose of these guidelines, “quality” is defined as consistently meeting predetermined technical and management standards, as defined by the World Health Organization (WHO). These standards are critical not only to establish and maintain test quality, but also to meet regulatory requirements needed to eliminate malaria in South Africa by 2018. Clear and detailed QA protocols will increase testing quality and consistency, resulting in improved patient and public health outcomes as well as decreasing the costs associated with misdiagnosis.

These guidelines were drafted by the National Malaria Control Programme (NMCP), using the WHO Malaria Diagnosis Manual as a guide, in consultation with the National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service (NHLS), and the Malaria Research Unit of the South African Medical Research Council (MRC). Topics covered in these QA guidelines include internal and external quality control, equipment and reagent quality, workload, workplace conditions, training and laboratory staff support. Guidelines were reviewed by the SA Malaria Elimination Committee (SAMEC) and the National Malaria Control Programme, in consultation with leaders from the NHLS.



## 1.1 GOAL AND PURPOSE

The goal of this manual is to define measurable standards together with operating procedures for every stage of the malaria diagnostic process in order to improve the quality and consistency of malaria diagnosis in South Africa.

The purpose of these guidelines is to:

- describe the quality policies, processes, and activities related to malaria diagnosis; and
- provide the operating procedures which must be implemented at every stage of the malaria diagnostic process; and
- standardise diagnostic procedures thus ensuring every patient receives timely and reliable malaria test results.

These QA Guidelines are applicable to **all** levels of laboratory services and health care establishments in South Africa that conduct malaria diagnosis. Every laboratory and health establishment must have a copy of the manual and all staff involved in any stage of the malaria diagnostic cycle, from sample collection to results delivery, must be familiar with and adhere to all applicable guidelines and procedures outlined within this manual. Laboratory and health care establishment managers will be responsible for ensuring that all the relevant staff members are familiar with the quality policy and procedures outlined in these guidelines.

## 1.2 STANDARD OPERATING PROCEDURES

In order to ensure these QA Guidelines are optimally implemented, standard operating procedures (SOPs) have been included as Annexures A-F for the following:

- Rapid diagnostic tests
  - A. Use and interpretation of RDTs

- Blood films for microscopy
  - B. Thick and thin blood film preparation
  - C. Blood film staining with Giemsa stain
  - D. Microscopic examination for malaria parasites
  - E. Malaria parasite quantification
- Supervisory laboratory visits
  - F. Checklist for laboratory supervisory visits

The above are to be followed in all testing facilities and should be used as the basis for laboratory-specific standard operating procedures.

## 2. QUALITY ASSURANCE STRUCTURES AND RESPONSIBILITIES

Quality assurance is defined by the WHO as the monitoring and maintenance of high accuracy, reliability and efficiency of laboratory services.

The three groups of laboratories that diagnose malaria in South Africa are:

- NHLS;
- Private sector laboratories; and
- Laboratories based at Provincial Malaria Control Programmes (PMCP).

The NHLS and private laboratories provide the technical expertise regarding the processing of samples and confirmation of results, whereas the NMCP oversees the rollout and implementation of sample collection and result reporting by provincial teams in endemic and non-endemic provinces. Each is responsible for enforcing and reinforcing protocol within their respective domains.

Laboratories are responsible for:

- Guaranteeing quick turn-around times in submitting results to facility/provincial team in the specified times.
- Planning and implementing training and competency activities for laboratory staff, as well as retraining for staff not meeting QA competency standards.
- Ensuring equipment is in good working order with no breakdowns in the diagnostic supply chain.
- Coordinating cross-checking of slides and participation in EQA programmes.

Given the cross-cutting nature of certain challenges and the interconnectedness of specimen collection and scientific diagnosis, these organisations must collaborate closely to identify and address any issues impacting the quality of diagnostic tests and/or the timely delivery of results throughout the country.

### **3. PERSONNEL ENGAGED IN MALARIA DIAGNOSTICS**

Under the Malaria Elimination Strategy, certain healthcare professionals, namely, phlebotomists, nurses, surveillance officers, doctors and laboratory staff will be expected to perform malaria RDTs. Only qualified malaria microscopists and/or laboratory staff (technicians, technologists or scientists) who have demonstrated adequate malaria microscopy proficiency will be required to review blood films for diagnostic and QA purposes.

#### **3.1 HEALTH CARE WORKERS AND PHLEBOTOMISTS**

Nurses, doctors and phlebotomists are generally responsible for collecting blood as stipulated in the Health Act No. 61 of 2010 (sections 55 and 56).

#### **3.2 MALARIA SURVEILLANCE OFFICERS**

Malaria surveillance officers, based at the NMCP, provincial offices or field offices, conduct active surveillance in response to all positive malaria cases detected at health facilities. The surveillance entails performing rapid diagnostic tests (RDT) in the community from which the patient came.

#### **3.3 MALARIA MICROSCOPISTS/LABORATORY TECHNOLOGISTS**

Laboratory staff (scientists, technologists, technicians) and malaria microscopists are responsible for routine preparation, staining and microscopic examination of blood film and/or performing RDTs.

Basic training in malaria microscopy, either during pre-service training or through an intensive malaria microscopy course, is required for all laboratory professionals performing microscopy.

### **3.4 MALARIA QUALITY ASSURANCE OFFICERS**

Certain staff, who have achieved a high level of malaria microscopy competency, as demonstrated in an external quality assurance (EQA) testing scheme or performance in an international microscopy accreditation programme, may be selected to become Malaria Microscopy Quality Assurance Officers.

These officers, in addition to performing routine clinical malaria microscopy at their respective health facilities, may also be called on to serve as:

- trainers on malaria diagnosis, and/or
- reference microscopists for slide cross-checking.

## 4. MALARIA DIAGNOSIS

### 4.1 IMPORTANCE OF ACCURATE AND TIMELY DIAGNOSIS

Malaria diagnosis, based on clinical symptoms and signs alone (primarily the presence of fever), is non-specific in areas of low malaria transmission like South Africa. Consequently, diagnosis based on clinical grounds alone may lead to misdiagnosis, resulting in true cases of malaria remaining untreated and unnecessary loss of life. Therefore, accurate, reliable and timely malaria diagnosis is essential. Although microscopy has historically been considered the gold standard for malaria diagnosis, its value is limited by factors intrinsic to blood film microscopy, such as the detection threshold, as well as the fact that many South African laboratories lack both the infrastructure and skilled staff to efficiently conduct microscopic analysis. These limitations, especially the logistical challenges, are overcome to some extent by the use of RDTs. Therefore, under the current National Malaria Diagnosis and Treatment Guidelines, both malaria microscopy and malaria RDTs are employed in South Africa for malaria diagnosis.

Other methods for malaria diagnosis are available and may be used; these include quantitative buffy coat concentration, nucleic acid methods such as polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP).

When possible, malaria diagnostic test results should be confirmed, e.g. by a second microscopist, or by a different method, e.g. RDT could be confirmed by microscopy. However, this will be constrained by the capacity of the testing facility/laboratory network. Confirmation of results must not compromise turnaround time for patient diagnosis. Confirmatory testing processes in each laboratory should be documented e.g. in a SOP.

Prompt and accurate malaria diagnosis is essential for effective malaria case management as well as the public health response to malaria. By employing this multi-element malaria diagnostics system, South Africa's health care system will be able to:

- definitively diagnose the disease;
- assess the severity of disease, especially in the case of severe malaria;
- identify the *Plasmodium* species responsible for the malaria infection.

Guidelines for malaria diagnosis and quality assurance have been developed by the NICD and MRC to assist laboratory staff to identify, monitor and minimise the occurrence of errors that lead to incorrect/invalid malaria test results and resource wastage. The standard operating procedures (SOPs) included as annexures are to be used as points of reference.

