Royal Centre for Disease Control
རྒྱལ་འཛིན་ནད་རིགས་བཀག་ཐབས་ལྷེ་བ།
Department of Public Health | Ministry of Health | Royal Government of Bhutan

Standard Operating Procedure
for
Malaria Microscopy

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Content

National Malaria Reference laboratory

SOP for Malaria Microscopy

SOP title:		**Microscope Use and Preventive Maintenance**			
SOP No:	**SOP-NMRL-01**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This Standard Operating Procedure outlines procedures for the proper use and maintenance of microscopes in all laboratories where malaria microscopy is performed.

2. Background

A good quality microscopy result depends mainly on the functional microscope in use. It is essential to know proper handling and manipulation, limitations, and ways to keep in good working conditions to ensure the high standards required for microscopy.

3. Application

The SOP applies to all laboratory

- Performing malaria microscopy and it can imply to other lab procedure handling with microscope.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. Microscope daily maintenance log

6. Sample requirement

6.1. Equipment:

- Compound microscope

6.2. Lab consumables

- Lens cleaning paper
- Soft tissue paper / gauze

6.3. Reagent & Chemical

- Absolute alcohol

7. Precautions

7.1. Never clean microscope objective lens with xylene to remove the oil.

7.2. Never exchange parts from one microscope to another. (Even some models by the same manufacturer have different specifications.)

7.3. Never try to dismantle or clean any part of the microscope that is difficult to reach unless you have been trained to do so.

8. Procedures

8.1. Parts of compound microscope

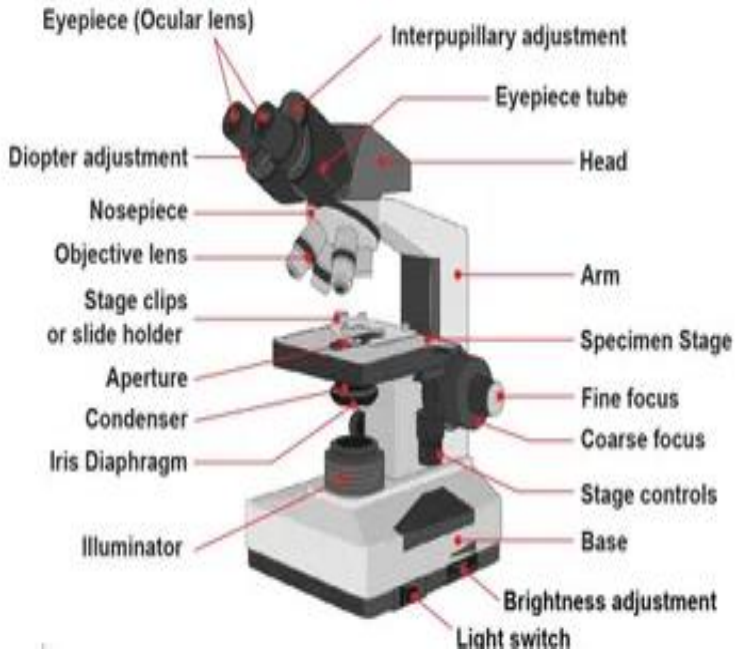


Figure 1: Part of Compound Microscope

8.2. Placement of microscope in lab

8.1.1. 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.

8.1.2. Place it in a position or bench with sufficient leg space for the microscopist; preferably use height-adjustable chairs

8.1.3. Do not place the microscope in front of a brightly lit window. Place it in front of a wall or a darkened window.

8.3. Microscope set up

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

8.3.1. Adjust the iris aperture to the setting recommended by the manufacturer to achieve maximum depth of field.

8.3.2. If the manufacturer provides an iris diaphragm setting on the condenser, set this to the 100x objective lens setting.

8.3.3. Use the following method if the eyepiece of the microscope can be removed:

8.3.4. raise the condenser

8.3.5. set the lamp to “low”

8.3.6. select the 40x objective

8.3.7. close the iris diaphragm

8.3.8. remove one eyepiece

8.3.9. 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.

