# STANDARD OPERATING PROCEDURES

## For Malaria Microscopy

## 2017


National Malaria Control Programme
Department of Public Health
Ministry of Health and Sports
The Republic of the Union of Myanmar

# FOREWORD

Myanmar has signed Asia Pacific Leaders Malaria Alliance (APLMA) declaration to eliminate malaria by 2030 in 9 East Asia Summit in 2014 along with other Greater Mekong Sub region countries. The National Malaria Control Porgramme is committed to pursue malaria elimination by 2030. "National Malaria Strategic Plan (NSP) for Intensifying Malaria Control and Accelerating Progress towards Malaria Elimination 2016-2020" and "National Plan for Malaria Elimination in Myanmar 2016-2030" have been developed.

Myanmar has made significant progress in reducing the malaria incidence by 49% in 2015 in comparison to 2012. The control programme has embarked into elimination aiming to eliminate *P. falciparum* by 2025 and malaria by 2030. Malaria control and elimination depends on parasitological diagnosis by microscopy or RDT to guide individual treatment. Ensuring universal quality assured malaria microscopy is a priority for the programme as we move towards malaria elimination. This will also reduce the over-use of malaria medicines- the Artemisinin-based Combination Therapies and reduce drug-resistance selection pressure on parasites. Malaria microscopy is a gold standard malaria diagnostic test which is currently deployed in the hospitals under the Department of Medical Services and some Public Health Centers under the Department of Public Health.

National Malaria Control Programme and National Health Laboratory have developed the Standard Operating Procedures (SOPs) for malaria microscopy with a technical support from WHO and URC-ADB project. This describes in detail the pre-analytic, analytic and post-analytic procedures for malaria microscopy services. The SOPs aim to provide the guidance to the malaria microscopists for performing the malaria microscopy in uniform, consistent and reliable manner that ensures the accuracy of the malaria microscopy procedures performed. The programme will distribute the SOPs to all microscopy centers (including private sectors) in the country to ensure the use of quality assured malaria microscopy procedures and services are offered effectively and efficiently.

I would fully endorse the malaria microscopy SOPs and ensure its full implementation.

Dr. Thar Tun Kyaw
Director General
Department of Public Health
Ministry of Health and Sports

# ACKNOWLEDGEMENTS

The SOP on malaria microscopy for every hospital in Myanmar has been revised by WHO and URC-ADB project under the guidance of the National Health Laboratory (NHL) and National Malaria Control Programme (NMCP), Ministry of Health and Sports, the Republic of Union of Myanmar.

Version 1 was prepared by URC-CAP Malaria Project with the support of Dr. Khin Mon Mon, former National Malaria Control Programme Manager in 2015. During 2016, "Malaria Surveillance and Quality Assurance System" project (URC-ADB project) was launched with the funding support from the Asian Development Bank (ADB). On 22nd July 2016 stakeholder meeting, H.E Union Minister for Health and Sports delivered speech on malaria laboratory quality assurance system that is essential for the malaria elimination by the year 2020. This project along with WHO provided technical support to NMCP to develop "Quality Assurance and Quality Control Manual" and "Standard Operation Procedures for Malaria Microscopy".

National Malaria Control Programme would like to express heartfelt thanks to ADB Team leader, Gerard Servais, ADB Public Health Specialist, Dr. Kyi Thar, URC Senior Vice President, Dr. Neeraj Kak and Team Leader of URC-ADB project, Dr. Kheang Soy Ty for playing important roles in providing technical advises throughout the knowledge product development.

Moreover, series of reviewing and fruitful technical advice were provided by Prof. Htay Htay Tin, Deputy Director General (NHL), Dr. Win Thein, Director (Laboratory) and Dr. Thi Thi Htoon, Head of parasitology section (NHL). Special thanks to Dr. Badri Thapa, Scientist Malaria Control (WHO) for providing draft documents and proofreading. Moreover, contributors would like to offer their sincere gratitude to Dr. Thandar Lwin, Deputy Director General (Disease Control), Dr. Aung Thi, Deputy Director (Malaria) and Dr. Zaw Lin, Deputy Director (Dengue & Filaria) for final proofreading. Acknowledge and thanks to Malaria Regional Officers, VBDC Team Leaders, Ms. Thiri San, Chief Medical Technologist, NMCP, Medical Technologists and Grade 1 Laboratory Technicians from NMCP, Dr. Myat Kyaw, Deputy Team Leader (URC-ADB project) and Dr. Mie Mie Han, Laboratory QA Specialist (URC-ADB project) and URC-ADB project team for providing helpful discussion and supportive participation. Last, but not the least, National Malaria Control Programme would not have completed this document with the support from the partners and contributors.

National Malaria Control Program
Department of Public Health
Ministry of Health and Sports

**Acronyms**

ADB - Asian Development Bank
APLMA- Asia Pacific Leaders Malaria Alliance
BHS - Basic Health Staff
ECA - External Competency Assessment
EQA - External Quality Assurance
EQAS - External Quality Assessment Scheme
IQC - Internal Quality Control
Lab - Laboratory
LHV - Lady Health Visitor
MLT - Malaria Laboratory Technician
MM - Malaria microscopy
MOHS - Ministry of Health and Sports
MP - Malaria Parasite
MRL - Malaria Reference Laboratory
NCA - National Competence Assessment
NGOs - Non government organizations
NHL - National Health Laboratory
NMCP - National Malaria Control Programme
NMRL - National Malaria Reference Laboratory
NRL - National Reference Laboratory
NSP - National Malaria Strategic Plan
OTSS - Outreach Training and Supportive Supervision
PPE - Personal Protective Equipment
QA - Quality Assurance
QC - Quality Control
QMS - Quality Management System
RDT - Rapid Diagnostic Test
RHC - Rural Health Centre
S&E - Supplies & Equipment
S/R - State/Region
SH - Station Hospital
SOP - Standard Operating Procedures
TP - True Positive
TN - True Negative
TMO - Township Medical Officer
URC - University Research Co., LLC
VBDC - Vector Borne Disease Control
WHO - World Health Organization

Contents

## OVERVIEW OF SOP

### 1. PURPOSE AND SCOPE

These SOPs provide an overview of the processes and procedures relevant to providing quality assured malaria microscopy services.

This procedure will be updated and modified as and when necessary with approval of the *National Malaria Program Manager and National Health Laboratory.*

All procedures specified herein are mandatory for all malaria microscopists working for Malaria Microscopy in public and private health facilities**.**

### 2. PRINCIPLE

Microscopy using Giemsa-stained blood films remains the gold standard procedure for malaria diagnosis in the National Malaria Control Programme. Therefore a quality assurance (QA) system for malaria microscopy is needed to ensure that there are good practices related to sample collection, staining, examination, recording and reporting and cross-checking of results both in the central and peripheral diagnostic facilities. SOPs will serve a reference document to follow by the microscopists that brings uniformity and consistency to perform the malaria microscopy. Training and supervision of the microscopists and laboratories are also important aspects of ensuring quality microscopy services.

### 3. DOCUMENT

Standard Operating Procedures (SOPs) of the different procedures for malaria microscopy.

| FLOWCHART | DESCRIPTION OF ACTIVITY |
|---|---|
| Use, Care and Maintenance of Microscope | NMCP-MM-SOP-01 |
| Cleaning and storing of glass slides | NMCP-MM-SOP-02 |
| Preparation of Giemsa stock solution | NMCP-MM-SOP-03 |
| Collection and preparation of blood films for malaria microscopy<br>Labeling of malaria blood films | NMCP-MM-SOP-04 |
| Staining of malaria blood films with Giemsa | NMCP-MM-SOP-05 |
| Examining thick and thin malaria blood films | NMCP-MM-SOP-06 |
| Recording and reporting of malaria results | NMCP-MM-SOP-07 |
| Cross- checking of malaria blood films | NMCP-MM-SOP-08 |
| Instruction for Laboratory aspect of Infection Prevention and Control | NMCP-MM-SOP-09 |
| Medical Laboratory waste management instruction | NMCP-MM-SOP-10 |
| Supervisory visits to laboratories providing malaria microscopy services | NMCP-MM-SOP-11 |

| | | |
|---|---|---|
| | National Malaria Control Program<br>Department of Public Health<br>Ministry of Health and Sports | Document Control No:<br>NMCP-MM-SOP-01 |
| | Republic of the Union of Myanmar | Version - 2 |
| | **Giemsa Malaria Microscopy** | Date of issue:<br>15 September 2017 |
| | Title:<br>**Use, Care and Maintenance of Microscope** | Total Page : 5 |

## 1. PURPOSE AND SCOPE

This SOP describes the general procedures for the use, care and maintenance of microscopes in laboratories where malaria microscopy is performed.

## 2. SUPPLIES, MATERIALS AND EQUIPMENT

- compound microscope fitted with paired 10x oculars (eye pieces), 10x, 40x, and 100x (oil immersion) objectives and a mechanical stage
- dust cover
- lens paper
- commercial microscope cleaning solution, and
- soft cloth

## 3. TRANSPORTING AND MOVING MICROSCOPES

- Transport in original container with the packing materials to prevent from moving within the container.
- If original packaging is not available, use a specifically designed microscope transport box supplied by the manufacturer or a fabricated container with foam or similar packing materials to prevent the microscope from moving within the container during transport.
- During transport, protect it from excessive movement or vibration; pack it so that it does not move around in the vehicle and cannot fall from the top of other items being transported.
- Carry a microscope with hands, one supporting the base and the other holding the arm.

## 4. PLACEMENT

- Place it on a flat, level, firm bench, free from vibration. At high magnification, very small movements of the bench will cause large movements in the image being viewed by the microscopist.
- Place it in a position or bench with sufficient leg space for the microscopist; preferably use height-adjustable chairs
- Do not place the microscope in front of a brightly lit window. Place it in front of a wall or a darkened window.

## 5. MICROSCOPE SET UP

Follow the manufacturer's directions for optimal set-up of the optical system and general use.

- Adjust the iris aperture to the setting recommended by the manufacturer to achieve maximum depth of field.
- If the manufacturer provides an iris diaphragm setting on the condenser, set this to the 100x objective lens setting.
- Use the following method if the **eyepiece of the microscope can be removed**:
  - raise the condenser
  - set the lamp to "low"
  - select the 40x objective
  - close the iris diaphragm
  - remove one eyepiece
  - Look down the tube, then adjust the condenser until the edge of the diaphragm is in sharp focus without showing an outer fringe ring in green or in red.

  - open the iris diaphragm until the objective lens completely fills with light
  - the shape of the light is usually octagonal; when the points of the octagon touch the outside of the objective lens, open the iris diaphragm until the light appears circular
  - replace the eyepiece.
- **An alternative method**, which does not require removal of the eyepieces, is to set the microscope into Kohler illumination:
  - Close the iris diaphragm on the condenser.
  - Close the light source at the base of the microscope, where the light is reflected up to the condenser.
  - Turn the light up as bright as possible.
  - Put a stained blood smear on the stage, add immersion oil, and use the 100x oil immersion objective lens.

- Look through the microscope oculars, and focus the condenser up and down until the edge of the field is sharp.
- Use the adjustment screws on the condenser to move the circle of light to the center of the field if necessary
- Turn the light down and open the iris diaphragm and the light source before use.

- Adjust the inter-pupillary distance.
- Adjust the diopter.

**6. Use of the microscope**

- Be familiar with the parts of the microscope and their function.
- Turn on the light source, and adjust the optimum light setting to ensure the correct level of brightness by turning or sliding the brightness adjustment knob at the base.
- Rotate the low power objective into position.
- Remove the eyepiece, look down the body tube and adjust the mirror and diaphragm setting so that light is reflected up the tube and a circle of evenly illuminated light is visible in the field of view.
- View the specimen with the 10x objective, then with the 40x and then with the 100x oil immersion objective.

**a. Prevention of damage to the 40x objectives**

- Remove immersion oil that comes into contact with the 40x objective. Because the 40x lens is located next to the 100x lens and because it is also a long lens, it is easy for it to come into contact with immersion oil accidentally. The 40x objective is not sealed against oil penetration, and any immersion oil left in contact with this objective will penetrate the lens and be deposited on the inside of the lower lens.
- Only the manufacturer can repair, after dismantling the objective lens.
- Lower the stage before removing a slide.
- After use, immediately clean the immersion oil from the objective with lens paper and commercial lens cleaner.
- If not done, the oil may thicken and harden over time and affect the optical performance of the lens.
- To protect the 100x objective lens, the objective should be rotated up when the microscope is not in use.

**b. Daily maintenance "Do's"**

- Inspect for damage or malfunction.
- Record any damage or malfunction in the daily microscopy maintenance sheet.
- Clean the parts of the microscope with a clean cloth.
- Clean the objectives after each day's work.
- Ensure that immersion oil residues are removed.

- Clean the objectives with lens cleaning tissue only.
- Cover microscope with a dust cover when not in use.
- Turn off the power and unplug the microscope at the end of the day, to protect it from a power surge.

**c. Daily maintenance "Don'ts"**

- Do not clean any part of the microscope with xylene, which will damage the microscope and is toxic.
- Never clean lenses with alcohol, ordinary tissues, cleaning paper, toilet paper, cotton wool or hand towels, which will scratch the lens surface.
- Do not leave lens ports uncovered; use the port cover or sealing tape.