---

## SAFETY

Universal safety precautions should be applied at all times including sample collection and testing. Personal protective equipment must be worn. Extra care must be taken when working with possibly infectious blood samples and sharps i.e. glass slides, lancets. As soon as test results have been recorded, all sharps and materials must be disposed of into appropriate waste containers. The test area must also be swabbed down with an appropriate disinfectant. Prior to leaving the test area staff must also wash their hands thoroughly with disinfectant.

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## WORKPLACE ENVIRONMENT

### **Microscopy**

The following are prerequisites for adequate microscopy in the workplace environment:

- Airflow/air-conditioning to keep the environment at a comfortable temperature for microscopists.
- Ambient light, at least that of 2000 - 5000 lux (provided by fluorescent overhead lighting).
- A receiving bench/table.
- Enough bench space for a microscope, 2 slide racks, writing equipment and log books, sharps disposal containers and sundries used while working per microscopist.
- Under-bench space for waste bins, both biological and normal waste.
- Wall space for bench aids and clock/timer.
- An adjustable chair per microscopist.
- Separate space for sinks and staining equipment on bench top.
- Storage spaces, one for chemicals and the other for records and slides.

## **RDTs**

The following are prerequisites for the laboratory environment:

- Ambient lighting and temperature as above.
- Cupboard for storage.
- Bench top/desk space to perform test (equipment below) and for record books, notification books, referral slips and pens.
- Seating for both tester and patient.
- Wall space for bench aids and information.

The following are prerequisites for field usage:

- Cooler box with ice-bricks in which to transport kits, preferably with thermometer (much like those used by EPI).
- Flat surface to act as a bench top as described above.
- Timer.
- User and patient information leaflets.
- Record and notification books and writing equipment.
- Referral slips.

## 5. MALARIA RAPID DIAGNOSTIC TESTS (RDTs)

The WHO malaria QA update, April 2008, reported on RDT use in a number of different malaria control programmes and revealed RDTs results varied greatly in the absence of a strong QA programme.

### 5.1 QA CHALLENGES FOR RDTs

Unlike other laboratory-based diagnostic tests, malaria RDT kit usage has its own unique set of challenges, which include:

- Healthcare workers with limited or no professional laboratory training or experience are expected to definitively diagnose malaria using the RDTs. To address this shortcoming, high-quality laboratory training involving specimen collection, equipment usage, storage conditions, results interpretation and laboratory safety must be provided to these non-laboratory-skilled staff members (see below).
- Quality control of laboratory tests. As RDTs are single-use devices, QA testing of the kits must entail batch testing, i.e. testing one RDT on a daily basis and/or each time a new box is opened against a known positive specimen. However, given the limited access to positive specimens, alternative means of ensuring RDT quality (e.g. checking a proportion of RDTs using a reference method) are acceptable.

### 5.2 RDT PURCHASING

Malaria RDTs either detect only *Plasmodium falciparum* or *P. falciparum* and other *Plasmodium* species (PAN-kits). In South Africa the use of *P. falciparum* specific kits is recommended as they are more sensitive than PAN-kits and *P. falciparum* infections are the most common type of malaria in our country. PAN-kits may be kept by the reference laboratories and larger testing sites.

### 5.3 RDT STORAGE

RDTs degrade rapidly when exposed to high temperatures and/or high humidity. Therefore, it is imperative that the RDT test kits are stored within the manufacturer's specified temperature ranges. This information is available on the package insert, which should remain in the kit box at all times.

1. All RDT storage rooms must be equipped with a reliable accurate thermometer and if possible, a hygrometer. These pieces of equipment must be serviced and calibrated on an annual basis.
2. Staff must familiarise themselves with the temperature specification of the chosen RDT kit and ensure the store room temperature is maintained within recommended range.
3. The temperature of the store room must be monitored on a daily basis. These temperature readings must be recorded on a log sheet, which is reviewed on a monthly basis and then archived.
4. If dramatic fluctuations in the storage room temperature are detected, the problem must be investigated and rectified.
5. All the RDTs exposed to the excess temperature fluctuations must be discarded and new lots of RDTs must be requisitioned.
6. RDTs may be taken into the field, provided that they are stored in a temperature-monitored cooler box, much like those used for vaccines. This procedure should also be documented, reviewed and archived.

### 5.4 RDT PRE-TEST PROTOCOL

#### INFORMED CONSENT

In cases where the healthcare worker is to perform the malaria RDT test, he/she must identify the patient to be tested and explain clearly the RDT procedure, ensuring they understand and give informed consent to taking the test.

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## INSPECTION PRIOR TO USE

1. Before opening the RDT packaging box, the healthcare worker must ensure that the RDT has not expired and that the RDT foil packaging of individual tests has not been damaged in any manner.
2. The box must be dated and signed and labelled as "Opened on ...(date) by...(name)." The RDT must be discarded if the expiration date has passed and/or if the packaging has been damaged.
3. The package insert should be examined to check that the manufacturers have not changed any of the testing requirements or procedures. The insert should be kept with the box until all RDTs have been used and then archived.

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## LABELLING OF THE RDT

1. The RDT test itself must be labelled with the patient details.
2. After the RDT is appropriately labelled, sample collection and testing can begin (see **Annexure A**).

## 5.5 RDT RESULTS

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### RDT INTERPRETATION

The RDT results must be interpreted in accordance with the manufacturer's instructions and SOP for interpreting RDT results. Possible results for a *P. falciparum* RDT are:

- **Negative:** only the control band develops after the appropriate incubation period.
- **Positive:** both the control band and test band develop after the appropriate incubation period. Note: faint bands are acceptable.
- **Invalid:** no control band develops, while a test band may or may not develop after the appropriate incubation period. This result is invalid

and a new RDT must be administered.

---

#### LIMITATIONS AND MISCONCEPTIONS ABOUT RDTs

1. Although the sensitivity of RDTs is 90-100% compared to light microscopy, the operational gold standard for malaria diagnosis is still a Giemsa-stained thick and thin film examined under a light microscope. Therefore if in doubt of the RDT result (in a correctly quality assured kit), always perform microscopy at the required standard.
2. Due to persistence of histidine-rich protein 2 (HRP2, the antigen detected by many *P. falciparum* RDTs) despite the clearance of parasites, the use of RDTs for monitoring treatment is limited.
3. Parasite load cannot be determined.
4. Mixed infections will not be detected when *P. falciparum*-specific RDTs are used. If a mixed infection is suspected, either microscopy on a thin film may be done or a blood sample be sent to a reference laboratory.
5. False-negative results, especially in the case of high parasitaemias, may occur. Hence if test negative but clinical index of suspicion high, repeat the test or perform standard microscopy.
6. False negatives may also result from very low parasitaemias i.e. parasite load <200 parasites/ $\mu$ l of blood.
7. False positives may occur, in patients who are positive for rheumatoid factor or other autoimmune markers.
8. Operator errors may produce false results.