8.3.10. open the iris diaphragm until the objective lens completely fills with light

8.3.11. 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

8.3.12. Replace the eyepiece.

8.4. Care and maintenance of compound microscope

8.4.1. After used, remove immersion oil with lens cleaning paper in one direction method

8.4.2. Rotate the nosepiece so to bring the lowest objective lens to mechanical stage.

8.4.3. Cover the microscope when not in use with the anti- static cover provided (fig:2).

Figure 2 covering the microscope with anti static cover

8.4.4. Rotate the nosepiece so to bring the lowest objective lens to mechanical stage.

8.4.5. Never apply strong physical force to an objective. To move another objective into position, move the revolving nose piece; do not grab the objective and pull on it.

8.4.6. Remove dust off of the microscope stage, base, and body with a clean soft lint free cloth. If necessary, clean off any immersion oil with ethanol (fig: 3).

Figure 3 cleaning the body parts of the microscope

8.4.7. Cleaning the eye pieces 6.2.15.1 Blow off dust particles using the rubber bulb (fig:4)

Figure 4 blowing off the dust particles from eye pieces

8.4.8. Prepare a clean lens tissue swab using a clean lens tissue and an applicator stick (fig:5).

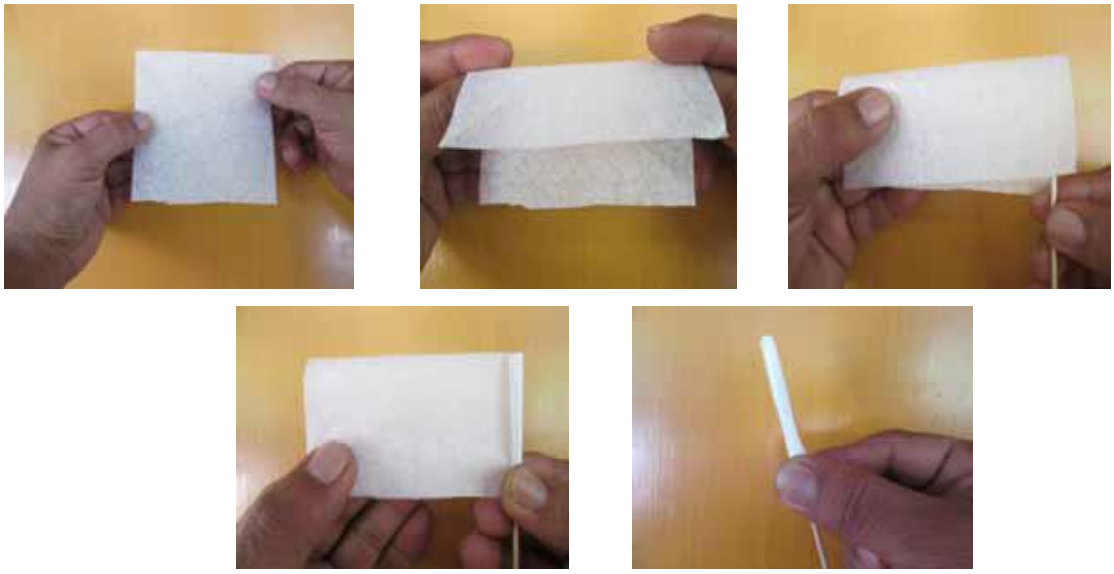


Figure 5: How to roll the lens tissue around a stick (lens tissue swab)

8.4.9. Wipe the upper lens with a lens tissue swab slightly moistened with absolute alcohol in circular motion starting at the center going outward (see figure) Repeat the process using dry tissue swab.

Figure 6: Cleaning the eye pieces of the microscope

8.4.10. Cleaning the objectives

- Never use ordinary paper or cotton wool to clean the lenses of the microscope.
- Always clean objectives by moving the lens tissue across the lens, not circularly. This can be done by placing a lens tissue between your finger and the lens and pulling the tissue without moving your finger to drag the lens tissue across the objective lens Repeat 3-4 times using a clean section of lens tissue for each pull.
- Before cleaning the dry objectives (x4,x10,x40), with lens tissue, blow off dust particles using the rubber bulb.
- To clean the oil Immersion objective, a lens tissue moistened with absolute alcohol can be used if needed.