**d. Storage**

- In humid places, store the microscope in dry conditions when not in use to prevent fungal growth on glass surfaces.
- Keep in a "warm cupboard" with a constant temperature and low humidity.
- Store lens and prism heads in airtight boxes with desiccant.

**e. Repairs**

- Staff can replace a broken bulb or replace a blown fuse by strictly following the manufacturer's instructions.
- Other repairs must be performed by a qualified service engineer or technician.
- Do not exchange lenses and ports with those of other microscopes.
- Routine servicing, such as realignment of optics, replacement of lens and maintenance and lubrication of the stage, should be performed by a qualified service technician.
- Record all repairs on the microscope maintenance sheet.

**f. Precautions and safety**

- Secure and stabilize the power source and connections to the microscope to avoid risk of electric shock.
- Do not expose the microscope and electrical connections to water.
- Care should be taken to prevent damage to the eyes from exposure to the high light-intensity halogen lamp.
- Use the microscope ergonomically to prevent back and neck strain.

## 7. Microscope Maintenance Chart (Attachment-1)

EXAMPLE OF MICROSCOPE MAINTENANCE CHART (Attachment-1)

**MICROSCOPE ID:#** _____... **Month/Year:** _____.

Microscope type: Model CX-23 Serial number: SN-0987-65 Manufacture: Olympus

Please tick √ the activities. This chart should be filed away at the end of each month.

| | Day of month | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Daily maintenance<br>Check all optics for damage | 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 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Check coarse and fine adjustments | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Clean surfaces of components | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Clean the external surface | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Remove oil | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Replace bulbs as needed | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Cover microscope after use | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Technician's initials | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Comments | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

QA check ..... QA review .....

Date: ..... Audit .....

**8. REFERENCES –** WHO Collaborating Center for Malaria Diagnosis

## 9. DOCUMENT HISTORY

| Date | Version | Comments | Responsible Person |
|---|---|---|---|
| January 2017 | 2 | Add use and repair of microscope and format change; flowchart introduced<br>Add the chart for microscope maintenance | Medical Technologist and Laboratory Technicians Grade I, NMCP |

| | | |
|---|---|---|
| | National Malaria Control Program<br>Department of Public Health<br>Ministry of Health and Sports<br>Republic of the Union of Myanmar | Document Control No:<br>NMCP-MM-SOP-02<br>Version – 2 |
| | **Giemsa Malaria Microscopy** | Date of issue:<br>15 September 2017 |
| | Title:<br>**Cleaning and Storing of glass slides** | Total Page: 3 |

## 1. PURPOSE AND SCOPE

This SOP describes the procedure for cleaning and storing glass slides that are to be used for preparing blood films for routine malaria microscopy.

## 2. PRINCIPLE

It is important to ascertain that the slides to be used are clean and scratch-free, because dirty and scratched slides can lead to poorly prepared blood films that can compromise the quality and integrity of the diagnosis.

## 3. SUPPLIES AND MATERIALS

a. Glass slides, 25 x 75 mm, preferably with one frosted end for labeling and with ground edges, and must be of superior quality
b. Two (2) basins, medium sized
c. Dishwashing or liquid detergent
d. Washing cloth or sponges
e. Clean water
f. Lint-free towels and paper
g. Slide boxes (made of hard plastic)
h. Latex disposal gloves (powder- free)
i. Marking pen
j. Silica gel

## 4. SAFETY PRECAUTION

Wear gloves to prevent accidental cuts or injury from the glass slides during washing.

**5. PROCEDURE**

| FLOWCHART | DESCRIPTION OF ACTIVITY |
|---|---|
| 1. Soak slides in water with detergent for 1-2 hours. | Soak slides for at least 1-2 hours in water mixed with a mild dishwashing or liquid detergent. |
| 2. Clean slides with cloth or sponge. | With a washing cloth or sponge, clean both sides of each slide. |
| 3. Rinse slides in clean water. | Rinse the slides individually in clean water to remove all traces of detergent. |
| 4. Dry slides thoroughly. | Dry each slide thoroughly with a lint-free towel. |
| 5. Wrap slides by 10's or 20's, in clean paper and place inside box. | Handling the dried slides by their edges only, wrap them in clean lint-free paper (by 10's or 20's), and return them in their original cardboard boxes, or in plastic slide boxes, each secured with a rubber band |
| 6. For long storage, place silica gel inside box. | If the slides will not be used immediately, put some silica gel inside the box to prevent fungal growths. |
| 7. Label box and document procedure In a record or logbook | With a marker pen, label the boxes cover with the date, box number (out of the total) and number of slides per box as in the example below, and document in a record or logbook**:**<br>1. *Example*: *05 Aug 2015* |
| 8. Store boxes inside the cabinet. | Store the boxes containing the cleaned slides inside a cabinet. |

## 6. PROCEDURE NOTES

a. Recycling of glass slides must NOT be done unless there is a shortage or stock out.
b. For newly purchased slides, ascertain that they are clean, scratch-free and greaseless. If they are, this SOP may be skipped.
c. Discard chipped or scratched slides.
d. Use cleaned slides following the “first in, first out” rule.

## 7. REFERENCES

WHO Malaria Microscopy Quality Assurance Manual. Version 1. 2009

## 8. DOCUMENT HISTORY

| Date | Version | Comments | Responsible Person |
|---|---|---|---|
| Oct 2014 | 1 | SOP introduced and formatted | NMCP |
| Feb 2016 | 2 | Minor revision and format change; flowchart introduced | NMCP |

| | | |
|---|---|---|
| | National Malaria Control Program<br>Department of Public Health<br>Ministry of Health and Sports<br>Republic of the Union of Myanmar | Document Control No:<br>NMCP-MM-SOP-03<br>Version – 2 |
| | **Giemsa Malaria Microscopy** | Date of issue:<br>15 September 2017 |
| | Title:<br>**Preparation of Giemsa Stock Solution** | Total Page: 4 |

## 1. PURPOSE AND SCOPE

This SOP describes the procedure for preparing stock solution of Giemsa which is used for routine staining of malaria blood films.

## 2. PRINCIPLE

Giemsa is the standard stain used for staining blood films for malaria diagnosis. It is commercially available as a ready to use product, but the quality varies according to source. To ensure quality, Giemsa is best made up at the Zonal Production Center and distributed to the different facilities providing malaria microscopy services.

## 3. SUPPLIES, MATERIALS AND EQUIPMENT

a. Giemsa powder/stain, 3.8g (*preferably BSC grade -Biological Stain Commission, to ensure a very good, standard quality of product)*
b. Methanol, pure, 250 Ml
c. Glycerol, pure, 250 mL
d. Solid glass beads, 3-5 mm in diameter, 50-100 pieces
e. Spatula or measuring spoon
f. Weighing paper
g. Graduated cylinder
h. Glass or plastic funnel
i. Screw-capped, dark or amber glass bottle, clean and dry, 500 mL capacity (*if one is not available, a chemically clean and dry, clear hard glass or polyethylene bottle of suitable size may be used, but should be fitted with a brown paper wrapping).*
j. Analytical balance, weighing in divisions of 0.01 g

## 4. SAFETY PRECAUTION

a. Methanol and Giemsa stain are highly flammable and toxic if inhaled or swallowed. Avoid contact and inhalation. Universal safety precautions, including use of relevant Personal Protective Equipment (PPE), e.g., gloves and laboratory coat, must be practiced.

**5. PROCEDURE**

| FLOWCHART | DESCRIPTION OF ACTIVITY |
|---|---|
| 1. Place 50 beads in a dark bottle. | Place about 50 glass beads into a dark or amber bottle. |
| 2. Add 3.8 g Giemsa powder. | Weigh 3.8 g of stain powder on an analytical balance, and pour into bottle containing the beads. |
| 3. Add 250 mL methanol. | Measure 250 mL methanol in a graduated cylinder and pour gently into the bottle. |
| 4. Allow stain to settle at the bottom. | Allow the stain powder to sink through the methanol until it settles to the bottom. |
| 5. Stopper bottle and shake for 2-3 minutes with circular motion. | Stopper the bottle tightly then shake it with circular motion for 2-3 minutes to dissolve the stain. |
| 6. Add 250 mL glycerol. | Measure 250 mL glycerol in a graduated cylinder and pour gently into the bottle. |
| 7. Stopper bottle and repeat shaking as above. | Stopper the bottle tightly before repeating the shaking process. |
| 8. Shake bottle as in above 3–5 times on first day. | Continue the shaking for 3–5 minutes for about 3-5 times during the first day. |
| 9. Repeat shaking as in above for 2-3 days. | Repeat the shaking every day for 2-3 days until the stain is completely mixed. |

| FLOWCHART | DESCRIPTION OF ACTIVITY |
|---|---|
| 10. Assign batch number and label bottle of stock Giemsa.<br>↓<br>11. Store Giemsa stock bottles in cool, dark area. | Assign batch number for each stock solution prepared. Label the bottle as in example below:<br>Giemsa Stock Solution<br>Batch No.: 2015-01<br>Date prepared: 5 Aug 2015<br>Batch #2015-01 stands for the year prepared and stock or lot number.<br>Store the bottle tightly stoppered to prevent absorption of water vapour from the air, and in a cool place away from direct sunlight. |

## 6. PROCEDURE NOTES

a. Do NOT shake, or filter the stock Giemsa bottle before use to avoid re-suspending the precipitates which settle on blood films during staining and obscure important details during microscopy.
b. Do NOT contaminate the stock Giemsa solution with water. Keep a small amount in a small bottle for daily use to avoid contaminating the whole stock.
c. Do NOT return unused or left-over stain to the stock bottle or to the bottle containing the working solution; stain once out of the bottle must be used quickly or discarded.
d. A single batch of Giemsa stain must not be made up for use, or re-use, throughout the day, or longer. Giemsa stain quickly absorbs water vapor in the air; and when diluted with de-ionized, distilled or any form of water, it rapidly loses its staining properties so that slides stain poorly after just a short time. The iridescent scum on the surface of made-up Giemsa stain adheres easily to the blood film making examination difficult.
e. If a clear stock bottle is used, wrap it with a thick dark paper to avoid light penetration.

## 7. QUALITY CONTROL AND DOCUMENTATION

a. Perform quality control check for every new batch/lot of stock solution prepared.
b. On logbook, record information as in the following example:

| Date of stock preparation | Batch number | Number of bottles prepared | Volume per bottle (mL) | Date of QC | pH of stain | Remarks on the quality of stain | Action taken | Name and signature of staff who performed QC |
|---|---|---|---|---|---|---|---|---|
| 5 Aug 2015 | 2015-001 | 5 | 50 | 5 Aug 2015 | 7.4 | *Good/ poor* | Adjusted pH to 7.2 | |

## 8. REFERENCES

Basic Malaria Microscopy. Part I. Learner's Guide, Second Edition. 2010

WHO Malaria Microscopy Quality Assurance Manual. Version 1. 2009

## 9. DOCUMENT HISTORY

| Date | Version | Comments | Responsible Person |
|---|---|---|---|
| Oct 2014 | 1 | SOP introduced and formatted | NMCP |
| Feb 2016 | 2 | Minor revision and format change; flowchart introduced | NMCP |

| | | |
|---|---|---|
| | National Malaria Control Program<br>Department of Public Health<br>Ministry of Health and Sports<br>Republic of the Union of Myanmar | Document Control No:<br>NMCP-MM-SOP-04<br>Version – 2 |
| | **Giemsa Malaria Microscopy** | Date of issue:<br>15 September 2017 |
| | Title:<br>**Collection of Finger- Prick Blood and Preparation of Thick and Thin Blood Films Labeling of Malaria Blood Films** | Total Page: 5 |

## 1. PURPOSE AND SCOPE

This SOP describes the preparation of thick and thin blood films using finger prick blood for malaria diagnosis by light microscopy and also describes the proper and correct labeling of malaria blood films

## 2. PRINCIPLE

Examination of blood films by microscopy is a basic technique, which remains the gold standard for the diagnosis of malaria. Blood films for malaria diagnosis are best prepared from capillary blood obtained by finger prick. Good quality blood films are essential to establish accurate diagnosis.

Proper and correct labeling of malaria blood films is important to ensure that the sample and the data correspond to the patient. The integrity of the diagnosis may be compromised by unlabeled or wrongly-labeled blood films.