## 6. MICROSCOPY

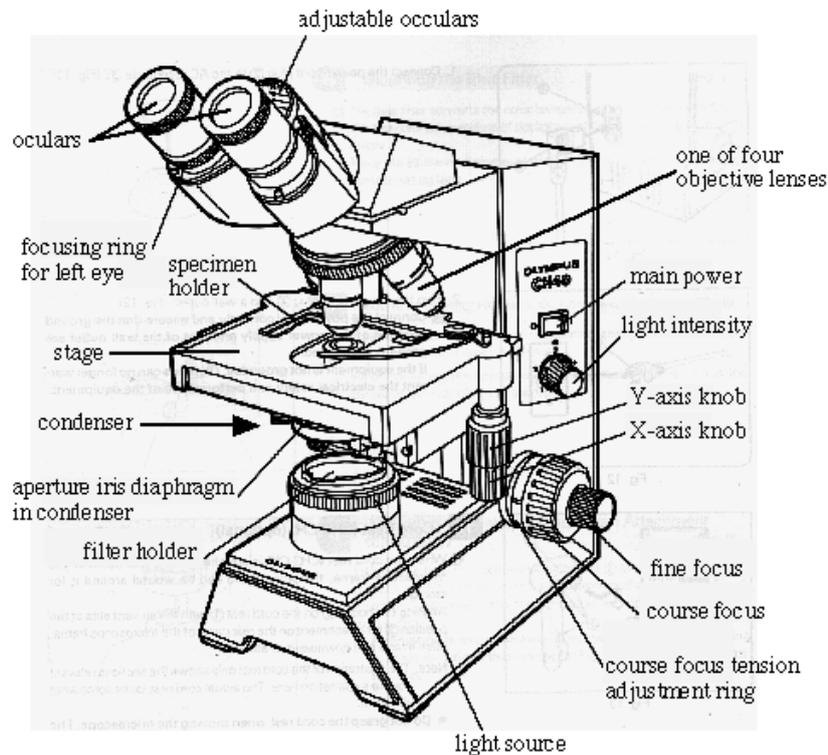
Both RDTs and blood films can be used to diagnose malaria, but information regarding parasitaemia level and malaria parasite species can only be obtained from the microscopic analysis of blood films. Although microscopic analysis is potentially both sensitive and specific, several human resource and operational short comings must be addressed in order to maintain high-quality testing. Maintaining skilled laboratory staff capable of efficiently reading blood films is extremely challenging in South Africa given the low national malaria incidence. Consequently, regular training and stringent competency testing is essential to ensure the required test quality level is maintained.

### 6.1 EQUIPMENT, REAGENTS AND CONSUMABLES NEEDED FOR MICROSCOPY

Good microscopes and lighting is essential for successful malaria diagnosis, as morphology and size of the red blood cells as well as the parasite are critical.

Your microscope should be equipped with the following parts (see Figure 1 below):

1. Head: either binocular (preferred) or monocular.
2. Ocular/s: 10x is essential.
3. Objectives: 10x (low power), 40x (high power) and 100x (oil immersion). For malaria microscopy 100x oil immersion lens is essential.
4. Stage: a mechanical stage for movement on the X and Y axis is important.
5. Condenser: a condenser with an iris diaphragm is required.
6. Light source: either reflected or illuminated.



**Figure 1. A compound microscope<sup>9</sup>**

**Maintenance of the microscope**

1. Always cover the microscope when not in use.
2. Remember: do not expose non-oil immersion lenses to oil.
3. Remove dust from all optical surfaces with a clean soft cloth or soft brush.
4. Remove oil and fingerprints on the lenses with clean soft cloth, alcohol swab or lens tissues. Do not use ordinary tissues or toilet paper as you may scratch the lens.
5. An alcohol swab or 70% isopropyl or ethyl alcohol on a tissue may be used to clean the stage, lamp (when cool) and substage condenser as needed.
6. Schedule a complete clean and calibration at least annually and record all data related to maintenance and repair.

Reagents and consumables needed for successful malaria microscopy include:

1. Pre-cleaned glass slides (slides should be cleaned with methanol).
2. Pipette/ graduated cylinders.
3. Spreaders (bevelled glass slides - these are more expensive but use may be limited to spreading of blood films).
4. Methanol\*.
5. Giemsa stain\* plus suitable diluent of pH 7.2\*.
6. Filter paper and funnel.
7. Wash bottles/ apparatus to wash stain off slides.
8. Staining and drying racks.
9. Timer.
10. Immersion oil and lens paper.
11. Slide boxes/ cabinets for storage.

\*These reagents must be dated and signed and labelled as "Opened on .....(date) by....(name)". Reagents must be discarded if the expiration date has passed and/or if the packaging has been damaged.

## 6.2 MICROSCOPY OF BLOOD FILMS

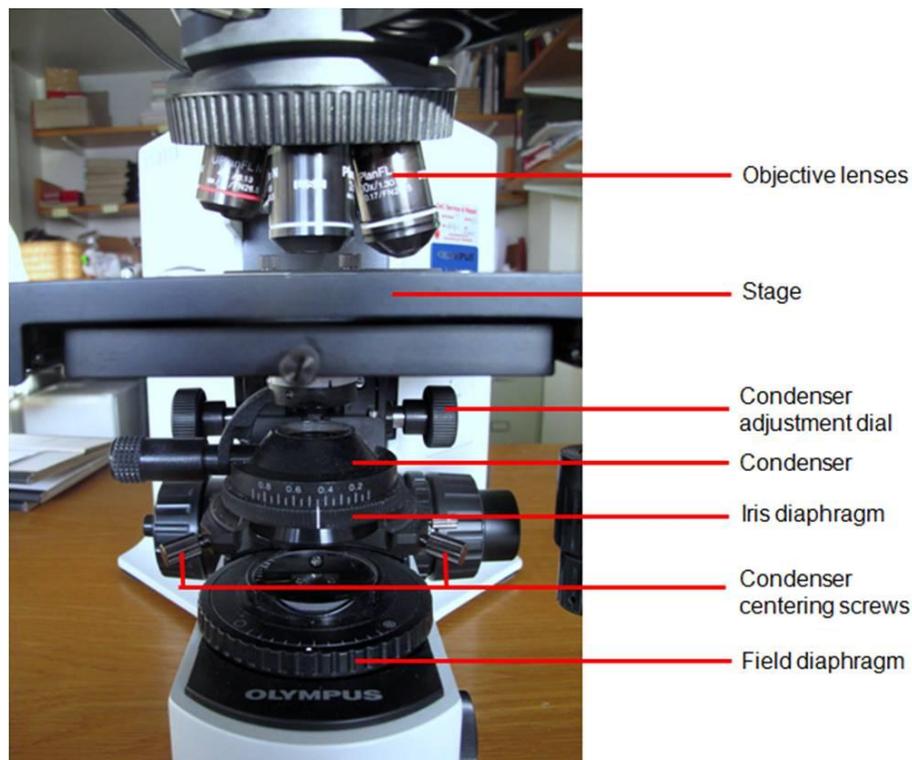
### KÖHLER ILLUMINATION

This method ensures that the specimen is optimally illuminated in a uniform fashion and any imperfections on the glass surfaces are minimised. See Figure 2.

1. Place the slide on the stage and focus on the blood cells using the 10x objective.
2. Open the condenser iris diaphragm fully.
3. Close the aperture of the field diaphragm to produce the smallest diameter field of light.
4. Slowly turn the condenser adjustment dial until the edge of the field diaphragm appears in sharp definition against the light. As you do this,

there may be a red fringe on one side, and a blue fringe on the other; select an even balance.

5. Use the condenser centering screws to centre the circle in your field of view.
6. Lastly, open the field diaphragm so that the circle enlarges until it is just inside your field of view (the black border of the circle is no longer visible).



**Figure 2. Components of microscope used for Köhler Illumination<sup>10</sup>**

## BLOOD FILM PREPARATION & EXAMINATION

In malaria microscopy, preparation of good quality blood films is crucial. For every patient suspected of having malaria, a thick and thin blood film must be prepared. No gaps must be present in the thick blood film preparation while the thin film must have a feathered edge (see **Annexure B**). Although there is a range of stains that can be used for malaria microscopy, in South Africa Giemsa stain is recommended. The buffer used to dilute the Giemsa stain must be at pH 7.2, to ensure accurate malaria species identification (see

**Annexure C).** Blood films from different patients should not be prepared on the same slide or stained in the same staining jar. A control slide should be stained with each batch to ensure that the reagents are acceptable – this serves as an internal quality control (IQC).

When reading blood films for malaria, **the thick film must be read first** as low parasitaemias may not have detectable levels of parasites on the thin film (see **Annexure D**). If *P. falciparum* parasites are found, then a parasite count on the thin blood film must be performed (see **Annexure E**) and the percentage of infected red blood cells (RBCs) reported.