8.4.11. Clean the condenser top lens the same way as the objectives using soft cloth or lens tissue. 6.2.18 Clean the mirror and light exit glass using a lens paper wrapped around a finger (figure 8)

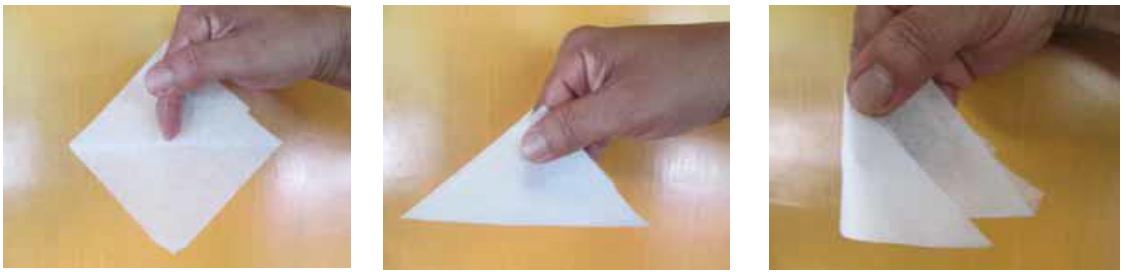


Figure 8: How to roll the lens paper around the finger

8.4.12. Never use xylene in cleaning any part of the microscope.

8.5. Storage

8.5.1. To prevent the growth of fungus, whenever possible, keep the microscope in a continuously air-conditioned room.

8.5.2. If the air conditioner is not used continuously for 24 hours, the microscope must be kept in a warm cupboard heated by 1 or 2 light bulbs (25 – 40 watts).

8.5.3. In Laboratories without electricity, the microscope should be kept in an airtight container 15-20 cm in diameter with no less than 250 grams of dry blue silica gel (It should not be stored in the original wooden box).

8.6. Transporting the microscope

8.6.1. When moving the microscope, carry it with one hand under the base and the other hand holding the arm. Do not hold the microscope by the stage, stage feed knobs, and observation tube to prevent damage

8.6.2. Loosen the observation tube clamping knob slightly, rotate the tube by 180 degrees, and tighten the knob.

8.6.3. Put the transport band, whenever available.

8.6.4. Secure the stage and other movable parts.

8.6.5. Use protective materials such as Styrofoam, pad and the packaging carton if available.

8.6.6. Use the securing device which screws the base inside its storage box.

9. Maintenance

9.1. Inspection and performance check

9.4.1. All outer surfaces of the instrument must be clean.

9.4.2. All electrical plugs, sockets, cables, etc. must be cleaned and free of corrosion or other visible damage; screws must be tightened securely.

9.4.3. The light bulb must be clean.

9.4.4. The field iris diaphragm must move through its full range of opening smoothly and easily; all blades of the iris must move properly.

9.4.5. The mechanical stage must move the specimen smoothly and without any play through its full range of adjustment in both directions

10. Annexure

10.1. Microscope daily maintenance log

11. Reference

11.1. WHO Standard Operating Procedure for Malaria microscopy.

11.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

	National Malaria Reference laboratory SOP for Malaria Microscopy				
SOP title:		**Cleaning and storing of glass slides**			
SOP No:	**SOP-NMRL-02**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

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. Background

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.

All slides must be thoroughly clean and free from scratches, grease or moisture. This will prevent most of the artifacts which confuse Malaria diagnosis and will avoid the detachment and washing away of thick blood films during the staining process. Poorly cleaned slides will lead to sub-standard blood films, in turn leading to imprecise microscopy.