## 3. SUPPLIES AND MATERIALS

a. Glass slides, 25 x 75 mm, with one frosted end for labeling, preferably with ground edges and must be of good quality
b. 70% ethyl alcohol or alcohol swabs
c. Sterile lancets, one per patient
d. Dry cotton
e. Latex protective gloves (powder free)
f. Sharps container
g. Slide tray or box and cover to dry slides horizontally protected from dust and flies
h. Drying rack
i. Record forms (Malaria microscopy registration form)
j. Lead pencil or permanent marker pen

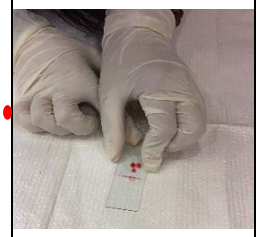
## 4. SAFETY PRECAUTIONS

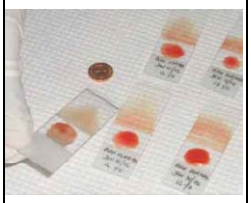
a. Wear gloves before starting the blood collection and when handling slides to avoid leaving behind oil on the slide that may interfere with the smear preparation
b. Always use a new lancet for each patient. **NEVER** re-use lancets

## 5. PROCEDURE

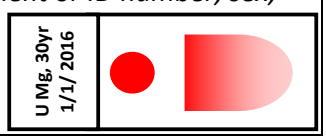
### (1)Collection of Finger- Prick Blood and Preparation of Thick and Thin Blood Films

| FLOWCHART | DESCRIPTION OF ACTIVITY | Illustration |
|---|---|---|
| 1. Prepare a pre-cleane glass slide and other materials needed. | Prepare a glass slide that has been pre-cleaned and the other materials needed for blood collection. | |
| 2-3. Clean the 3rd or 4th finger from the thumb with 70% ethanol or alcohol swab. Let the finger dry. | Select the 3rd or 4th finger from the thumb (or big toe for infants, not the heel; do not use thumb, for either children or adults).<br>Hold the patient's hand, palm facing upwards, and clean the selected finger with 70% ethanol or alcohol swab. Use firm strokes to remove dirt and oils from the ball of the finger and to stimulate blood circulation. Let the alcohol dry from the finger. |  |
| 4. Prick finger with a new, Sterile lancet. | Prick the finger (or big toe) with a lancet. Use a new, sterile lancet for every patient. |  |
| 5-6. Express and wipe first drop of blood with dry cotton. | Apply gentle pressure to the finger (or toe) and express the first drop of blood. Wipe the first drop of blood with dry cotton, making sure that no cotton strands remain on the finger that might stick to the blood. |  |
| 7. Make the thick and thin blood films on the same slide. | Make both thick and thin blood films (for one patient) on the frosted side of the same slide. | |

| FLOWCHART | DESCRIPTION OF ACTIVITY | Illustration |
|---|---|---|
| 8. Collect 2-3 more small drops of blood, and use them to make the thick film. | Apply further gentle pressure to express more blood, and collect two or three drops on the slide similar to these ∴ , about 1 cm away from the drop intended for the thin film. |  |
| 9. Touch the blood with the slide to collect a small drop of blood, and use it to make the thin film. | Apply gentle pressure to the finger and by touching the slide to the blood, collect a single small drop of blood about this • size on the middle of the slide. This is for the thin film. |  |
| 10. Wipe clean the blood from the finger. | Wipe the remaining blood off the finger with a clean, dry cotton. | |
| 11. Place the slide with the blood facing up on a flat surface. | Do not delay between applying and spreading the drops. Prepare the blood films with the slide lying on a flat surface. | |
| 12-14. Using a clean "spreader" slide, make the thin film first by pushing forward the one drop of blood in a smooth, continuous motion. | - To prepare the thin film, place the edge of a clean "spreader" slide at a 45$^{o}$ angle in front of the blood drop intended for the thin film.<br>- Slowly pull the "spreader" back until it touches the drop of blood and it spreads along the edge of the "spreader".<br>- Rapidly push the "spreader" forward (away from the center) in a smooth, continuous motion, until the spreader leaves the bloody part of the slide leaving a "feathery" end for the thin film. | |

| FLOWCHART | DESCRIPTION OF ACTIVITY | Illustration |
|---|---|---|
| 15. With the corner of a "spreader, make the thick film by swirling together the 3 drops of blood to form a circle. | With the corner of the same "spreader" used for making the thin film, make the thick film by swirling the 3 drops of blood together forming a circle of about 1.2 cm diameter in size similar to this  |  |
| 16. Label the slide with the patient's name/ ID, sex, age and date of collection. | Using a permanent marker or lead pencil, label the frosted end of the slide with the patient's unique identifier (name/ ID number, sex, age and date of collection). |  |
| 17. Air dry the films in a horizontal position. | After preparation of the thin and thick blood films, allow them to air dry in a horizontal position on a slide tray. If a rapid drying is required, dry films with low heat from a hair drier for few seconds. Do not place the slides too close to the drier as the films might be heat fixed. |  |

**(2) Labeling of Malaria Blood Films**

| FLOWCHART | DESCRIPTION OF ACTIVITY |
|---|---|
| 1. Label the slide immediately | Label the slide immediately after the blood film preparation to avoid mix ups especially if there are more than one slides to prepare. |
| 2. Check the patient information on the test request form. | Verify the details against the test request form to ensure that they are correct and correspond to the sample or patient**.** |
| 3. On the frosted end, write the patient's name/ID number, sex and age of patient and date of collection. | Using a permanent marker or lead pencil, write the following information on the frosted end of the slide: *Name of patient or ID number, sex, age, date of collection* Example:   |

## 6. REFERENCES

Basic Malaria Microscopy. Part I. Learner's Guide, Second Edition. 2010

WHO Malaria Microscopy Quality Assurance Manual. Version 1. 2009

## 7. DOCUMENT HISTORY

| Date | Version | Comments | Responsible Person |
|---|---|---|---|
| Oct 2014 | 1 | SOP introduced and formatted | NMCP |
| Feb 2016 | 2 | New, separate SOP introduced; minor revision and format change; flowchart introduced; blood collection photos change | NMCP |

| | | |
|---|---|---|
| | National Malaria Control Program<br>Department of Public Health<br>Ministry of Health and Sports<br>Republic of the Union of Myanmar | Document Control No:<br>NMCP-MM-SOP-05<br>Version – 2 |
| | **Giemsa Malaria Microscopy** | Date of issue:<br>15 September 2017 |
| | Title:<br>**Staining of malaria blood films with Giemsa** | Total Page: 7 |

## 1. PURPOSE AND SCOPE

In order to prepare staining of malaria blood films with Giemsa, there are three steps to follow: First, prepare the buffered water to pH of 7.2 for use in the preparation of Giemsa stain solution. Second, prepare the working solution of Giemsa stain for routine staining of malaria blood film. Then, malaria blood films are staining with Giemsa stain.

## 2. PRINCIPLE

Freshly-prepared working solution of Giemsa, made from well-prepared stock and diluted with water buffered to pH 7.2 is recommended to achieve optimal staining quality of malaria blood films. Giemsa stock solution prepared for the national programme is standardized to minimize the need for frequent adjustment of SOPs for staining.

Freshly-prepared working solution of Giemsa, made from well-prepared stock and diluted with water buffered to pH 7.2 is recommended to achieve optimal staining quality of malaria blood films. Giemsa stock solution procured for the national programme is standardized to minimize the need for frequent adjustments to staining SOPs.

A properly stained blood film is critical for malaria diagnosis especially for precise identification of malaria species. The use of Giemsa stain is the recommended and most reliable procedure for staining thick and thin blood films. Giemsa solution is composed of eosin and methylene blue (azure). The eosin component stains the parasite nucleus red, while the methylene component stains the cytoplasm blue. The thin film is fixed with methanol. Dehemoglobinization of the thick film and staining takes place at the same time during the process. An ideal pH of 7.2 is required to demonstrate stippling of the parasites to allow proper species identification.

## 3. SUPPLIES AND MATERIALS

1. Commercial phosphate buffer tablets for 1 L of water (pH 7.2)
2. Commercial distilled or deionized bottled water, 1 L
3. Graduated cylinder, 1 L capacity
4. Conical flask or beaker, 1 L capacity
5. Measuring cylinder, clean, 10ml, 50ml & 100mL capacity
6. Coplin jar for stain immersion
7. Methanol, absolute
8. Gloves, latex, disposable
9. Pasteur pipette, 3ml, Brand®
10. Drying rack
11. Staining platform or tray
12. Timer

## 4. SAFETY PRECAUTIONS

Methanol (methyl alcohol) is highly toxic and flammable if inhaled or swallowed; it can cause blindness and even death if swallowed in any quantity. Avoid contact and inhalation. Universal safety precautions, including use of relevant Personal Protective Equipment (PPE) - gloves and laboratory coat – must be practiced.

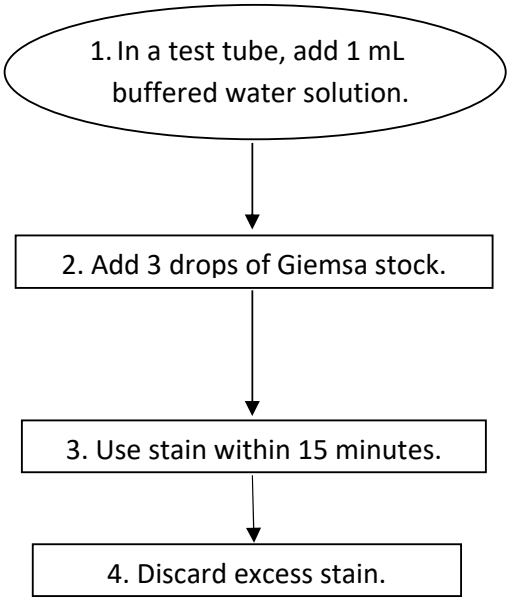
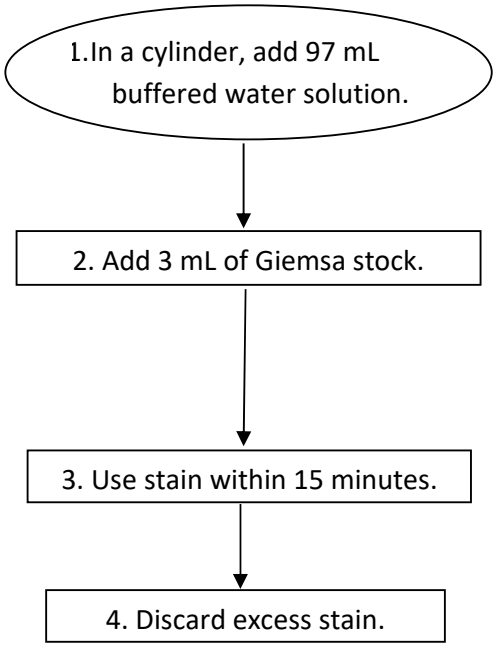
## 5. PROCEDURE

### (1) Procedure of preparation of buffer solution

| FLOWCHART | DESCRIPTION OF ACTIVITY |
|---|---|
| 1. Pour 1 L of distilled or de-ionized water into flask or beaker. | 1. Measure 1 L of distilled or deionized water into a flask or beaker. |
| 2. Add 1 buffer tablet. | 2. Add the phosphate buffer tablet. |
| 3. Mix solution by swirling. | 3. Mix by gentle swirling until the tablet is completely dissolved. Should be tested with pH paper to get correct pH |
| 4. Label the bottle of buffered water solution. | 4. Label the bottle of buffer solution as below:<br>Buffer solution pH 7.2<br>Date prepared: 05 Aug 2015 |
| 5. Use buffered water solution pH 7.2 to prepare Giemsa working solution. | 5. Use buffered water solution with pH 7.2 for preparing working solution of Giemsa stain. |

**Remark**: If buffer tablet is not available, deionized water with pH 7 – 7.2 can be used.

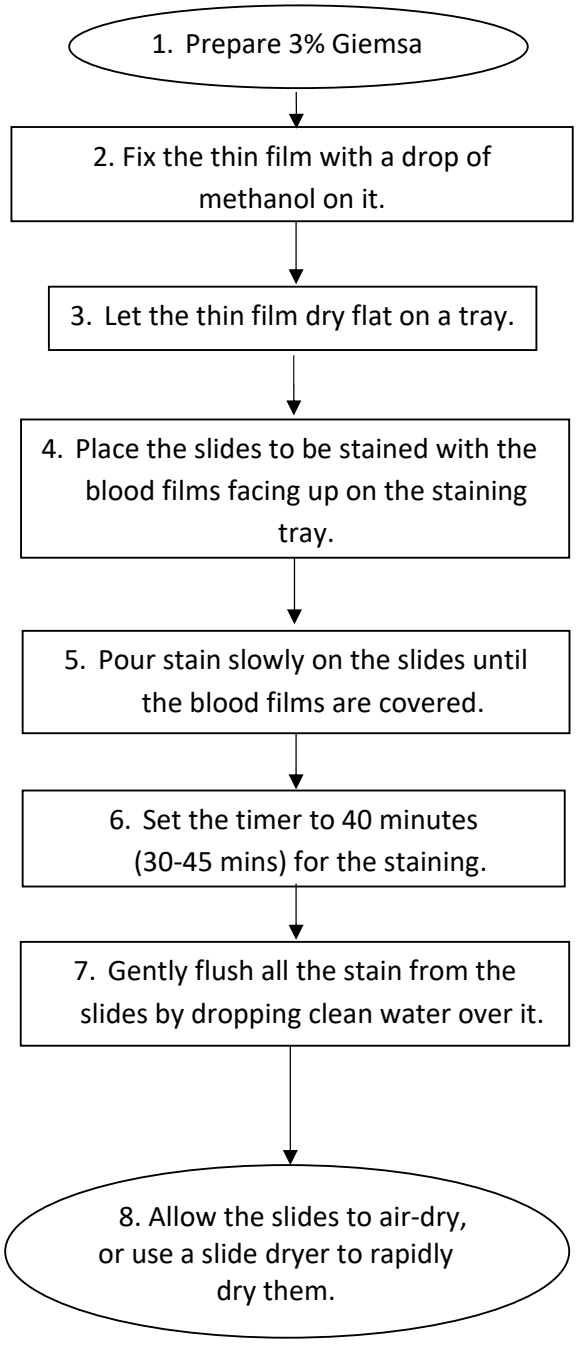
**(2) Preparation of Giemsa Working Solution**

| FLOWCHART | DESCRIPTION OF ACTIVITY |
|---|---|
| Preparation of 10% Giemsa working solution<br>  | Preparation of fresh 10% Giemsa working solution from stock solution for rapid method of staining a few number of slides. **Each slide with blood film needs approximately 3 mL of stain to cover it.**<br>1. In a clean container or test tube, add previously prepared 1 mL buffered water, pH 7.2.<br>2. Using a Pasteur pipette, add 3 drops of Giemsa stock solution previously transferred into a small container. Do NOT get this from the big bottle containing the stock to avoid contaminating it.<br>3. Prepare the Giemsa working solution just before staining the blood film(s), and use it within a maximum of 15 minutes after preparation.<br>4. Discard any excess stain. |
| Preparation of 3% Giemsa working solution<br>  | Preparation of fresh 3% Giemsa working solution from stock solution for slow staining of a batch of slides (20 - 100 slides).<br>1. In a clean measuring cylinder, add 97 mL buffered water, pH 7.2 that has been previously prepared.<br>2. Using measuring cylinder, measure 3 mL of Giemsa stock solution previously transferred into a small container. Do NOT get directly from the big bottle containing the stock to avoid contaminating it.<br>3. Prepare the Giemsa working solution just before staining the blood film(s), and use it within a maximum of 15 minutes after preparation.<br>4. Discard any excess stain. |