All laboratories performing malaria microscopy will participate in several quality assurance activities, including laboratory supervision, participation in EQA programmes, and rechecking of malaria microscopy slides, either within or between different laboratories. These programs are described in detail below.

### 6.3 SLIDE STORAGE

All slides examined and reported, (including slides for initial diagnosis and patient follow-up) must be stored for at least month. Positive patient slides should be kept for a year. Slides should be kept in secure slide boxes/cabinets, protected from excessive heat and humidity (desiccant bags may be used). Slides must be well labelled and stored systematically.

### 6.4 RECHECKING OF MICROSCOPY

Microscopy results should be cross-checked as described below. Rechecking is distinct from test confirmation (as detailed in 4.1) and is done primarily for quality assurance purposes.

- PMCPs should consider having microscopists dedicated to quality assessment/assurance including rechecking of slides.

- Routine diagnostic laboratories (NHLS and private) should conduct rechecking either by microscopy or an alternative identification method, e.g. PCR or QBC. Reference laboratories and large testing sites must be identified as cross-checking facilities, and should have Malaria QA Officers i.e. slide re-checkers.

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## STRUCTURE OF CROSS-CHECKS

Cross-checking must be blinded to ensure objectivity, meaning the Malaria QA Officer checking the slides must not know the initial result. Therefore slide selection (and mixing of positive and negatives) for quality control is done by the sending laboratory. Once the slides have been selected, the laboratory must pack and transport slides to the cross-checking facility on a monthly basis. The responsible Malaria QA Officer reviews the slides and completes a report with results. Cross-checking of slides should occur as soon as possible after receipt of slides from the facilities. Ideally, cross-checking is performed on a monthly basis and results and slides sent back to sites within two weeks.

---

## SIZE OF CROSS-CHECK SAMPLE

Initially, all positive blood films and 10% of negative films undergo cross-checking by a Malaria QA Officer. In the future, the proportion of slides that are cross-checked and the frequency of cross-checking may be adjusted.

---

## ANALYSIS OF RESULTS AND FOLLOW-UP

Once the slides have been read and recorded, the results are compared to the initial results. Discrepancies should be re-checked by a further un-blinded reader before reporting the result as discrepant.

The false positives and false negatives will be recorded to calculate the overall percent agreement between the laboratory's microscopy results and the re-checking method. The Malaria QA Officer issues a report with the results of the cross-checking to the laboratories, and also sends back the slides. As experience with the cross-checking is gained, reports to laboratories should also include past performance and information on

performance of all participating laboratories (e.g. averages and ranges). For discrepant results, the Malaria QA should provide feedback, including likely explanations for the discrepancy and suggested corrective actions. For discrepant results, a corrective action form is completed by the laboratory and should be available for review by supervisors during visits. Follow-up visits to sites and remedial training should be provided to laboratories as needed.

Results of cross-checking should be analysed for several months, as a poor result in one month may not be representative of overall performance due to the small number of samples selected for cross-checking.

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## STANDARDS FOR CROSS-CHECKING

At the central level, it is desirable to establish performance targets and thresholds for follow-up. These should reflect reasonably achievable standards and should take into consideration the results of all participating laboratories. It is best to set targets and thresholds based on actual results and experience with cross-checking. Until South Africa has additional experience with cross-checking, local targets and thresholds must be flexible, but at the start of cross-checking programme, the following thresholds for each laboratory may be considered:

When previous cross-checking results have been good to satisfactory:

- 2 errors out of 10 is an alert.
- $\geq 3$  errors out of 10 results requires immediate investigation.

When previous cross-checking results have been poor:

- A result that is better than the previous result is encouraging.
- A persistently static or declining percent agreement between the facility results and the cross-checking results indicates that corrective actions have not been effective and should be reviewed.

When reviewing cross-checking results and setting thresholds, it is imperative

to maintain focus on the greater goal of microscopy cross-checking: continuous improvement in the quality of malaria diagnosis in South Africa. A collaborative spirit between the laboratory and cross-checking facility should be maintained. Results of re-checking can have a considerable impact on staff morale. Furthermore, the interpretation of cross-checking results must be taken in context with the number of slides cross-checked, previous performance of the laboratory, and the overall level of accuracy of testing required to support effective clinical management and surveillance. The relatively small number of slides examined may not be representative of a laboratory's overall performance. In case of questionable results, additional slides should be cross-checked and past performance considered.

### **6.5 PROFICIENCY TESTING SCHEMES (PTS)**

All laboratories must be enrolled in an appropriate PT Scheme; ideally such that all malaria diagnostic tests used by the laboratory are covered. Laboratories will be responsible for: receiving PT samples; completing and dispatching the assessment within the stated timeframe; receiving performance results; and taking the necessary actions to correct poor performance.

A Blood Parasite PT Scheme is produced by the NHLS. PT samples are couriered three times a year to participating laboratories and results are generally due back three to four weeks after shipping. Results are assessed and individual reports and commentaries are sent back to participants. Within the NHLS, the Quality Assurance Managers/Coordinators are responsible for monitoring PT performance and ensuring corrective actions are taken by their respective laboratories.

## 7. MALARIA RESULT REPORTING

Test results must be documented on patient health cards (or laboratory requisition form/work card) and should be reported to the healthcare worker (HCW) that ordered the test. The HCW is responsible for informing the patient of the test outcome.

The test results must also be recorded in the appropriate malaria case register, which are forwarded to the NMCP at regular predetermined intervals.

The malaria case registers that need to be completed include:

- **Weekly Notifiable Diseases Summary Report:** This form is completed by all clinics and outpatient departments on a weekly basis and includes information on the numbers of suspected malaria cases, confirmed malaria cases, treated malaria cases, malaria-related referrals and malaria-related deaths
- **Health Management Information Systems (HMIS) Monthly Summary Sheet:** This form is completed by all clinics and outpatient departments on a monthly basis and contains aggregate data for all diseases detected at health facility.
- **Positive Case Report Form:** This form is filled in by selected health facilities and only reports on confirmed malaria cases.

Completion of all the above are the responsibility of the HCW, not the laboratory staff. Please note reporting requirements of the regulations regarding Notifiable Medical Conditions.

It is required by law, as stipulated in the Health Act No 63, 2003, to notify the NDoH of all malaria cases detected. This provides the NDoH with the information needed to monitor malaria trends in South Africa and to take the necessary actions when required. Such surveillance is especially important to achieve malaria elimination.

## 8. SUPERVISION

### 8.1 ROUTINE SUPERVISORY LABORATORY VISITS

Supervisory visits to laboratories performing malaria diagnosis strengthen communication between the different levels of the QA programme. They also provide an opportunity to identify reasons for poor performance and recommend appropriate actions to correct these challenges. Many issues (e.g. poorly maintained microscopes, stock-outs, excessively high workload), which may contribute to poor microscopy performance, can be identified and addressed during a supervisory visit.

The NHLS began a programme of routine quality assurance supervisory visits to all laboratories in late 2008. These visits are conducted by the trained auditors/QA coordinators, who employ a standardised checklist to review all sections and functions of the laboratories. The NDoH will arrange similar visits to the PMCP laboratories to ensure criteria for malaria diagnosis are met. The WHO has developed a useful checklist that can be used for malaria supervisory visits; this can be found in the WHO Universal Access to Malaria Diagnostic Testing Operators Manual version one, as Annex 12, (<http://www.who.int/malaria/publications/atoz/9789241502092/en/>). See **Annexure F**.

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#### STRUCTURE OF VISITS

Site visits generally last a day. The targeted frequency is 4 visits per year, although the minimum recommended frequency for malaria is 2 visits per laboratory per year.