3. Application

The SOP applies to all Laboratory Technicians doing Malaria Microscopy.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. NA

6. Sample requirement

6.1. Lab consumables

- Glass slides- preferably frosted end for labeling.
- Plastic bowls or basins
- Good quality liquid detergents
- Washing clothes or soft sponges

- Clean, lint free cotton cloths (the kind used to dry glassware)
- Good supply of clean water
- Sheets of clean paper cut to 11 cm. x 15 cm.
- Empty slide boxes
- Clear adhesive tape
- Desiccators /silica gel
- Gloves

7. Precautions

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

8. Procedure

8.4. Cleaning of slides

8.4.1. Soak slides for at least 1-2 hours in water mixed with a mild dishwashing or liquid detergent.

8.4.2. With a washing cloth or sponge, clean both sides of each slide.

8.4.3. Rinse the slides individually in clean water to remove all traces of detergent.

8.4.4. Dry each slide thoroughly with a lint-free towel.

8.4.5. 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

8.4.6. If the slides will not be used immediately, put some silica gel inside the box to prevent fungal growths. 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:

8.4.7. Example:

05 Aug 2015

8.5. Storing of slides

8.5.1. Glass slides should not be kept in the ambient climate of the humid tropics for more than a few weeks. Otherwise, they will adhere to each other due to entrapped moisture and there will be a loss of transparency due to 'frosting'

8.5.2. It is recommended that cleaned slides be stored in packages of 10, wrapped in thin paper and secured with cellulose adhesive tape or rubber bands, ready to be used when required

8.5.3. Packages of slides can be put in the original cardboard boxes or other suitable boxes with desiccant.

06 Annexure

9.1. Microscope daily maintenance log

07 Reference

10.1 WHO Standard Operating Procedure for Malaria microscopy.

10.2 VDCP Standard Operating Procedure for malaria microscopy- first edition

National Malaria Reference laboratory

SOP for Malaria Microscopy

SOP title:		**Preparation of Giemsa Stock Solution**			
SOP No:	**SOP-NMRL-03**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

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

2. Background

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. Application

The SOP applies to all Laboratory Technicians doing Malaria Microscopy.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. NA

6. Sample requirement

6.1. Reagent/ chemical

- Giemsa powder- preferably biological stain commission grade
- Methanol
- Glycerol

6.2. Equipment

- Analytical balance
- Electromagnetic starrier

6.3. Lab consumables

- Solid glass beads / magnetic beads, 3-5 mm in diameter
- Spatula or measuring spoon
- Weighing paper
- Graduated cylinder
- Glass or plastic funnel
- 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)

7. Precautions

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

7.2. 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.

8. Procedure

8.1. Place about 50 glass beads into a dark or amber bottle.

8.2. Weigh 3.8 g of stain powder on an analytical balance and pour into bottle containing the beads.

8.3. Measure 250 mL methanol in a graduated cylinder and pour gently into the bottle.

8.4. Allow the stain powder to sink through the methanol until it settles to the bottom.

8.5. Stopper the bottle tightly then shake it with circular motion for 2-3 minutes to dissolve the stain.

8.6. Measure 250 mL glycerol in a graduated cylinder and pour gently into the bottle.

8.7. Stopper the bottle tightly before repeating the shaking process.

8.8. Continue the shaking for 3–5 minutes for about 3-5 times during the first day.

8.9. Repeat the shaking every day for 2-3 days until the stain is completely mixed.

8.10. Assign batch number for each stock solution prepared. Label the bottle as in example below:

Giemsa Stock Solution
Batch No: 2015-01
Date prepared: 5 Aug 2015

8.11. Batch #2015-01 stands for the year prepared and stock or lot number.

8.12. Store the bottle tightly stoppered to prevent absorption of water vapour from the air, and in a cool place away from direct sunlight.

9. Procedure Note

9.1. 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.

9.2. 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.

9.3. 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.

9.4. 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.