**(3) Staining of malaria blood films with Giemsa (For 10%)**

| FLOWCHART | DESCRIPTION OF ACTIVITY |
|---|---|
| 1. Prepare 10% Giemsa solution. | 1. Estimate the amount of 10% Giemsa working solution needed for the number of slides to be stained. |
| 2. Fix the thin film with a drop of methanol on it. | 2. Using a Pasteur pipette, fix the thin film by dropping absolute methanol into it. Avoid contact between the thick film and methanol, to avoid accidentally fixing the thick film. |
| 3. Let the thin film dry flat on a tray. | 3. Place the blood film in the drying rack. Allow the slide to air-dry. |
| 4. Place the slides to be stained with the blood films facing up on the staining tray. | 4. Place the slides to be stained with the blood films facing up on the staining tray or staining platform. |
| 5. Pour stain slowly on the slides until the blood films are covered. | 5. Pour the stain gently on the slides until each is covered with stain, or gently pour the stain onto the top of the slide lying face upwards on the staining rack. |
| 6. Set the timer to 10 minutes (8-15 mins) for the staining. | 6. Stain the blood films for 10 minutes (8-15 min). Set the timer for 10 minutes. |
| 7. Gently flush all the stain from the slides by dropping clean water over it. | 7. Gently flush the stain off the slide by adding drops of clean water until all stain are washed away. Do not pour the stain directly off the slides, or the metallic green surface scum will stick to the film. |
| 8. Allow the slides to air-dry, or use a slide dryer to rapidly dry them. | 8. Place the slide in the drying rack, film side downwards, to drain and dry. Drying of the blood films can be hastened by using low heat from a slide dryer. |

**Staining of malaria blood films with Giemsa (For 3%)**

| FLOWCHART | DESCRIPTION OF ACTIVITY |
|---|---|
|  | 1. Estimate the amount of 3% Giemsa working solution needed for the number of slides to be stained. Prepare the stain according to SOP.<br><br>2. Using a Pasteur pipette, fix the thin film by dropping absolute methanol into it. Avoid contact between the thick film and methanol, to avoid accidentally fixing the thick film.<br><br>3. Place the blood film in the drying rack. Allow the slide to air-dry.<br><br>4. Place the slides to be stained with the blood films facing up on the staining tray or staining platform.<br><br>5. Pour the stain gently on the slides until each is covered with stain, or gently pour the stain onto the top of the slide lying face upwards on the staining rack.<br><br>6. Stain the blood films for 40 minutes (30-45 mins). Set the timer for 40 minutes.<br><br>7. Gently flush the stain off the slide by adding drops of clean water until all stain are washed away. Do not pour the stain directly off the slides, or the metallic green surface scum will stick to the film.<br><br>8. Place the slide in the drying rack, film side downwards, to drain and dry. Drying of the blood films can be hastened by using low heat from a slide dryer. |

## 6. REFERENCES

Basic Malaria Microscopy. Part I. Learner's Guide, Second Edition. 2010
WHO Malaria Microscopy Quality Assurance Manual. Version 1. 2009

## 7. DOCUMENT HISTORY

| Date | Version | Comments | Responsible Person |
|---|---|---|---|
| Oct 2014 | 1 | SOP introduced and formatted | NMCP |
| Feb 2016 | 2 | New, separate SOP introduced; minor revision and format change; flowchart introduced | NMCP |

| | National Malaria Control Program<br>Department of Public Health<br>Ministry of Health and Sports | Document Control No:<br>NMCP-MM-SOP-06 |
|---|---|---|
| | Republic of the Union of Myanmar | Version – 2 |
| | **Giemsa Malaria Microscopy** | Date of issue:<br>15 September 2017 |
| | Title:<br>**Examining thick and thin malaria blood films** | Total Page: 7 |

## 1. PURPOSE AND SCOPE

This SOP describes the procedure for proper examination, detection, identification and quantification of malaria parasites in Giemsa-stained blood films under light microscopy; and correctly recording and reporting results.

## 2. PRINCIPLE

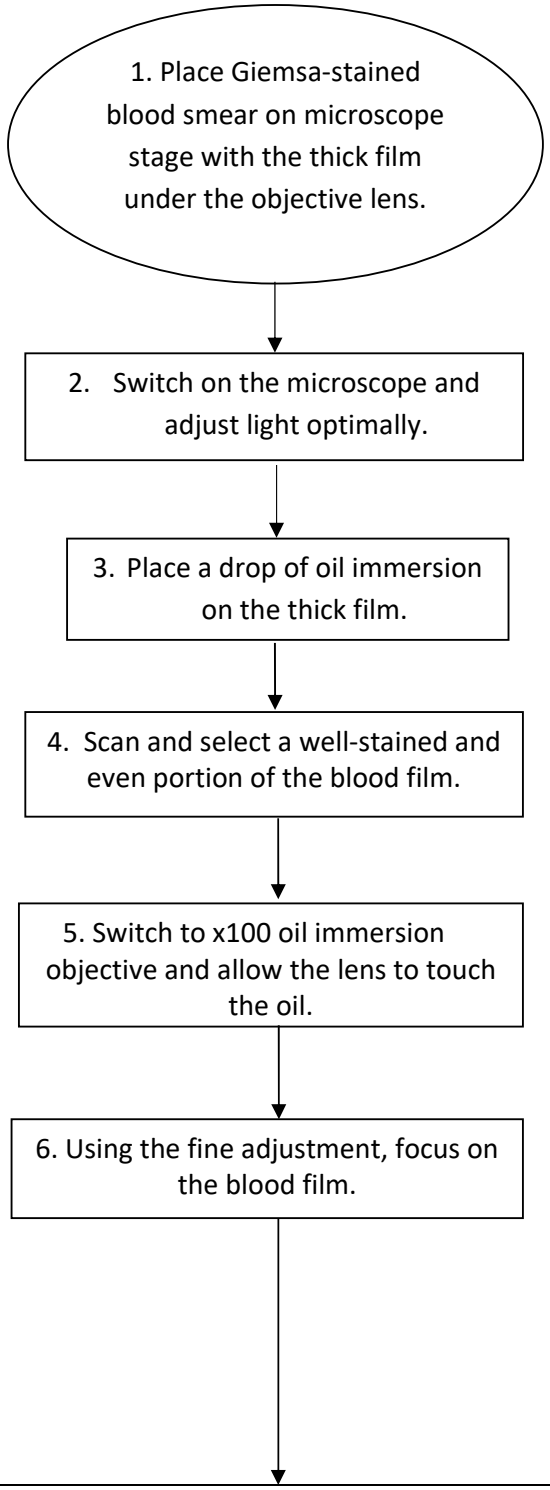
In the thick film, the red blood cells (RBCs) are lysed and dehemoglobinized while the malaria parasites are left intact and concentrated allowing their proper detection and identification. In the thin film, when fixed with absolute methanol, the RBCs retain their original morphology, and if malaria parasites are present, become visible inside the cells. It is critical that malaria diagnosis is based on well-prepared thick and thin malaria blood films to ensure correct speciation and accurate estimation of parasite density.

Detection and identification of the *Plasmodium* species and stages, and estimating parasitaemia is very important in the clinical management of patient, drug efficacy studies and malaria epidemiological surveys.

## 3. SUPPLIES, MATERIALS AND EQUIPMENT

a. Giemsa-stained blood film
b. Non-drying Immersion Oil for microscopy (Type A)
c. Compound microscope with 100x objective and 10x paired oculars
d. Cell counter with multiple tallies (piano type)
e. Calculator, handheld
f. Slide tray
g. Lens paper
h. Malaria registry book or results record book
i. Patient result form
j. Pens

## 4. PROCEDURE

| FLOWCHART | DESCRIPTION OF ACTIVITY |
|---|---|
| **4.1. Examining the thick film**<br>  | 1. Place the Giemsa-stained blood film to be examined on the microscope stage. Position the thick film in line with the objective lens.<br><br>2. Switch on the microscope and adjust the light source optimally by looking through the ocular and the x10 objective. (low power)<br><br>3. Place a drop of immersion oil on the thick film and allow it to spread. To avoid cross contamination, ensure that the immersion oil applicator never touches the slide.<br><br>4. Scan the blood film for parasites and blood elements. Select part of the film that is well stained and has evenly distributed white blood cells.<br><br>5. Switch to x100 oil immersion objective over the selected portion of the thick film. Raise the mechanical stage until the objective lens gently touches the immersion oil but not the slide.<br><br>6. Using fine adjustment, focus on the cell elements and confirm that the portion of the film is acceptable for routine examination: 15-20 white blood cells per thick film field will give a satisfactory film thickness. Films with fewer white blood cells per field will require more extensive examination. |

7. Start at the field on the top left end part of the film, then move the slide to the right, field by field.

8. When the other end of the film is reached, move the slide downward, then to the left, field by field, and so forth.

7. Examine the slide in a systematic manner. Start at the top left end of film (marked with vertical green arrow on **Figure 1** below) and begin at the periphery of the field, then move horizontally to the right, field by field.

8. When the other end of the film is reached, move the slide a little bit downward, then to the left, field by field, and so forth (see illustration below). For efficient examination, continuously focus and refocus using the fine adjustment throughout examination of each field.

Alternatively, from the top left end of the film, move vertically downwards to the next adjacent fields. When the other end of the film is reached, move the slide to the right, then go upwards to the adjacent fields, and so forth.

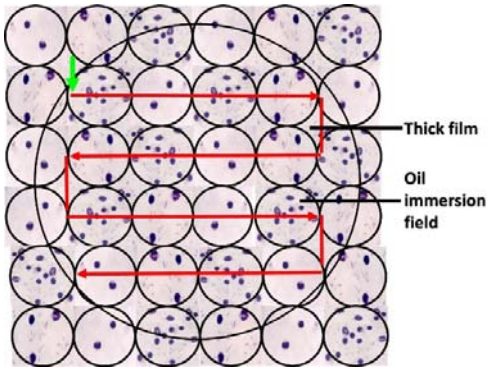
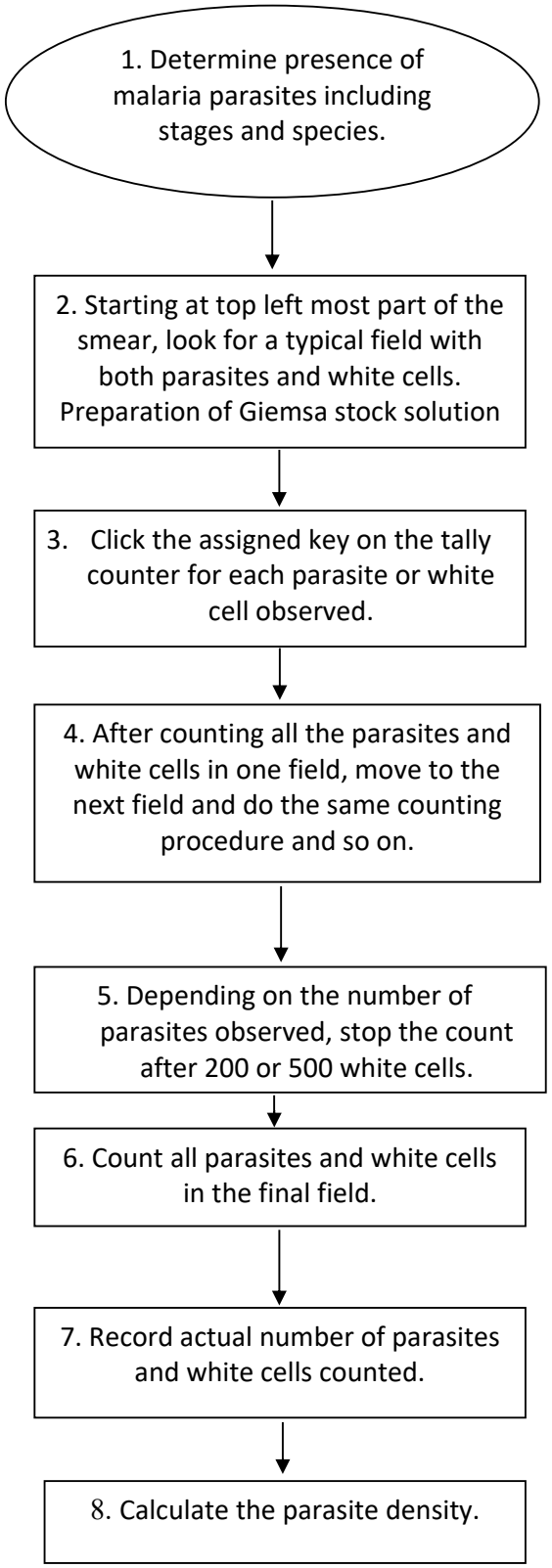