Key aspects of malaria microscopy and malaria RDT quality assurance are covered during the routine quality assurance supervisory visits. The QA checklist should include the following sections relevant to malaria diagnosis:

- Corrective actions (from previous quality assurance visit, cross-checking results, EQA results).

- Equipment maintenance, including a well-maintained microscope.
- Internal QC of stains performed at required intervals, and availability of control slides.
- Receiving and storage of reagents and RDTs.
- Inventory management of malaria products.
- Expired reagents and RDTs.
- Malaria sample management.
- Housekeeping (e.g., waste disposal).
- Safety.
- Malaria laboratory logs.
- Malaria SOPs filing and storage.
- Procedures for blood film preparation, staining and examination are in accordance with SOPs.
- Slides are available and properly stored for cross-checking.
- Staff have received adequate training.
- Workload.

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## POST-VISIT FOLLOW UP

The results of the QA visit are discussed with the laboratory supervisor before the assessor leaves the site. In addition, the results and need for corrective action as they relate to malaria are shared with the NMCP. Laboratories are expected to undertake and document corrective actions as needed. Records of corrective action should be maintained at the laboratory for review by supervisors during visits.

## 8.2 CASE MANAGEMENT MONITORING AND MENTORING SITE VISITS

The Case Management Coordinator and other NMCP/Laboratory Services staff periodically visit sites to mentor healthcare workers on malaria case management, including diagnosis. The objective of site visits is to mentor staff on implementation of case management policies, adherence to the 100% definitive diagnosis policy, use of RDTs, and management of patients based on diagnostic test results. Mentoring will primarily be performed by the case management coordinator.

### STRUCTURE OF VISITS

The frequency of visits depends on the facility:

- For facilities in the malaria endemic regions of South Africa, the recommended number of visits is one per quarter during the malaria season.
- For all other facilities, one visit per year is recommended.

With regards to malaria diagnosis QA, visits include the following:

- Check on the ability of the healthcare worker to set up a testing environment consistent with that described in the SOP, including temperature monitoring and storage conditions, labelling and recording, and safety considerations.
- Observation of RDT performance to ensure that all steps are performed correctly.
- Observation of preparation of blood films to ensure proper preparation and storage.
- Check on ability to properly carry out all record keeping procedures.
- Review of clinic registers to assess adherence to National Guidelines for Malaria Diagnosis and Treatment.

- Check to ensure good blood safety practices are maintained.
- Review of stocks to ensure sufficient supplies are in place, including medications for management of malarial and non-malarial fever.

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#### POST-VISIT FOLLOW-UP

Most problems or deficiencies identified during visits are discussed immediately and rectified before the Case Management Coordinator or other mentor leaves the site. At times, additional training or follow-up visits might be warranted. The Case Management Coordinator tracks site visits completed and discusses progress and major problems with NMCP and NHLS during regular Malaria Diagnostic Quality Assurance meetings.

## 9. PERSONNEL QUALITY ASSURANCE: TRAINING & COMPETENCY ASSESSMENTS

One of the most important factors in ensuring accurate and reliable malaria test results is the availability of appropriately trained staff to perform microscopy, RDT testing and the various quality assurance activities. Training sessions organised by provincial/regional laboratories are to be held on an annual basis to maintain skill levels.

### 9.1 TRAINING ON RDTs AND MICROSCOPY

Healthcare workers and surveillance officers must receive training in RDT administration and interpretation of results, prior to administering RDTs at health facilities and/or in communities. Although malaria RDTs are relatively simple to use, operational research has demonstrated that health workers who receive training and job aids outperform fellow health workers who rely solely on the RDT manufacturer's instructions, as mentioned in the WHO link <http://www.wpro.who.int/sites/rdt/copyright.htm>.

Malaria microscopy requires thorough training and experience/practice. Training may include how to prepare, stain and examine blood films, and how to perform parasite counts. To maintain quality and accuracy, a microscopist should **not** examine slides for the presence of malaria parasites for more than **4 (four) hours** per day **nor** examine more than 50 slides per day.

Malaria RDT and microscopy training should include result recording and reporting.

### 9.2 RDT COMPETENCY ASSESSMENT

Prior to a trainee being permitted to perform RDT testing unsupervised, his or her ability to conduct the test efficiently and accurately must be assessed through practical demonstration. The RDT competency assessment includes:

- **Technique in performing RDTs:** the staff member is observed in performing the finger prick, transferring blood with the blood transfer device to the RDT, and performing the test procedure in accordance with the SOP.
- **Reading RDT results:** the healthcare worker is required interpret a set of photographs of prepared RDTs, including negative results, weak positive results and invalid results.

### 9.3 MICROSCOPY COMPETENCY ASSESSMENT

At the end of training, laboratory technologists and malaria microscopists are required to demonstrate competence in:

- **Preparing and staining blood films:** the laboratory technologist/malaria microscopist is test-witnessed in preparing and staining thick and thin blood films.
- **Reading microscopy results:** the laboratory technologist/malaria microscopist is required to correctly interpret a set of prepared microscopy slides, which include blood films with no parasites and low positive results.

## 10. REFERENCES

1. WHO Malaria Elimination Manual; Global Malaria Programme, WHO 2009.
2. South Africa Draft Malaria Elimination Strategy, 2010 -2018.
3. National Health 2003 (Act No 63, 2003)
4. National Health Act,2010(Act No 61 of 2010, sections 55 and 56)
5. Versalovic, J. Manual of Clinical Microbiology. 10th edition (2011), ASM Press, Washington.
6. Clinical Microbiology Procedures Handbook (2007) ASM Press
7. WHO malaria QA update, April 2008
8. <http://www.wpro.who.int/sites/rdt/copyright.htm>
9. [www.bio.davidson.edu](http://www.bio.davidson.edu), accessed 11/02/14
10. Frean J, Poonsamy B, Shandukani B, *et al.* Case management of malaria: diagnosis. S Afr Med J 2013; 103 (10 Suppl 2): 789-793
11. World Health Organization. Malaria Light Microscopy: Creating a Culture of Quality. Geneva: WHO, 2008.
12. [www.bitesizebio.com](http://www.bitesizebio.com), accessed 06/08/2014

## 11. ANNEXURES

### ANNEXURE A: PERFORMING A MALARIA RDT

Prior to administering a RDT, ensure the testing area is clean, that the RDT expiry date is valid, and that the package is undamaged. The following illustrates the procedure using a finger prick and the First Response RDT as *an example*.



Open the RDT package and label the RDT appropriately. Explain the test procedure to the patient again.



Select the third or fourth finger.



Apply pressure to assist with blood flow. Clean the finger with the alcohol swab, starting from the middle and moving to the tip. Allow skin to dry.



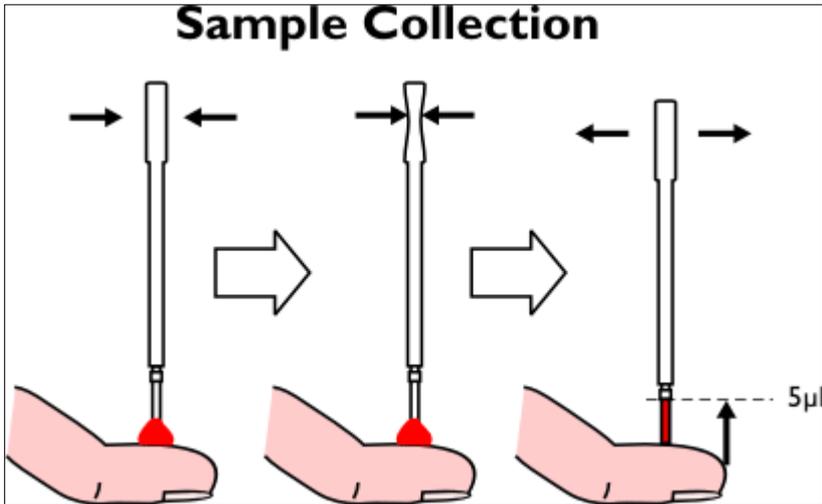
Hold finger firmly and prick side of finger (off-centre) with lancet firmly. Dispose of the lancet safely in a sharps container.