9.5. If a clear stock bottle is used, wrap it with a thick dark paper to avoid light penetration.

10. Quality Control

10.1. Perform quality control check for every new batch/lot of stock solution prepared.

10.2. On logbook, record QC finding and if pass can be used for staining.

11. Maintenance

11.1. NA

12. Annexure

12.1. Record sheet for reagent preparation

13. Reference

13.1. WHO Standard Operating Procedure for Malaria microscopy.

13.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

National Malaria Reference laboratory

SOP for Malaria Microscopy

SOP title:		**Preparation of Giemsa Working Solution**			
SOP No:	**SOP-NMRL-04**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This SOP describe the procedure for preparing a working solution of Giemsa stain from the stock solution for routine staining of malaria blood films

2. Background

A freshly prepared working solution of Giemsa, made from well-prepared stock and diluted with buffered water to pH 7.2 is recommended to achieve optimal staining of malaria blood films.

Staining with Giemsa stain can be done with a rapid (10% working solution) method or a slow (3% working solution) method. The rapid method is used in outpatient clinics and busy laboratories where a quick diagnosis is essential for patient care. The slow method is used for staining large numbers of slides, such as for preparation of panel slides for panel testing and malaria slide bank

3. Application

The SOP applies to all Laboratory Technicians doing Malaria Microscopy.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. NA

6. Sample requirement

6.1. Reagent/ chemical

- Giemsa stock solution
- Methanol
- Buffered water -pH 7.2 / Distilled water

6.2. Equipment

- Pasteur pipettes

- Graduated cylinder
- Drying Rack
- Staining trough
- Clean tap water
- Timer
- Wash bottle

7. Precautions

8. Procedure

Two methods of staining with Giemsa stain:

- The Rapid (10%) method
- The Routine or Regular (3%) method

8.1. Preparation of 10% Giemsa working solution for rapid staining

8.1.1. Pour 90 ml of buffered water / distilled water (pH7.2) into a 100ml graduated cylinder

8.1.2. Filter the giemsa stock using whatman #1 and transfer to clean container

8.1.3. Using a Pasteur pipette, draw 10 ml of Giemsa stock solution. Add the stain to the buffered distilled water in the graduated cylinder.

8.1.4. Cover the top of the graduated cylinder with parafilm. Gently invert the cylinder several times until completely mixed. If parafilm is not available, mixing can be done by gently using pasture pipette.

8.1.5. Preparation of 10% Giemsa stain solution for individual slide staining

8.1.6. For individual slide staining, each slide needs approximately 3 mL of Giemsa working solution to cover it.

8.1.7. Using a Pasteur pipette add 9 drops of Giemsa stock solution to 3ml of buffered distilled water in a 10ml graduated cylinder.

8.2. Preparation of 3 % giemsa working solution for slow staining

8.2.1. Place 97 mL of previously prepared buffered water, pH 7.2, into a clean measuring cylinder.

8.2.2. Filter the Giemsa stock solution through paper Whatman #1 and transfer to a 25 to 50 mL container.

8.2.3. Using measuring cylinder or a pipette, measure 3 mL of Giemsa stock solution. Do **not** take the aliquot directly from the large bottle containing the Giemsa stock solution, to avoid contaminating it.

8.2.4. Prepare the Giemsa working solution just before staining the blood film(s) and use it within a maximum of 15 minutes of preparation. Discard any excess stain.

8.3. Procedure Note

8.3.1. 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.

8.3.2. 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.

8.3.3. 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.

8.3.4. 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.

8.3.5. If a clear stock bottle is used, wrap it with a thick dark paper to avoid light penetration.

9. Quality Control

9.1. Perform quality control check for every new batch/lot of stock solution prepared.

9.2. On logbook, record QC finding and if pass can be used for staining.

10. Maintenance

10.1. NA

11. Annexure

11.1. Record sheet for reagent preparation

12. Reference

12.1. WHO Standard Operating Procedure for Malaria microscopy.

12.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

	National Malaria Reference laboratory **SOP for Malaria Microscopy**				
SOP title:		**Preparation of Buffered Water to pH 7.2**			
SOP No:	**SOP-NMRL-05**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This SOP describe the procedure for preparing buffered water to pH 7.2 for use in the preparation of a working solution of Giemsa stain for routine staining of malaria blood films.

2. Background

Malaria parasites can be seen clearly under the microscope on correctly stained blood films. Using buffered water at the correct pH helps to ensure good staining.