Figure 1. Examining the thick film

| FLOWCHART | DESCRIPTION OF ACTIVITY |
|---|---|
| **4.2. Determining if a thick film is negative or positive for malaria parasites and identifying the species**<br><br>1. Examine the thick film under oil immersion objective, field by field, horizontally or vertically.<br>↓<br>2. Read a minimum of 100 fields before declaring a negative diagnosis for malaria.<br>↓<br>3. If parasites are found, scan the rest of the film to rule out possible mixed infections.<br>↓<br>4. Examine the thin film if doubtful of the species, or if mixed infection is suspected.<br>↓<br>5. Determine all species and stages observed. | 1. Continue to examine the thick film under oil immersion objective, moving from one field to the next, either horizontally or vertically, following the pattern shown in Figure 1. Use the fine adjustment for focusing.<br><br>2. A minimum of 100 fields must be examined before the thick film is considered negative (no malaria parasites), but if time permits, the whole thick film should be scanned.<br><br>3. If parasites are observed, scan the rest of the blood film to ensure that there is little possibility of a mixed infection being overlooked.<br><br>4. When doubtful with the parasite species, or if mixed infections are suspected, carefully examine the thin smear under oil immersion objective. The thin film has the advantage of providing detailed parasite and RBC morphology over the thick film.<br><br>5. Identify all species present. Refer to the WHO Bench Aids for the Diagnosis of Malaria for morphological confirmation of each species. |

**4.3 Performing a parasite count on the thick film and calculating parasite density (parasitaemia)**




1. If malaria parasites are present, count asexual forms (in either single or mixed species infections) as well as sexual (gametocyte) forms. If different species are observed, this should also be recorded.

2. Start counting in a field where parasites and white cells are observed together. A typical field (at 100x magnification) should have approximately 12–15 white cells.

3. Using a piano type tally counter, count parasites and white cells simultaneously by clicking on the assigned key as parasites or white cells are observed.

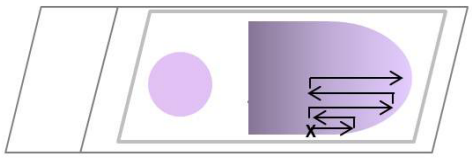
4. After counting all the parasites and white cells in one field, move to the next field following the pattern of movement shown in Figure 1 (examining the thick film) and do the same counting procedure and so on.

5. Stop the count by following these rules:

- If after 200 white cells, **100 or more parasites have been counted,** stop the count and record the results in terms of number of parasites per 200 white cells.
- If after 200 white cells, **only 99 or less parasites have been counted,** continue the count until 500 white cells, then stop and record the results in terms of number of parasites per 500 white cells.

8. Count all parasites and white cells in the final field even if the white cell count exceeds 200 or 500. Record actual number of parasites and white cells counted in an appropriate worksheet.

| FLOWCHART | DESCRIPTION OF ACTIVITY |
|---|---|
| **Parasites**<br>**µL blood = No of parasites counted x 8000 WBCs/µL**<br>**No. of WBCs counted** | When counting is completed, calculate the parasite density using an arbitrary white cell count of 8000/µL. Use the patient's actual white cell count if available. Use the following formula in the calculation: |

| FLOWCHART | DESCRIPTION OF ACTIVITY |
|---|---|
| **4.4 Examining the thin film for confirmation of species and mixed infections**<br>1.To confirm species and mixed infections, move the objective over the thin film to examine it.<br>2. Place a drop of oil on the center of the film.<br>3. Touch the objectives on the oil and focus on the film.<br>4. Read on the thin or feathery edge of the film, moving from one field to the next, horizontally or vertically.<br>5. Scan the film until all the species have been confirmed. | 1. If doubtful of the parasite species or suspicious of mixed infections after examining the thick film, move the x100 oil immersion over the middle of the thin film.<br>2. Place a drop of immersion oil on the middle of the thin film.<br>3. Rack the mechanical stage up until the objective lens touches the immersion oil, but not the slide.<br>4. Perform examination at the feathery end/edge of the thin, following the pattern of movement shown in Figure 2. Move along the edge of the film, then move the slide inwards by one field, returning in a lateral movement and so on.<br>  <br>**Figure 2. Examining the thin**<br>5. Continue examining the thin film until the presence and species of malaria parasites have been confirmed. |

## 6. REFERENCES

Basic Malaria Microscopy. Part I. Learner's Guide, Second Edition. 2010

WHO Malaria Microscopy Quality Assurance Manual. Version 1. 2009

## 7. DOCUMENT HISTORY

| Date | Version | Comments | Responsible Person |
|---|---|---|---|
| Oct 2014 | 1 | SOP introduced and formatted | NMCP |
| Feb 2016 | 2 | Major revision; new/separate SOP; flowchart introduced | NMCP |

| | | |
|---|---|---|
| | National Malaria Control Program<br>Department of Public Health<br>Ministry of Health and Sports<br>Republic of the Union of Myanmar | Document Control No:<br>NMCP-MM-SOP-7<br>Version – 2 |
| | **Giemsa Malaria Microscopy** | Date of issue:<br>15 September 2017 |
| | Title:<br>**Recording and reporting of malaria results** | Total Page: 3 |

## 1. PURPOSE AND SCOPE

This SOP describes the procedure for proper documentation and correct reporting of microscopy results.

## 2. PRINCIPLE

The accuracy of reporting of microscopy results has an impact on the way a patient's illness is managed by the clinician or health worker. Similarly, documentation of cases affects the reliability of epidemiological data that serves as basis for control policies. Therefore, it is imperative to ensure that laboratory reports and malaria registry are accurate and complete.

## 3. SUPPLIES AND MATERIALS

a. Malaria registry book or results record book
b. Patient result form
c. Calculator (for estimating parasite density)
d. Pens

## 4. PROCEDURE

a. After identifying all the species present and calculating the parasite density, record results immediately on the malaria registry or results logbook, along with other relevant information about to the patient.

The most commonly used method of recording of findings after microscopic examination of malaria in NMCP are as follows:

| Malaria Species | Results |
|---|---|
| Plasmodium falciparum<br>- Ring only<br>- Ring and gametocytes<br>- Gametocyte only | <br>Pf<br>Pf + g<br>Pfg |
| Plasmodium vivax<br>- All stages at once | <br>Pv |
| Plasmodium ovale<br>- All stages at once | <br>Po |
| Plasmodium malariae<br>- All stages at once | <br>Pm |
| Mixed infection<br>(More than one species) | <br>Seen (Eg. F + V) |
| No malaria parasite seen<br>(After 200 thick film fields) | <br>NMPS |

b. Prepare a report for the clinician and/or patient. Report all species and stages seen and the parasite density as in the following examples.

***Example 1:***

*Plasmodium falciparum* trophozoites counted = 155
White cells counted relative to parasites = 208
Using the following formula:

$$\text{Parasites per µL blood} = \frac{\text{Number of parasites counted} \times 8000 \text{ WBCs/µL}}{\text{Number of WBCs counted}}$$

Parasite count: $\frac{155 \times 8{,}000}{208}$ = 5,962 parasites/µl blood

**Report as:**
*P. falciparum* trophozoites = 5,962 p/µl blood

***Example 2:***

*P. vivax* trophozoites counted = 88
White cells counted relative to parasites = 505
Actual white cell count of patient = 6500
Parasite count: $\frac{88 \times 6{,}500}{505}$ = 1,133 parasites/µl blood

**Report as:**
*P. vivax* trophozoites = 1,133 parasites/µl blood

c. In mixed or more than one species infections, count all the species together (sexual and asexual stages) and express the results as in the example below:

***Example 3:***

*P. falciparum* gametocytes + *P. vivax* trophozoites = a total of 360 parasites (all stages) counted against 202 white cells

**Report as:**

*P. falciparum* gametocytes + *P. vivax* trophozoites = 14,257 parasites/µl blood

d. Report also the presence of the following:

- Gametocytes - a separate count is made of the gametocytes of *P. falciparum* but when this is done, they are still included in the general parasite count. It is rarely possible to separate the gametocytes of *P. vivax* or *P. malariae* from the asexual parasites with sufficient accuracy to justify a gametocyte count.
- Schizonts - since they might be an indication of disease severity.

e. If no malaria parasites are observed in the blood film, report as "Negative for malaria" or "no malaria parasites seen".

The 'plus system' is an old method, which is simple but far less accurate for establishing parasite density in thick blood films. Because of its unreliability, it has been replaced by the method described above and is no longer recommended. Its use persists in places where the quantitative method cannot be used. Studies have shown that many workers forget the finer details of the system and mix up the code (the number of plus signs) and the count (the number of parasites per field or per 100 fields), resulting in unreliable information on parasite density. In this system,

\+ = 1–10 parasites per 100 oil-immersion thick film fields

++ = 11–100 parasites per 100 oil-immersion thick film fields

+++ = 1–10 parasites per single oil-immersion thick film field

++++ = more than 10 parasites per single oil-immersion thick film field

## 5. REFERENCES

Basic Malaria Microscopy. Part I. Learner's Guide, Second Edition. 2010
WHO Malaria Microscopy Quality Assurance Manual. Version 1. 2009

## 6. DOCUMENT HISTORY

| Date | Version | Comments | Responsible Person |
|---|---|---|---|
| Oct 2014 | 1 | SOP introduced and formatted | NMCP |
| Feb 2016 | 2 | Separate SOP introduced | NMCP |

| | | |
|---|---|---|
| | National Malaria Control Program<br>Department of Public Health<br>Ministry of Health and Sports<br>Republic of the Union of Myanmar | Document Control No:<br>NMCP-MM-SOP-8<br>Version – 2 |
| | **Giemsa Malaria Microscopy** | Date of issue:<br>15 September 2017 |
| | Title:<br>**Cross-checking of malaria blood films** | Total Page:8 |

## 1. PURPOSE AND SCOPE

This SOP describes the procedure for regular cross-checking of malaria blood films from the provincial hospitals.

## 2. PRINCIPLE

Cross-checking or validation of malaria blood films plays a vital role in monitoring the accuracy of diagnosis to ensure that high quality microscopy services are provided and maintained in the hospitals.

## 3. SUPPLIES AND MATERIALS

a. Giemsa-stained blood films (selected form the provincial hospitals)
b. Non- drying Immersion Oil for microscopy (Type A)
c. Compound microscope with 100x objective and 10x paired oculars
d. Cell counter with multiple tallies
e. Calculator, handheld
f. Slide tray
g. Lens paper
h. Forms
   - QA Form 1 (Microscopist's Results Form)
   - QA Form 2 (Validator's Results Form)
   - QA Form 3 (Validation Report Form)
i. Pens
j. Slide boxes (for transporting slides)

## 4. SAFETY PRECAUTIONS

Slides to be submitted must be packed and shipped according to applicable national and international standards for transporting infectious materials.

## 5. PROCEDURE

### 5.1. Selection and prequalification of validator(s)

a. A validator must be prequalified and must satisfy the following requirements:

- Must possess a Level 1 certificate as malaria microscopist obtained from a WHO External Competency Assessment within the last 3 years
- Preferably a regular staff of the Ministry of Health (MOH) and willing to accept responsibility as validator
- Must have achieved the performance benchmarks for basic malaria microscopy training and/or refresher training within the past 3 years
- With at least 3-5 years' experience working as malaria microscopist

b. A validator(s) must always satisfy the standard requirements of training and assessment listed above, and participate in a WHO ECA every 3 years to maintain the validity of his/her certificate. If he/she fails the assessment, another potential validator should be recruited, assessed, and if qualified, designated.