Gentle apply pressure to the finger until a new drop of blood appears.



Squeeze the top of the pipette and place the open end on the blood drop. Gently release pressure drawing the blood into the pipette.

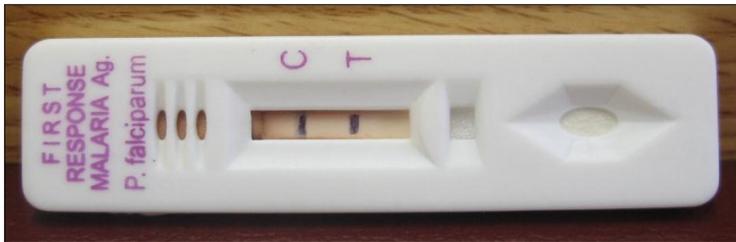


Transfer 5µl of blood from the pipette to the sample well on the rapid diagnostic test cassette.

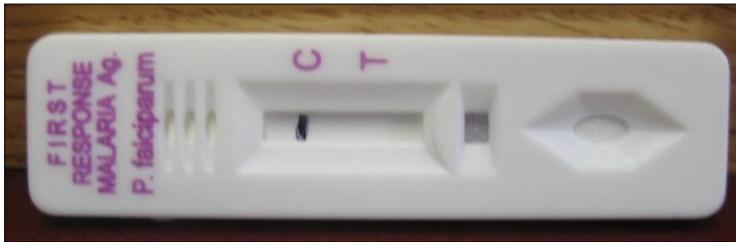


Add 2 drops of buffer into the buffer well. After 15 minutes, record the RDT result on the register/OPD card. Do not interpret results after 20 minutes.

**Interpretation of results:**



*P. falciparum* positive



*P. falciparum* negative



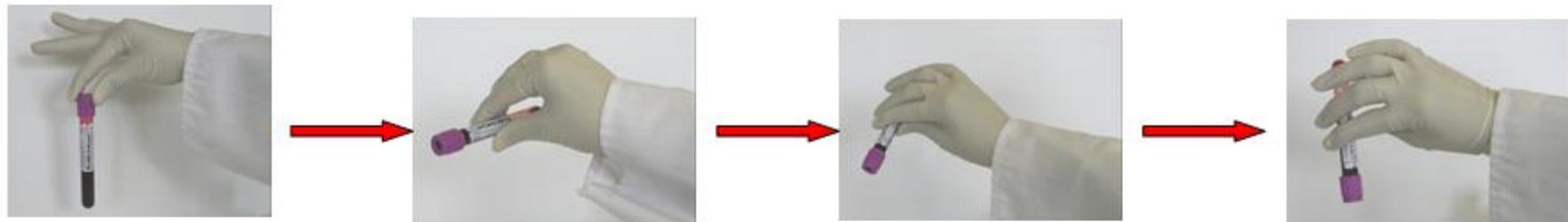
Invalid test



Invalid test

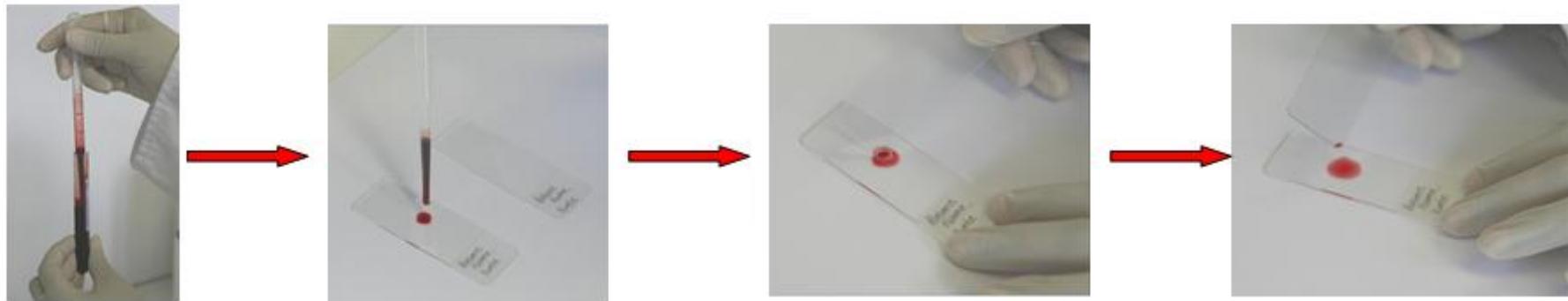
## ANNEXURE B: PREPARATION OF BLOOD FILMS FOR MALARIA MICROSCOPY

Mix EDTA blood slowly by inverting the tube prior to blood film preparation.

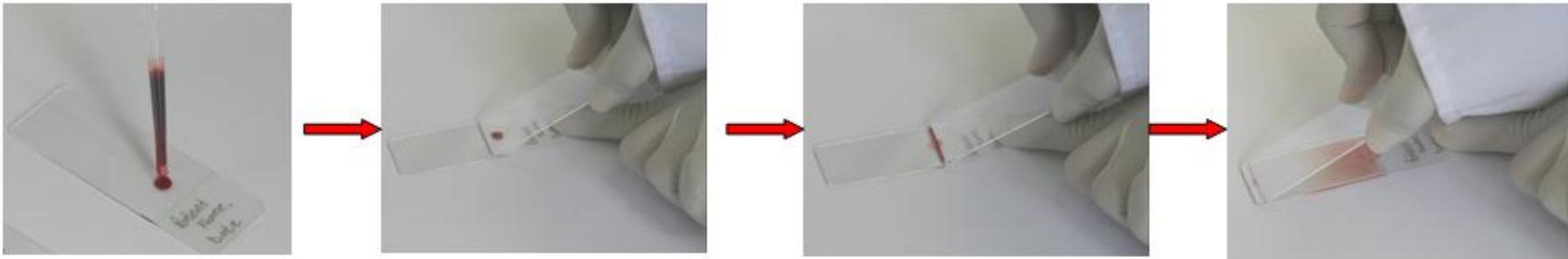


Open blood tubes away from the eyes, preferably behind a shield. Prepare at least one thick and one thin film, per specimen.

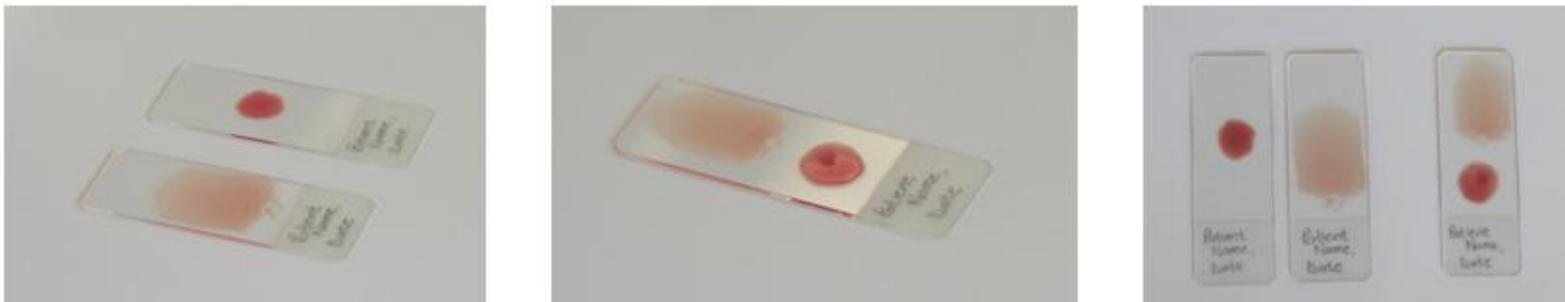
**Thick film:** place a drop of blood ( $\pm 6\mu\text{l}$ ) in the centre of a clean, labelled slide. Using the corner of a bevelled slide, spread the drop in a circular spiral pattern until it is about 10-15mm in diameter (the size of a 10 cent coin). Lay the thick films flat to air dry for about 1 hour. A fan can be used to accelerate drying.