This SOP has three components:

- Preparation of water buffered to pH 7.2,
- Preparation of 2% correcting fluid and
- Checking and adjusting the pH of buffered water.

3. Application

The SOP applies to all Laboratory Technicians doing Malaria Microscopy.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. NA

6. Sample requirement

6.1. Reagent/ chemical

- Potassium dihydrogen phosphate (anhydrous) – KH2PO4
- Disodium hydrogen phosphate (anhydrous) – Na2HPO4
- Distilled water- preferably sterile

6.2. Equipment

- Analytical balance accurate to 0.01 g

6.3. Lab consumables

- Spatula
- Weigh boat / clean paper
- Filter paper whatman #1
- Conical flask
- Beaker
- Labelling paper
- Screw capped glass bottle

7. Precautions

7.1. NA

8. Procedure

8.1. Preparation of buffered water to pH 7. 2

8.1.1. Weigh 0.7 g of potassium dihydrogen phosphate (KH2PO4)

8.1.2. Transfer the weighed KH2PO4 to the glass beaker, add about 150 mL of distilled water, and stir with a clean spatula until the salt dissolves.

8.1.3. Weigh 1 g of Na2HPO4.

8.1.4. Add the Na2HPO4 to the solution in the beaker and stir as in step 2.

8.1.5. When the salts have dissolved, add the solution to the conical flask, and top up to the 1-L mark with water.

8.1.6. Pour the buffered water into a glass bottle. Label it clearly and document it in quality control log-book with the date of preparation, date of expiry and name of the person who prepared the buffer.

Buffered water, pH 7.2

Prepared by:

Date prepared:

Expiry date:

8.1.7. Store the buffered water for a maximum of 7 days, tightly stoppered in a cool place away from sunlight. Use of a dark or amber bottle is recommended.

8.2. Preparation of 2 % Na2 HPO4

8.2.1. Weigh 2 g of Na2HPO4.

8.2.2. Add it to 100 mL of water in a clean beaker and stir with the spatula until the salts have dissolved.

8.2.3. Pour the solution into one of the glass bottles, and label it “2% Na2HPO4”. Write the date of preparation and the name of person who prepared the correcting fluid on the label.

8.2.4. Store the bottle of 2% Na2HPO4 in a cool place away from sunlight.

8.3. Preparation of 2 % Na2 HPO4

8.3.1. Weigh 2 g of KH2PO4 .

8.3.2. Add it to 100 mL of water in a clean beaker. Stir with spatula until the salts have dissolved.

8.3.3. Pour the solution into one of the glass bottles and label it “2% KH2PO4 ”. Write the date of preparation and the name of person who prepared the correcting fluid on the label.

8.3.4. Store the bottle of 2% KH2PO4 in a cool place away from sunlight.

8.4. Checking and adjusting the pH of buffered water

8.4.1. Check the pH of buffered water routinely before use. To adjust the pH, add small quantities of the correcting fluids to the buffer: 2% Na2HPO4 if the pH is below 7.2 (too acid) or 2% KH2PO4 if the pH is above 7.2 (too alkaline). Adjustments can be made as outlined below.

8.4.2. Adjust the pH of the water in the conical flask by adding two or three drops of the relevant correcting fluid: Na2HPO4 to make it alkaline, KH2PO4 to make it acid. Stir with a clean spatula.

8.4.3. Check the pH of the buffered water by repeating steps. Continue until the correct pH of 7.2 is reached.

8.5. Procedure Note

8.5.1. It is best to store buffered water in a cool place away from direct sunlight. Use of a dark bottle or a clear glass bottle wrapped in brown paper is recommended to prevent bacterial, fungal and algal growth.

8.5.2. Check continually for contamination.

8.5.3. Do not keep buffer solution for more than 7 days to avoid change in the pH and to prevent contamination.

8.5.4. Check the pH of buffered water routinely before use, and record it in the quality control log-book.

9. Quality Control

9.1. Perform a quality control check on every new batch of buffered water prepared and before every use and record the information in the log-book. See MM-SOP 3c: Quality control of Giemsa stock solution and buffered water.