### 5.2. Slide validation

a. Slide validation is done on a pre-determined number of slides initially examined by the microscopists, and sent to the validator to be re-examined in a completely independent manner.

b. All malaria blood films examined in the hospitals should **NOT** be discarded until validation is done.

c. Selection of slides by the hospital microscopists

- For the hospitals every month, if the number of slides examined is more than 50 per month, the hospital microscopist must select **ALL POSITIVE cases** and **20% NEGATIVE cases** randomly selected from the hospital malaria register. Alternatively, if the number of slides per month is 50 or less, all slides must be submitted for validation.
- For the "elimination" areas, **ALL slides** examined (positive and negative) within the month must be selected.

d. Submission of slides by the microscopist

- All slide submission must be accompanied with **QA Form 1 (Microscopist's Results Form).**
- For the hospitals submission of slides will be <u>every quarter</u>, and must be within 2 weeks after the last month of the preceding quarter
  - *<u>Example:</u> For the first quarter of 2016 (April-June), submit all selected slides for this quarter on the first 2 weeks of July.*

- For the “elimination” province , submission of slides is every month, and within 1 week after the end of the previous month
  - *Example: For June 2015, submit ALL slides examined in June on the first week of July 2015.*
- If the hospital microscopist did not examine any slide for the quarter or month, the microscopist must still submit QA Form 1 (Microscopist Results Form) every quarter or month.
- If no blood films were examined during the quarter or month, indicate in the QA Form 1: “*No blood films examined”,* to differentiate from a situation where the microscopist did not submit his/her slides for validation.

e. Validation of the slides and analysis of results by the validator

- The validator must record the validation results on **QA Form 2 (Validator's Results Form).**
- For the hospitals, the validator must examine the slides and analyze the results within 2 to 4 weeks after receiving them.
- For the “elimination” area, the validator must examine the slides and analyze the results within 1 to 2 weeks after receiving them.
- The validator must analyse the validation results and determine the % agreement between the microscopist and the validator using the analysis tables and tool (Excel?) (Annex). The acceptable agreement must be at least 80%.
- The validator must also assess and comment on the quality and staining of the thick and thin blood films submitted.
- The results and findings of the validation, including recommendations, must be reported by the validator on **QA Form 3 (Validation Report Form).** The validator must provide feedback to the microscopy center specifying the level of accuracy, smear and staining quality including recommendations.

f. Sending of validation reports to the province and NMCP-MOHS

- For the hospitals, the validator must send out the validation report (**QA Form 3)** to the head or supervisor of the hospital laboratory, with a copy provided to the microscopist, within 1-2 months after receiving the slides.
- For the “elimination” province, the validator must send out the validation report (**QA Form 3)** to the head or supervisor of the hospital laboratory, with a copy provided to the microscopist, within 2 weeks after receiving the slides.
- All validation reports must be signed by the validator and head of the NMCP and NMRL before they are sent out.
- All validation reports must be summarized every quarter and submitted to the NMCP-MOH and NHL.

**Feedback and resolution of discordant results between the microscopist and validator**

- Discordant validation results must be discussed during supervisory visits, with the questionable slides available for mentoring purposes.
- For the microscopist, if the % agreement between the validator and the microscopist is 80% or above, the validator must schedule supervisory visit at least once anytime during the year, to include discussion of discordant results.
- If % agreement between the validator and the microscopist is <80% in any quarter, the validator must schedule supervisory visit within 2 months after the report, and institute corrective mentoring to the microscopist.
- For the "elimination" area, any discordant validation result must be officially reported immediately to the proper authorities in the hospital and NMCP-MOHS for rapid case investigation. A supervisory visit must be scheduled immediately.

A summary of the proposed slide validation scheme is presented in **Table 1**.

**Table 1. Slide Validation Scheme**

| Process/ Activity | Areas reporting cases to or more than 30 slides/ month (Control Areas) | Areas reporting of less than 30 slides/ month ( Elimination Areas) | Related Forms/ Documents |
|---|---|---|---|
| Selection of slides by the hospital microscopist | **Every month,**<br>If total slides examined is **>50,** select<br>**ALL POSITIVE cases**<br>**20% NEGATIVE cases**<br>randomly selected<br>If total slides examined is **≤50**, select **ALL slides**.<br>*Note: All malaria blood films examined should **NOT** be discarded until validated.* | **Every month,**<br>Collect **ALL slides** examined<br>*Note: All malaria blood films examined should **NOT** be discarded until validated.* | Hospital microscopist will randomly select negative slides based on the hospital **Malaria Register.** |
| Submission of slides by the hospital microscopist to the NMCP or NMRL | **Quarterly**<br>▪ Submit within 2 weeks after the last month of the preceding quarter<br>*Example:*<br>*For the 2nd quarter of 2015 (April-June), submit all selected slides for this quarter (based on above) on the first 2 weeks of July.*<br>▪ If no blood films were examined during the quarter, indicate in QA Form 1: ***"No blood films examined"*** | **Monthly**<br>▪ Submit within 1 week after the previous month<br>*Example:*<br>*For June 2015, submit ALL slides examined within the month on the first week of July.*<br>▪ If no blood films were examined during the month, indicate in QA Form 1: ***"No blood films examined"*** | Hospital microscopist will accomplish and submit to the NMRL the **QA Form 1 (Microscopist's Results Form),** with or without slides examined/ submitted |

| | | | |
|---|---|---|---|
| Validation/ Cross-checking of the slides by the NMCP or NMRL validator | ▪ 2 to 4 weeks after receiving the slides from the hospitals | ▪ 1 to 2 weeks after receiving the slides | Validator will accomplish **QA Form 2 (Validator's Results Form)** and **QA Form 3 (Validation Report Form)** |
| Analysis results and preparation of reports by the NMCP or NMRL validator | ▪ Analysis:*(develop a simple Excel tool for this)*<br>▪ % agreement between microscopist and validator<br>Acceptable agreement is 80%. | ▪ Analysis:*(develop a simple Excel tool for this)*<br>▪ % agreement between microscopist and validator<br>▪ Acceptable agreement is 80%. | |

**% agreement w/o species ID = (A+D) x 100**
**A + B+ C + D**

| **Microscopist results** | **Validator's result** | |
|---|---|---|
| | **Positive** | **Negative** |
| Positive | **A**: number of slides reported as positive by both readers | **B**: (False Positives) number of slides reported as positive by microscopist but reported as negative by validator |
| Negative | **C**: (False Negative) number of slides reported as negative by microscopist but reported as positive by validator | **D**: number slides reported as negatives by both readers |

**% agreement w/ species ID = (A+D) x 100**
**A + B+ C + D**

| **Microscopist results** | **Validator's result** | |
|---|---|---|
| | **Positive** | **Negative** |
| Positive | **A**: number of slides reported as Pf (single/ mono or mixed infection) by both reader | **B**: number of slides reported as Pf by microscopist but Pf was not reported by validator (incorrect species ID) |
| Negative | **C**: number of slides reported as Pf negative by microscopist but found to be positive by validator (single/mono or mixed infection) | **D**: number slides reported as Pf negatives by both readers |

| | | | |
|---|---|---|---|
| Sending of validation reports to the S/R and NMCP-MOHS | ▪ 1 to 2 months after receiving the slides | ▪ 2 weeks after receiving the slides | Validator will send out **QA Form 3 (Validation Report Form)** |

| | | | |
|---|---|---|---|
| Feedback and resolution of discordant results between the microscopist and validator | ▪ If % agreement is 80% or above, discuss results during scheduled supervisory visits (1-2 times per year)<br>▪ If % agreement is <80% in any quarter, schedule a supervisory visit within 1-2 months after the report to discuss results and do corrective action | ▪ For every discordant result in any quarter, 2nd validator should be checked for correct result. If the result is discordant, officially notify authorities immediately and schedule a supervisory visit within 1 month of the report and do corrective action. | Validator will schedule visit and accomplish **(Supervisory Visit Checklist)** |

**For the "elimination" province, any discordant validation result must be officially reported immediately to the proper authorities in the hospital and NMCP-MOHS for rapid case investigation. A supervisory visit must be scheduled immediately or within 1 month after the report.**

## Form 1: Malaria Blood Films for Validation

(1) Month: …… (2) Year …….. (3) State/Region ……..……

(4) Township …… (5) Station ……..

(6) Health Facility Name: ……..

(7) Total No. of slides submitted: /  /  /  /

(8) Total No. of Positive for the month: ……

(9) Total No. of slides examined for this month: ……

| | (10) Slide ID No. | (11) Date Examined (mm/dd/yyyy) | Malaria Smear Result | | | Remarks |
|---|---|---|---|---|---|---|
| | | | (12) Species | (13) Parasites/µl blood (t,s) | (14) Parasites/µl blood (gametocytes | |
| 1 | | | | | | |
| 2 | | | | | | |
| 3 | | | | | | |
| 4 | | | | | | |
| 5 | | | | | | |
| 6 | | | | | | |
| 7 | | | | | | |
| 8 | | | | | | |
| 9 | | | | | | |
| 10 | | | | | | |

Examined by: …….  Date of Submission: ……

Name of Examined microscopist: ……..

Noted by: ……

Township Medical Officer: ……..

## Form 2: Malaria Blood Films Report By Validator

(1) Month …….. (2) Year …… (3) State/Region……..(4) Township ……

(5) Station ……..(6) Health Facility Name: ……..

(7) Total No. of blood films received: / / / (8) Date blood films examined: ……

| | (9) Slide ID No. | (10) Microscopist's results | | | (11)Blood Films results by Validator | | | (12) Percent agreement | | (13) Smear and staining Quality Assessment | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (10a) Type of Species | (10b) Parasite count (asexual) | (10c) Parasite count (Sexual) | (11a) Species | (11b) Parasites/µl blood (t,s) | (11c) Parasites/µl blood (gametocytes) | (12a) Accuracy of crosschecking without species differentiation | (12b) Accuracy of crosschecking with species differentiation | (13a) Smear **G**-good **P**-poor smear | (13b) Staining **G**-good , **U**-under , **O**-over stained | (13c) Remarks (Describe smear assessment) |
| 1 | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | |
| 10 | | | | | | | | | | | | |
| Total | | | | | | | | | | | | |

Examined by: ……..

Name of Examined microscopist: ……..

Noted by: ……..

Township Medical Officer: ……..

Signature

Name of Validator ……..

Date ……..

## Form 3: Summary validation report

| VALIDATION SUMMARY REPORT | | |
|---|---|---|
| Report prepared by: | Date of report prepared: | |
| For TMO: | | |
| For the attention of: | | |
| SUMMARY OF RESULTS: | | |
| No. of blood films received: …… malaria blood smears | | |
| Date received: | | |
| Date of validation: | | |
| % Agreement in parasite detection = $\frac{(A+D) \times 100}{A+B+C+D}$ | | |
| Routine laboratory result | Cross-check | |
| | Positive | Negative |
| Positive | A | B |
| Negative | C | D |
| % Agreement in falciparum detection = $\frac{(A+D)x100}{A+B+C+D}$ | | |
| Routine laboratory result | Cross-check | |
| | Positive | Negative |
| Positive | A | B |
| Negative | C | D |
| Interpretation of results: Microscopist had a false positive result, with a % agreement of ……% on species detection and ……% on species identification, …% of smear assessment are in good quality. | | |
| Validator's comments and recommendation: | | |
| Schedule for next validation: | | |
| Validator: | | |

## 6. REFERENCES


Basic Malaria Microscopy. Part I. Learner's Guide, Second Edition. 2010
WHO Malaria Microscopy Quality Assurance Manual, Version 1. 2009.
WHO Malaria Microscopy Quality Assurance Manual, Version 2. 2015.


## 7. DOCUMENT HISTORY

| Date | Version | Comments | Responsible Person |
|---|---|---|---|
| August 2017 | 1 | New SOP developed and introduced | NMCP |

| | | |
|---|---|---|
| | National Malaria Control Program<br>Department of Public Health<br>Ministry of Health and Sports<br>Republic of the Union of Myanmar | Document Control No:<br>NMCP-MM-SOP-9<br>Version – 2 |
| | **Giemsa Malaria Microscopy** | Date of issue:<br>15 September 2017 |
| | Title:<br>**Instruction for Laboratory aspect of Infection Prevention and Control** | Total Page: 7 |

## 1. PURPOSE AND SCOPE

This SOP describes the instruction for laboratory aspect of infection prevention and control for routine staining of malaria blood films.

## 2. STANDARD PRECAUTIONS IN MEDICAL LABORATORIES

1. Hand washing
2. Housekeeping practices and general sanitation
3. Cough etiquettes
4. Use of PPE
5. Laboratory water quality monitoring
6. Biomedical waste management
7. Health checkup and screening of all laboratory staff and vaccination for Hepatitis B, etc.
8. Use of Post-exposure prophylaxis (PEP) by health staff in case of potential exposure to infected blood and other body fluids
9. Collaboration and communication with hospital infection control committee

## 3. COMPONENTS OF UNIVERSAL PRECAUTION

1. Use of protective barriers, PPE (gloves, gowns, aprons, face-mask, goggles, etc.)
2. Prevention of accidents particularly injuries by sharps
3. Proper use of disinfection and sterilization techniques to render contaminated material, instruments and surfaces safe
4. Safe discard and disposal of contaminated waste

## 4. BIOSAFETY

Biosafety aims to protect all those who are exposed, directly or indirectly to infectious agents while handling laboratory specimens

**Biosafety level of risks with organisms**

It depends on the basis of risks to laboratory staff, spread in the community, pathogenicity and availability of effective prophylaxis and treatment.

**Risk group (1) organisms**

Risk group (1) organisms are harmless or pose a minimal hazard to laboratory staff and community.

**Risk group (2) organisms**

Risk group (2) organisms pose moderate potential hazard for laboratory staff but limited risk for community. Effective preventive measures and treatment are available.

**Risk group (3) organisms**

Risk group (3) organisms cause serious human disease and pose serious hazards to laboratory staff.

These organisms are transmitted through aerosol but do not readily spread from one infective individual to another. They are low risk for the community. Effective prophylaxis and treatment are normally available.

**Risk group (4) organisms**

Risk group (4) organisms pose severe human disease and are high risk for laboratory personnel. These organisms readily spread from one infected individual to another in the community. There is no effective treatment or prophylaxis, and maximum containment facilities during handling are required.