**Thin film:** place a small drop of blood ( $\pm 2\mu\text{l}$ ) towards the frosted end of a clean, labelled slide. Bring a bevelled spreader slide at a 30-45° angle up to the drop, allowing the drop to spread along the contact line of the 2 slides. Push the spreader smoothly but quickly forward toward the unfrosted end of the lower slide to form a film with a feathered edge. Leave the thin films to dry for about 30 minutes.



The thick and thin blood film can be made on separate slides or on one slide.



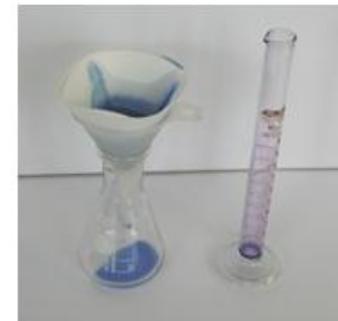
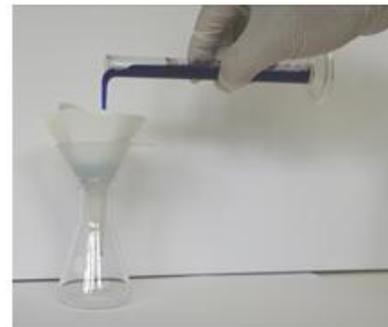
After the blood films dry, stain them with Giemsa as per Annexure C.

## ANNEXURE C: STAINING OF BLOOD FILMS FOR MALARIA MICROSCOPY

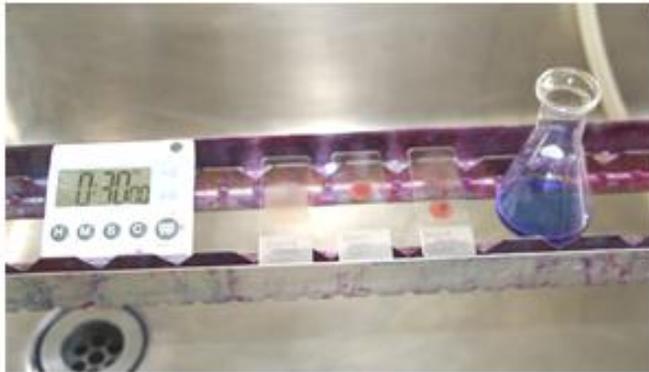
Fix thin blood films in methanol for less than 5 seconds. Thick blood films are **not** fixed prior to staining! If the thick and thin are on one slide, be extra careful that the methanol/ methanol fumes do not come into contact with the thick film.



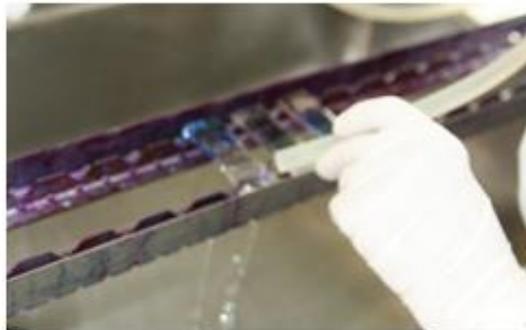
Prepare working Giemsa stain by mixing 1 part stock Giemsa to 9 parts buffered diluent (pH 7.2) or the current dilution established by the Giemsa quality control procedure. The stain should be made fresh, just before use. Filter the stain through filter paper before use.



Flood the blood films with Giemsa stain and leave to stain for 30 minutes.



Rinse the stain gently off the slides with water, being especially careful not to wash the thick films off the slides. It is preferable to rinse the back of the slide bearing the thick film with slow running water, to avoid washing it off.



Allow films to air dry before examining microscopically as per Annexure D.

## ANNEXURE D: MICROSCOPY OF BLOOD FILMS

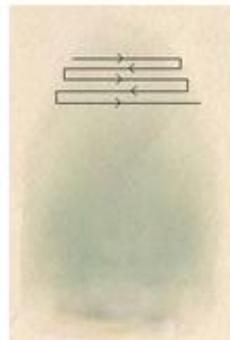
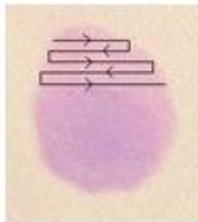
Microscopes must be well-maintained, serviced regularly and the light focused and centred.



**ALWAYS view the thick blood film first.** Scan under 10x objectives to detect microfilariae and trypanosomes. Then examine with 50x and 100x oil immersion objectives. Examine at least 100-150 fields of the thick blood film before reporting a negative result.  
Note: low parasitemias will **ONLY** have malaria parasites on the thick blood film.



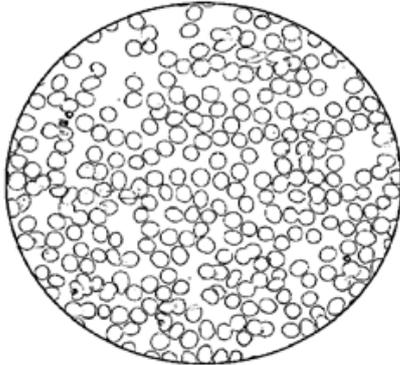
If malaria parasites are present on the thick film, examine the feathered edge of the thin blood film with 50x and 100x oil immersion objectives to determine the species present.



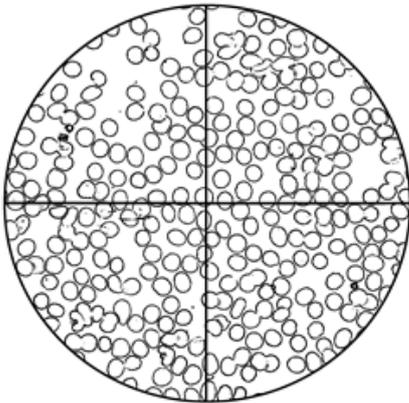
Examine blood films using the 'battlement' method.

If *Plasmodium falciparum* is present, perform a parasite count according to Annexure E.

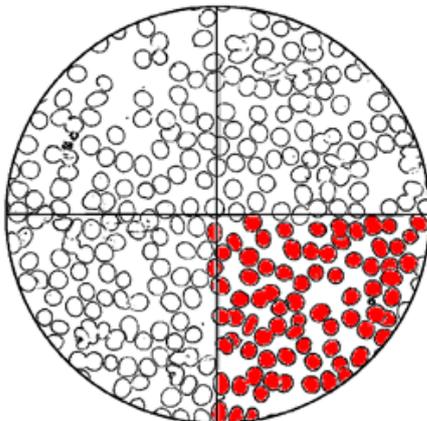
## ANNEXURE E: QUANTITATION OF *P. FALCIPARUM* PARASITES



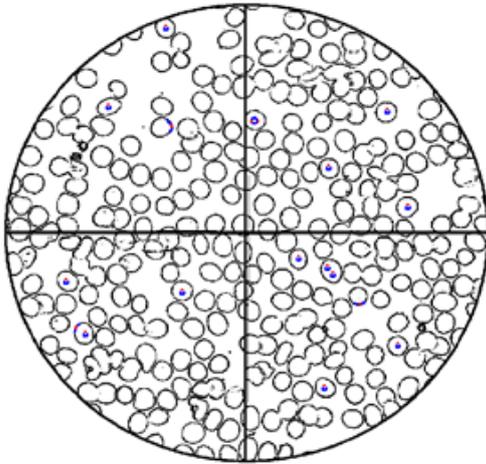
Using the oil objective, find a suitable high power field (HPF) by avoiding the edges and moving to an area on the thin blood film close to the feathered edge, where the red blood cells (RBC) are evenly distributed and not overlapping.