10. Maintenance

10.1. NA

11. Annexure

11.1. Record sheet for reagent preparation

12. Reference

12.1. WHO Standard Operating Procedure for Malaria microscopy.

12.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

National Malaria Reference laboratory

SOP for Malaria Microscopy

SOP title:		**Collection of finger-prick blood and preparation of blood film**			
SOP No:	**SOP-NMRL-06**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This SOP describe the procedure for collecting blood from a finger prick and preparing thick and thin blood films for malaria diagnosis by light microscopy.

2. Background

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 a finger prick. Good-quality blood films are essential to establish accurate diagnoses.

3. Application

The SOP applies to all Laboratory Technicians doing Malaria Microscopy.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.2. WHO smear preparation template

6. Sample requirement

6.1. Reagent

- 70% alcohol

6.2. Lab consumables

- cleaned glass slides, 25 x 75 mm, with one frosted end for labelling
- sterile lancets, one per patient
- dry cotton (cotton ball, swab, or gauze)
- protective latex gloves (powder free)
- a biohazard container or any puncture-resistance sharps container (See MM-SOP 13: Management of wastes generated from malaria diagnostic tests);

- an infectious wastes container (See MM-SOP 13: Management of wastes generated from malaria diagnostic tests)
- a slide tray or box and a cover to dry slides horizontally, protected from dust and flies;
- a drying rack;
- a lead pencil or permanent marker pen.

7. Precautions

7.1. Wear protective latex gloves before starting blood collection and when handling slides, for personal protection and to avoid leaving oil on the slide that may interfere with the smear preparation.

7.2. Always use a new lancet for each patient. Never re-use lancets.

7.3. Discard the infection waste as per the waste disposal protocol.

7.4. Sharps such as lancets and broken glass must be discarded in a “sharps” container for safe disposal by incineration or autoclaving.

7.5. The thick film should be dried flat and be protected from dust and flies.

7.6. The thick film may auto fix if exposed to extreme heat and should therefore be stained immediately.

7.7. The thick film can be dried gently with a hair-dryer set at warm but care must be taken to avoid heat fixation, which can occur quickly.

7.8. Do not use a ballpoint or gel pen to label slides, as the ink will spread when the film is fixed.

8. Procedure

8.1. Finger prick

8.1.1. Label the frosted end of the glass slide with the patient’s details

8.1.2. Select the third finger from the thumb of the non-dominant hand (or big toe for infants, not the heel)

8.1.3. Hold the patient’s hand, palm facing upwards, and clean the selected finger with a piece of cotton soaked lightly in 70% ethanol or alcohol swab. Use firm strokes to remove dirt and oil from the ball of the finger and to stimulate blood circulation. Make sure the finger is warm by applying gentle massage if required. Let the alcohol dry from the finger.

8.1.4. Using a new, sterile lancet and a quick rolling action, puncture the centre of the ball of the finger or toe.

8.1.5. Apply gentle pressure to the finger (or toe) and express the first drop of blood.

8.1.6. Wipe the first drop of blood off with dry cotton, making sure that no cotton strands remain on the finger that might stick to the blood.

8.1.7. Quickly collect blood by applying gentle pressure to the finger and touching the slide to the blood; collect a single small drop of blood on the middle of the slide for the thin film

8.1.8. Apply further gentle pressure to express more blood and collect three drops on the slide about 1 cm from the drop intended for the thin film.