# How to Handwash?

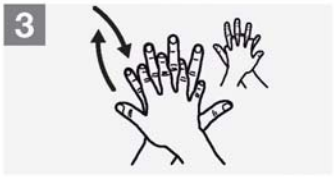
**WASH HANDS WHEN VISIBLY SOILED! OTHERWISE, USE HANDRUB**

**Duration of the entire procedure:** 40-60 seconds

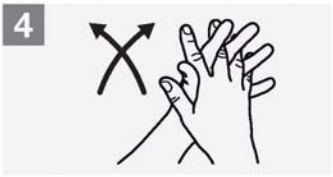
Wet hands with water;

Apply enough soap to cover all hand surfaces;

Rub hands palm to palm;



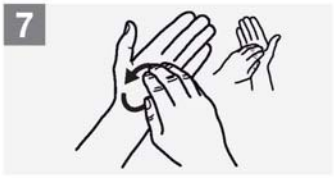
Right palm over left dorsum with interlaced fingers and vice versa;



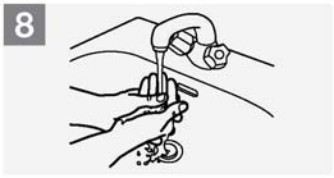
Palm to palm with fingers interlaced;

Backs of fingers to opposing palms with fingers interlocked;

Rotational rubbing of left thumb clasped in right palm and vice versa;

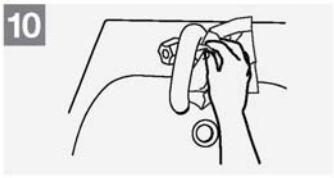


Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa;

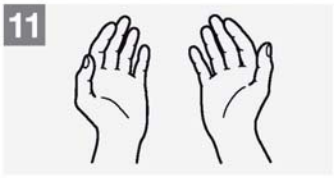


Rinse hands with water;

Dry hands thoroughly with a single use towel;



Use towel to turn off faucet;



Your hands are now safe.

World Health Organization | Patient Safety — A World Alliance for Safer Health Care | SAVE LIVES Clean Your Hands



WHO acknowledges the Hôpitaux Universitaires de Genève (HUG), in particular the members of the Infection Control Programme, for their active participation in developing this material.

May 2009

**Biosafety level**

Biosafety level is divided into four levels depending on risk of organisms.

**Biosafety level (1)**

- Organisms which are not known to cause disease in healthy adult humans
- Conducted on open benches with no special containment equipment

**Biosafety level (2)**

- Work involving agents of moderate potential hazard to staff and environment
- Staff take universal precaution and follow good microbiological techniques
- Procedure which create infectious aerosols are performed in biological safety cabinets

**Biosafety level (3)**

- Work with agents that may cause serious or potentially lethal disease as a result of exposure by inhalation
- All procedures are conducted within biological safety cabinets by wearing protective clothing

**Biosafety level (4)**

- Organisms that cause life-threatening disease
- Apart from level (3), include airlock entry, shower exit and special waste disposal facilities, class III biosafety safety cabinets, positive pressure suits, double-ended autoclaves and filtered air are essential safety requirement

## 5. LABORATORY PROCEDURES AND MODE OF TRANSMISSION OF HIV, HBV, HCV AND OTHER BLOOD BORN AGENTS

| Procedure | Mode of transmission |
|---|---|
| Collection of blood sample | - Skin puncture by needle or broken specimen container<br>- Contamination of hand by blood |
| Transfer of specimen (within laboratory) | - Contaminated exterior of specimens container<br>- Broken specimen container<br>- Spill or splash of specimen |
| HIV serology | - Skin puncture or contamination of mucous membrane<br>- Contaminated exterior of specimens container<br>- Contaminated work surface<br>- Broken specimen container<br>- Perforated gloves |
| Cleaning and maintenance | - Skin puncture or skin contamination<br>- Spill or splash<br>- Contaminated work surface |
| Waste disposal | - Contact with contaminated waste<br>- Puncture wounds and cuts |
| Shipment of specimens (to other centres) | - Broken or leaking specimen containers |

## 2. GUIDELINES FOR COLLECTION OF BLOOD SAMPLE

- Gloves should always be worn while taking blood
- If blood spills on the gloves, they should be discarded
- Care should be taken to avoid contamination of hands while taking blood
- Hands should be washed with soaps and water immediately after any contamination with blood and after work is completed
- Laboratory gowns should be worn
- Used needle and syringes should be placed in puncture resistant container, used needles should not be recapped, nor should they be removed from syringes
- Specimen containers should be sealed securely, and outside of the container should be wiped clean of blood contamination with disinfectant.

**Dos and Don'ts**

**Dos**

- Do keep your hair tied
- Do wash your hands after completing your work
- Do wear protective clothing including gloves
- Do disinfect the workplace after completion of every activity as well as at the end of every working day
- Do keep your nails trimmed all the times

**Don'ts**

- Do not eat or smoke or apply cosmetics in the laboratory
- Do not lick labels, but instead use gum or adhesive
- Do not touch your eyes, nose or other exposed parts while working with infected material
- Do not pipette by mouth
- Do not wear loose clothes while in the laboratory or while engaged in handling infectious material anywhere

## 3. BASIC REQUIREMENTS FOR LABORATORIES

- For handling of infectious material, clearly identified working area should be provided in the laboratory
- Walls, ceilings and floor of the laboratory should be smooth, easy to clean, impermeable and resistant to the chemicals and disinfectants normally used in the laboratory
- Floors should be non-slippery
- The bench tops should be impervious and resistant to disinfectants, acids, alkalis, organic solvents and moderate heat and laboratory furniture should be sturdy and easy to clean
- Wash basins should be provided in each laboratory, preferably near the exit
- Doors to the laboratory should be self-closing and have vision panels and windows should be fitted with fly screens

- Autoclave for decontamination of infectious laboratory material and waste should be available in the same building
- Facilities for storing outer clothing and personnel items and space for eating and drinking should be provided outside the workroom

*How to do when exposed by HIV specimens*

1. **Management system**

- Post-exposure prophylaxis manual

2. **Procedure for exposure**

- Use soap and water to wash any wound or skin contact with infected blood or fluid
- Flush exposed mucous membrane with water
- Irrigate an open wound with sterile saline or disinfectants
- Eye should be irrigated with water, saline or sterile eye irritants
- Counseling and HIV antibody testing to rule out pre-existing seropositive
- Use anti-retroviral therapy

3. **Follow up**

- HIV antibody testing is repeated 3 month, 6 month, 1 year after exposure

4. **Information flow**

- Responsible person / biosafety officer
- Laboratory supervisor
- Laboratory In-charge
- Hospital infection control committee

5. **Preventive measures for exposure to HIV specimens**

- Use of universal precaution
- Use of gloves, face mask, gowns, shoe cover and goggle (PPE)
- Avoiding recapping of needle
- Use of impervious needle-disposal container
- Transport of samples in triple container
- Availability of ART on side or easily
- Training personnel for post-exposure

**Decontamination of spills**

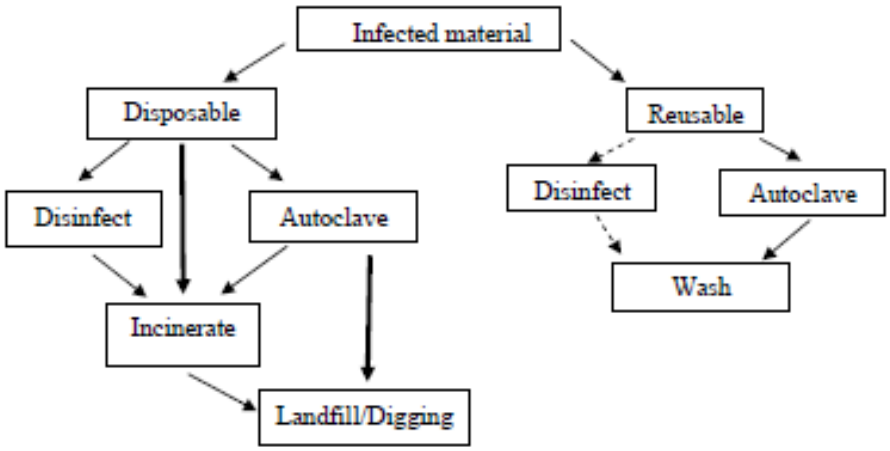
- Wear PPE
- The affected area is covered with absorbent paper towels and flooded with an appropriate disinfectant (5-10% sodium hydrochloride or available chlorine) and leave it for 30 minutes
- Other workers are warned to avoid this area
- Autoclavable dustpan and forceps are used to pick up absorbent paper towels and any solid materials with gloved hands and put in a bag. This area is swabbed with fresh disinfectant
- Absorbent Paper towels, dustpan and forceps are decontaminated by autoclaving

## 4. DECONTAMINATION

- Autoclave
- If not available, pressure cooker should be used the highest possible pressure
- Use of boiling water for 30 minutes is also effective for decontamination
- Appropriate concentration of sodium hypochlorite, formaldehyde or glutaraldehyde is desirable for disinfection
- Sodium hypochlorite is used universally as a disinfectant for disinfection of blood spillages and organic matter

## 5. DISPOSAL OF WASTE AND CONTAMINATED MATERIAL

- Specimens, cultures, gloves, gowns, needles, syringes, used pipettes, slides, tissues, paper towels, tissue used to wipe benches, equipment and surfaces
- Different for disposables and reusable




## 6. DOCUMENT HISTORY

| Date | Version | Comments | Responsible Person |
|---|---|---|---|
| January 2017 | 1 | Use instruction of infection control from NHL | National Health Laboratory |

| | | |
|---|---|---|
| | National Malaria Control Program<br>Department of Public Health<br>Ministry of Health and Sports<br>Republic of the Union of Myanmar | Document Control No:<br>NMCP-MM-SOP-10<br>Version - 2 |
| | **Giemsa Malaria Microscopy** | Date of issue:<br>15 September 2017 |
| | Title:<br>**Medical Laboratory Waste Management Instruction** | Total Page: 3 |

## 1. PURPOSE AND SCOPE

This SOP describes the instruction for medical laboratory waste management for routine examination of malaria blood films.

## 2. MEDICAL LABORATORY WASTE

- Waste generated within health-care services related to medical laboratory procedures. (including those contaminated with biological agents and all sharps, whether contaminated or not)

## 3. CATEGORIES OF MEDICAL LABORATORY WASTE

1. Non-hazardous waste
   - General waste
   - Communal waste
   - Domestic waste
2. Hazardous waste
   - Infectious waste
   - Sharp waste
   - Chemical waste
   - Pathological waste

1. *Non-hazardous waste*

   **Two types**
   - Dry- paper, leaves, plastic, can, wood
   - Wet- chemicals, washing solutions Sources - laboratory room, staff room, admin room
2. *Hazardous waste*

   **Infectious waste**
   - Gloves, blood and blood products, used alcohol pads

   Sources - laboratory, sample collection room

   **Sharp waste**
   - Needles and syringes, vacutainer, blades, contaminated broken glass, blood collection tubes / bottles

   Sources - laboratory, sample collection room

**Chemical waste**

- Solvents, reagents, corrosive or explosive chemicals and acids Sources - laboratory room

**Pathological waste**

- Tissues, organs, body parts, cytology fluids

Sources - Histopathology section, Cytopathology section

## 4. FIVE ELEMENTS OF MEDICAL LABORATORY WASTE MANAGEMENT

(1) Separation
(2) Identification
(3) Handling: - Collection
- Measurement
- Storage
- Transport

(4) Treatment
(5) Disposal

**Separation**

- Laboratory waste shall not be mixed with other wastes.
- They must be separated at the point of waste generation.

**Identification**

- WHO color coding
  - Yellow - infectious wastes and sharps
  - Red - highly infectious waste
  - Black or Blue - non-hazardous (communal waste)
  - Green - anatomical waste

**Handling**

Handling includes collection, measurement, storage and transport.

*Collection of Waste*

- Start immediately after laboratory procedure.

*Measurement*

- Daily output of waste should be measured to estimate future waste load.

**Storage and Transport**

Transport of waste from sources to interim storage.
Should be completely closed system.

- Interim storage area

• Storing waste within the facility for final treatment of disposal.
• Should not be stored beyond 48 hours.

**Treatment**

There are four principal medical laboratory waste treatment technologies.

1. Incineration
2. Autoclave
3. Boiling

4. Chemical disinfection
    a. 5% Lysol solution (lycresol)
    b. Hypochlorite solution (0.5%, 1%, 10%).

**Disposal**

Final disposal is done by following methods

1. Incineration
2. Digging – must be 3 meter deep and 50 meter away from water source
3. City Development Committee

## 5. SUMMARY OF GUIDELINES FOR MEDICAL LABORATORY WASTE

### I. Sharps (needles, syringes, scalpel blades, etc.)

1. Place intact "sharps," whether contaminated or not, in a puncture resistant "sharps" container. The containers can be purchased from the Chemistry Stores or laboratory safety equipment vendors.
2. Do not recap needles.
3. Fill 3/4 full, snap the lid closed and secure with tape. Overfilling or forced filling may result in puncture wounds. Do Not Overfill.