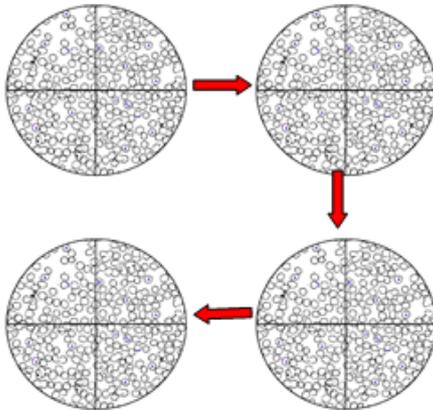
Divide the field into imaginary quarters.



Count the number of RBCs in 1 quarter. Multiply this number by 4 to get the total number of RBCs in that field.  
*Example: 77 RBCs (coloured in red) are present in 1 quarter, therefore 308 (77 x 4) RBCs are in the entire field.*



Count the number of infected RBCs, not parasites, in that entire field and write it down.  
*Example: 15 infected RBCs are present in the entire field.*



Move on to the next acceptable field and repeat the steps above.  
 For high parasitaemias (>2 parasites per field) – continue until a minimum of 2000 RBCs have been counted.  
 For low parasitaemias (~1-2 parasites per field) – continue until a minimum of 4000 RBCs have been counted.  
**Note: the more RBCs counted, the more accurate the count!**

HPF number	RBCs in HPF	Infected RBC
1	308	15
2	321	18
3	306	16
4	297	15
5	301	11
6	323	13
7	<u>306</u>	<u>10</u>
	2162	98



Calculate the percentage of infected RBCs using the following formula:

$$\frac{\text{Number of infected red blood cells} \times 100}{\text{Number of RBCs counted}}$$

Example:  $\frac{98 \times 100}{2162} = 4.5\%$



<b>C. Performance (Review of slides)</b>	
<b>Cross-checking or Validation</b>	
No. of slides cross-checked by validator	<input type="checkbox"/> <input type="checkbox"/>
Agreement (%)	<input type="checkbox"/> <input type="checkbox"/>
False positives (%)	<input type="checkbox"/> <input type="checkbox"/>
False negatives (%)	<input type="checkbox"/> <input type="checkbox"/>
Thick films too thin (%)	<input type="checkbox"/> <input type="checkbox"/>
Thick films too thick (%)	<input type="checkbox"/> <input type="checkbox"/>
Slides poorly stained (%)	<input type="checkbox"/> <input type="checkbox"/>
Slides containing stain precipitate (%)	<input type="checkbox"/> <input type="checkbox"/>
<b>Reference Slides provided by the validator and read by the laboratory</b>	<b>microscopist</b>
	1      2      3      4      5
No. of slides read	<input type="checkbox"/>
Agreement (%)	<input type="checkbox"/>
False positives (%)	<input type="checkbox"/>
False negatives (%)	<input type="checkbox"/>

### III. Laboratory Set-up

	Good	Poor	Remarks
Bench space			
Sink/washing area/staining area			
Access to clean water supply			
Natural lighting			
Power source			
Ventilation			
Storage space for of supplies and materials			
Waste management system			

### IV. Microscopes

	Yes	No	Remarks
The microscope(s) is binocular			
The microscope lamp(s) has sufficient power to provide good illumination when the condenser aperture is set at the correct setting for the x100 objective.			
Blood films can be brought into sharp focus at x100 oil immersion magnification			
The stage movement mechanism is precise and stable			
The microscope(s) is regularly serviced.			
The microscope(s) is protected with a cover when not in use.			
Xylene is never used to clean the microscope(s), objectives or eye pieces.			
Spare bulbs are stored in the laboratory.			

#### V. Microscope slides

	Yes	No	REMARKS
Microscope slides are good quality and thoroughly clean before use			
Microscope slides do not have scratches or surface aberrations			
Microscope slides do not give a blue background colour after staining			
Microscope slides do not have fungal contamination.			
Microscope slides that have been damaged by fungus are discarded and not used again.			
In programmes with high humidity the microscope slides are protected against fungal contamination.			

#### VI. Stain solutions

	Yes	No	REMARKS
Stock staining solutions are stored in the dark and not close to a heat source.			
Commercial stain solutions are within the manufacturer's expiry date.			
Commercial stain solutions do not contain excessive stain precipitate			
The cap of the reagent bottle is always tightly sealed except when stain is being removed for use.			
Stain is always removed from the reagent bottle using a clean pipette or similar			
Water is never added to the stock stain solution.			
Unused stain is never returned to the stock bottle			

#### VII. General Laboratory Supplies

	Yes	No	REMARKS
Alcohol and cotton (or similar) for cleaning skin prior to blood collection.			
Lancets			
Methanol			
Buffer salts or buffer tablets			
pH meter that reads to 2 decimal places			
pH calibration solutions			
Staining jar			

	Yes	No	REMARKS
Staining rack			
Drying rack			
Graduated cylinders of the correct size			
Wash bottles			
Timers – sufficient number for staining and for each microscopist			
Immersion oil			
Tally counters – sufficient number for the number of staff			
Lens paper			
Slide boxes for storage			
For laboratories that prepare stain from powder – sufficient glycerol, methanol, powder, beakers, measuring cylinders, filter paper, funnels, stirring rods, scales, spatulas and storage bottles.			

#### VIII. Documentation

	Yes	No	Remarks
Log book/record book located in the lab			
Patient's name and details recorded in an organized and legible manner			
Date smear collected/received recorded?			
Species identification performed?			
Parasite counting performed? (If yes, specify method used under remarks column)			
<b>Forms</b>			
Pathology request forms used?			
Result/Report forms completed correctly?			
Referral forms used?			
<b>Analysis</b>			
The laboratory prepares monthly analysis including at least the number of slides examined, positivity rate, percentage of species identified (as appropriate) and quantitation.			
Stain QC Register			

	Yes	No	Remarks
There is a Stain QC Register recording the brand, batch numbers, quantity, date of receipt and the date of expiry (if applicable) of all stain powders or solutions, methanol and glycerol used by the laboratory. For stains prepared from powders - the preparation date each batch of stock stain is prepared. For all stains - the staining time and pH for each new batch, and any problems encountered.			
4 Reference Materials			
SOPs located in the laboratory			
Technical Manual and Bench Aids located in the laboratory			
Operating and Maintenance Manuals available (if appropriate)			
Microscope maintenance logbook available			

#### IX Biosafety

	Yes	No	Remarks
The laboratory staff wear protective laboratory coats/gowns			
Staff wear gloves when collecting blood samples			
Hand washing facilities with soap (or similar) available			
The power supply for the microscope(s) and laboratory lighting in good condition and safe			
Proper disposal units			
1. Containers for dry waste			
2. Containers for infectious materials			
3. Puncture resistant container for sharps			

#### X. Quality and Performance Indicators

	Yes	No	Remarks
The laboratory fully complies with the national QC Protocol.			
There is a formal protocol available for the analysis of QC results and for taking corrective action if the results are not satisfactory.			
The laboratory performance in the national QC protocol is satisfactory.			

	Yes	No	Remarks
The laboratory has an additional internal QC program for monitoring the quality of slide preparation and staining.			
The laboratory measures workload.			
There is a protocol to manage workload during periods of staff absences (sickness, holidays etc.).			

**XI. Drug Dispensing**

	Yes	No	Remarks
1. Performs drug dispensing to positive cases?			
How?			
Why?			

**VIII. General Findings and Recommendations**

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**IX. Supervisor/Validator's Comments:**

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Accomplished by:

-----  
 Name of Validator

-----  
 Signature of Validator

Date: -----