### II. Non-Sharp Solid Waste for Autoclave

1. Collect non-sharp solid biological waste in autoclavable bags. It is preferable that autoclave bags be white or clear and without the word "biohazardous" or the universal symbol for biohazardous material.
2. Place filled bags into the autoclave pan for transport from the laboratory to the autoclave.
3. Add 250 mL of water to the bag and close loosely to allow the steam to escape and air to enter.
4. Autoclave the pan and bag at 121°C, 15 lb/cm3 for 15 minutes.
5. Allow the pan and autoclaved material to cool.
6. Put autoclaved bags into trash cans lined with heavyweight, opaque plastic bags, and then transport them to the building dumpster.

### III. Non-Sharp Solid Waste for Incineration

1. Twist the plastic bag(s) at the top; bend the twisted portion to form a loop and seal with tape.
2. Seal bags individually when double bagging.
3. Place the waste material in a plastic liner inside a biohazardous waste box ("burn box"). Double bag wet material with absorbent material in the inner bag.
4. Secure the waste box with tape.

## 6. DOCUMENT HISTORY

| Date | Version | Comments | Responsible Person |
|---|---|---|---|
| January 2017 | 1 | Use instruction of waste management system from NHL | National Health Laboratory |

| | | |
|---|---|---|
| | National Malaria Control Program<br>Department of Public Health<br>Ministry of Health and Sports<br>Republic of the Union of Myanmar | Document Control No:<br>NMCP-MM-SOP-11<br>Version – 2 |
| | **Giemsa Malaria Microscopy** | Date of issue:<br>15 September 2017 |
| | Title:<br>**Supervisory Visits to Laboratories Providing Malaria Microscopy Services** | Total Page: 8 |

## 1. PURPOSE AND SCOPE

This SOP describes the procedure for conducting regular supervisory visits to provincial hospital laboratories providing malaria microscopy services.

This procedure is to be modified only with approval of the National Malaria Programme Manager or supervisor/head of the National Malaria Reference Laboratory (NRL)

All procedures specified herein are mandatory for all malaria microscopists working in the laboratories.

## 2. PRINCIPLE

A key element of any quality assurance scheme is effective supervision and monitoring to ensure that high quality services are sustained. During these visits, actual working conditions are observed and necessary corrective actions are implemented immediately. It provides an environment for dialogue between the microscopist and validator/supervisor where they can identify and discuss problems or difficulties that are encountered in their work, so that technical advice or supplementary coaching may be provided by the supervisor, where necessary.

One major disadvantage of these visits is the considerable resources they entail, i.e. travel costs and time. These activities should therefore be organized and integrated into an overall plan, with funds provided for and approved by the authorities in the NMP-MOH and /or NRL. The person designated to conduct these visits must be adequately trained and have considerable expertise on malaria microscopy.

## 3. SUPPLIES AND MATERIALS

a. Form 1-3 (Supervisory Visit Checklist)
b. Blood films with discordant results (from recent validation)
c. Blood films for on-site mentoring
d. Pen
e. Camera (if available)
f. Laboratory equipment and reagent (necessary supplies)

## 4. PROCEDURE

a. Prepare a schedule of the supervisory visits depending on the extent of errors (% disagreement) detected between the validator's and microscopist's results.

- For the hospitals, if % disagreement is≥20%, on-site supervision must be done within 1-2 months after the report.
- If % disagreement is<20%, on-site supervision must still be done at least once a year.
- For the "elimination" area, any discordant result in a supervisory visit as soon as possible to implement rapid case investigation and corrective action.
- Any microscopist who consistently report and submit negative cases every 6 months and confirmed correct after validation will undergo panel testing consisting of 15 panel slides (any species, preferably of low parasite density). This is to maintain the skill of the microscopist in detecting and identifying malaria parasites.
- For laboratories that did not submit slides for validation for 2 consecutive quarters, a supervisory visit must be done during the next quarter to assess the situation.

b. Communicate the schedule of the visits in advance to the microscopist and his/her supervisor to ensure their presence during the activity.
c. During the visit, observe the working conditions of the laboratory and practices of the microscopist using the standard checklist for conducting supervisory visits (**Form 1: Supervisory Visit Checklist**).
d. Review the malaria records (registry for logbook) for completeness and accuracy.
e. Randomly select 10 at least blood films from the slide box and assess their quality and staining. (**Form 2: Accuracy of microscopic examination of blood slides**)
f. Do a rapid on-site cross-checking of these slides and comment on the results.
g. Before leaving the laboratory, summarize and discuss important findings to the microscopist and with his/her supervisor in a positive and non-confrontational manner. Provide a feasible solution or recommendation to observed problems, as necessary.
h. Comment also on the observed positive aspects of the workplace and the staff.
i. An official report signed **(Form 3: Supervisory Visit Feedback)**, by the validator/supervisor, must be forwarded to the laboratory, addressed to the head/supervisor of laboratory or Township Medical Officer and copied to the microscopist, within 2 weeks after the visit.
j. Key findings from all supervisory visit reports must be summarized every 6 months and reported to the NMP-MOH.

## 5. REFERENCES

Basic Malaria Microscopy.Part I. Learner's Guide, Second Edition. 2010
WHO Malaria Microscopy Quality Assurance Manual, Version 1. 2009.
WHO Malaria Microscopy Quality Assurance Manual, Version 2. 2015

## 6. DOCUMENT HISTORY

| Date | Version | Comments | Responsible Person |
|---|---|---|---|
| February 2017 | 1 | New SOP developed and introduced | NMCP |

**Form – 1 CHECKLIST FOR SUPERVISION (OTSS) OF MALARIA MICROSCOPY LABORATORY STATUS**

Region/ State ……Township ……Date:……

General Hospital/Station Hospital/RHC ……

Name of staff……..Designation……

| Name of Technicians | Years of experience | Training | | | |
|---|---|---|---|---|---|
| | | Frequency | Days | Last date | Topic |
| | | | | | |
| | | | | | |
| | | | | | |

Slides examined/day – ( ) No. Slides examined/year – ( )

Y = 1, N = 0

GENERAL CONDITION OF LABORATORY

| | Checklist Question | Y/N | Remark |
|---|---|---|---|
| 1 | Is the room or work place kept neat and tidy? | | |
| 2 | Are the equipment, chemical and utensil kept properly? | | |
| 3 | Is the room well ventilated with enough light? | | |
| 4 | Electricity | | |
| 5 | Water supply | | |
| 6 | Waste management system - properly set up? | | |
| | Total | | |

CHEMICALS FOR STAINING

| | Checklist Question | Y/N | Remark |
|---|---|---|---|
| 1 | Is the staining solution (working solution) used within one hour? | | |
| 2 | Are the solution and chemical bottles kept in cool, dry place and away from sunlight? | | |
| 3 | Are the staining solution and chemicals enough in stock? | | |
| 4 | Is the stock Giemsa stain bottle properly closed when it is not in use (Screw tight)? | | |
| 5 | Do you use (10%) Giemsa staining solution? | | |
| 6 | Is working solution prepared properly? | | |
| | Total | | |

MICROSCOPIC SLIDES

| Checklist Question | | Y/N | Remark |
|---|---|---|---|
| 1 | Are the glass slides cleaned before use? | | |
| 2 | Are the glass slides well packed and kept properly before use? | | |
| 3 | Are the steps performed in taking blood of patients correct? | | |
| 4 | Are the steps performed in making blood films on the slides correct? | | |
| | Total | | |

SAMPLE PREPARATION AND EXAMINATION

| Checklist Question | | Y/N | Remark |
|---|---|---|---|
| 1 | Do you prepare both thick and thin smears from one patient? | | |
| 2 | Do you prepare both thick and thin smears on one glass slide? | | |
| 3 | Do you observe (200) fields in thick smear before giving the result? | | |
| 4 | Do you report result from one patient within (45) minutes? | | |
| 5 | Do you report the MP result in species? | | |
| 6 | Do you report MP result in parasite's stage? | | |
| 7 | Do you report the parasites count in the result? | | |
| 8 | Do you send slides to Region/State VBDC or Central VBDC (or any other facilities) for CROSS-CHECKING? | | |
| | Total | | |

RDT

| Checklist Question | | Y/N | | | | | | Remark |
|---|---|---|---|---|---|---|---|---|
| 1 | Do you examine RDT along with Microscopic examination? | | | | | | | |
| 2 | If YES, who request for RDT examination? (Medical Doctor-1, Nurse-2, Lab tech-3, Microscopist –4,Patient -5, Other -6)(Tick according to the answer) | 1 | 2 | 3 | 4 | 5 | 6 | |
| 3 | Brand of RDT | | | | | | | |

STORAGE AND MAINTENANCE OF EQUIPMENT AND REAGENTS

| | Checklist Question | Y/N | Remark |
|---|---|---|---|
| 1 | What do you use to wipe out immersion oil on objective lens? (Cloth-1; No need to wipe because we use Anisole-2; Others -3) | 1 2 3 | |
| 2 | Do you cover the microscope with Plastic Dust Cover when the lab is closed (especially at night, weekend, national holidays) | | |
| 3 | Do you put the microscope back in the box or cabinet when the lab is closed (especially at night, weekend, national holidays) | | |
| 4 | Do you use special method /items to avoid mold? | | |
| 5 | Do you have special container, box or shelf to store glassware? | | |
| 6 | Do you have special container, box or shelf to store reagents? | | |
| 7 | Who manages the Key of the lab? | | |
| | Total | | |

SUPPLY SYSTEM

| | Checklist Question | Y/N | Remark |
|---|---|---|---|
| 1 | Do you have a file for stock in and out? | | |
| 2 | Do you have stock request form/ indent form? | | |
| | Total | | |

| ITEMS | Source & Frequency of SUPPLIES | | | | | |
|---|---|---|---|---|---|---|
| | VBDC | CMSD | NHL | OTHERS | HOW OFTEN | Remark |
| Lancets | | | | | | |
| Glass Slides | | | | | | |
| Giemsa stain | | | | | | |
| Immersion Oil | | | | | | |
| Methanol | | | | | | |
| RDT | | | | | | |

REPORTING SYSTEM

| | Checklist Question | Y/N | Remark |
|---|---|---|---|
| 1 | Do you use malaria microscopy? How many blood smears did you examine in last year? | | ……. slides |
| 2 | Do you have a format of **reporting form** to fill the result (on Malaria Microscopy)? | | |
| 3 | Do you report malaria microscopy data? **Where and when do you report** your routine malaria slides examination? | | ……. ……. |
| 4 | Do you report parasite species with staging and parasite count? | | |
| | Total | | |

General findings and recommendations

---

---

---

---

---

---

---

---

---

Supervisor / Validator's comments:

---

---

---

---

---

---

---

---

**FORM -2 ACCURACY OF MICROSCOPIC EXAMINATION OF BLOOD SLIDES**
**(Checking with Standard Slide Set during Supervision (OTSS) Visit)**

Region/State ……Township ……Date……
VBDC/General Hospital/Township Hospital/ Station Hospital/RHC
……
Name of staff…… Designation……

| Sr | Slides No | Parasite Species | No of asexual stages Count | Correct Species | Correct Count | Scoring | Remark |
|---|---|---|---|---|---|---|---|
| 1 | | | | | | | |
| 2 | | | | | | | |
| 3 | | | | | | | |
| 4 | | | | | | | |
| 5 | | | | | | | |
| 6 | | | | | | | |
| 7 | | | | | | | |
| 8 | | | | | | | |
| 9 | | | | | | | |
| 10 | | | | | | | |

**MICROSCOPE**

| Sr | Microscope Brand | Model | Bi/Monocular | Light/Electric | Function +/- |
|---|---|---|---|---|---|
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |

**PARTS OF MICROSCOPE**

| Parts EXD: | Microscope Brand/Model | Any defect identified | Repaired during visit |
|---|---|---|---|
| Eye Pieces | | | |
| Objectives | | | |
| Condenser | | | |
| Mirror | | | |
| Light Source | | | |

Signature of Supervisor……..
Name of Supervisor.....
Designation ……

**FORM – 3 FEEDBACK FOR SUPERVISION ASSESSMENT (OTSS) OF LABORATORY**
**(For Feedback to the TMO or concerned authority immediately after supervision)**

Region/State……Township……Date of Supervision……

Place of supervision - VBDC/ General Hospital/Township Hospital/ Station Hospital/RHC

.....

Name of laboratory in-charge ……..Designation……

| CHECKLIST | MAX SCORE | RESULT SCORE | REMARKS |
|---|---|---|---|
| GENERAL CONDITION OF LABORATORY | 6 | | |
| CHEMICALS FOR STAINING | 6 | | |
| MICROSCOPIC SLIDES | 4 | | |
| SAMPLE PREPARATION AND EXAMINATION | 8 | | |
| MICROSCOPE FUNCTIONING | 1 | | |
| STORAGE AND MAINTENANCE OF EQUIPMENT AND REAGENTS | 5 | | |
| SUPPLY SYSTEM | 2 | | |
| REPORTING | 4 | | |
| TOTAL | 36 | A | |

Score on observation of laboratory = ……A……

% of scoring result of laboratory = ……A…/36 = …….

| No | Name | Designation | No of Slide | True (+) ve | False(+) ve | False (-)ve | True (-)ve | Malaria knowledge (Theory) | % of Agreement result |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |

Result of Proficiency testing = ……..
(% of agreement result formula= TP+TN/Total slides)
General findings and recommendations

---
---
---
---

Signature of Supervisor……..
Name of Supervisor……
Designation.....

Page 8 of 8



UNIVERSITY RESEARCH CO., LLC