8.1.9. Wipe the remaining blood from the finger with clean, dry cotton.

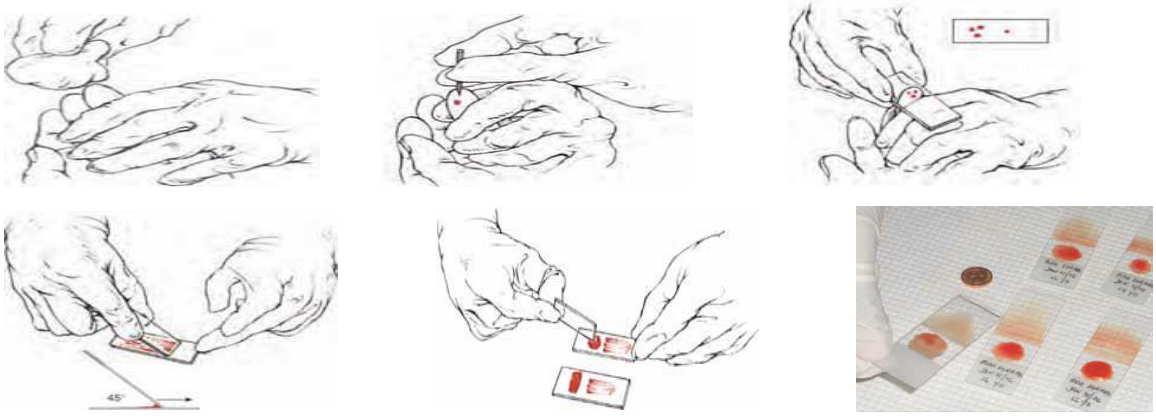
8.1.10. To prepare the thin film, place the edge of a clean “spreader” slide at 45o in front of the blood drop intended for the thin film.

8.1.11. Slowly pull the “spreader” back until it touches the drop of blood and the blood spreads along the edge of the “spreader”.

8.1.12. Rapidly push the “spreader” forwards (away from the centre) in a smooth, continuous motion, until the spreader leaves a “feathery” end for the thin film.

8.1.13. With the corner of the same “spreader” used for making the thin film, make the thick film by swirling the three drops of blood together forming a circle of about 1 cm in diameter size.

8.1.14. After preparing the thin and thick blood films, allow them to dry in air in a horizontal position on a slide tray.



9. Quality Control

9.1. Used WHO blood film preparation template to prepare good quality of thick and thin blood film

10. Maintenance

10.1. NA

11. Annexure

11.1. WHO template for preparation of thick and thin blood film

12. Reference

12.1. WHO Standard Operating Procedure for Malaria microscopy.

12.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

National Malaria Reference laboratory

SOP for Malaria Microscopy

SOP title:		**Collection of Venous blood and preparation of blood film**			
SOP No:	**SOP-NMRL-07**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This SOP describe the procedure for collecting blood by venipuncture and for preparing thick and thin blood films from venous blood collected in tubes containing anticoagulant

2. Background

Venous blood samples are collected for multiple analyses, which may include malaria diagnosis by microscopy. Larger volumes of venous blood are collected than in tubes containing anticoagulant (preferably ethylenediaminetetraacetic acid [EDTA]). Venous blood samples treated with EDTA can be used to prepare thin and thick blood films for malaria diagnosis, for preparing malaria panel slides and other molecular purposes.

3. Application

The SOP applies to all Laboratory Technicians doing Malaria Microscopy.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. WHO Smear preparation template

6. Sample requirement

6.1. Reagent

- 70% alcohol

6.2. Lab consumables

- cleaned glass slides, 25 x 75 mm, with one frosted end for labelling
- sterile syringe with needle (gauze 21 or 23) , one per patient
- Vacuum tube containing an EDTA anticoagulant
- Tourniquet

- Micro pipette / pasture pipette
- Micro tips
- dry cotton (cotton ball, swab, or gauze)
- protective latex gloves (powder free)
- a biohazard container or any puncture-resistance sharps container (See MM-SOP 13: Management of wastes generated from malaria diagnostic tests);
- an infectious wastes container (See MM-SOP 13: Management of wastes generated from malaria diagnostic tests)
- a slide tray or box and a cover to dry slides horizontally, protected from dust and flies;
- a drying rack;
- a lead pencil or permanent marker pen.

7. Precautions

7.1. Wear protective latex gloves before starting blood collection and when handling slides, for personal protection and to avoid leaving oil on the slide that may interfere with the smear preparation.

7.2. Always use a new lancet for each patient. Never re-use lancets.

7.3. Discard the infection waste as per the waste disposal protocol.

7.4. Sharps such as lancets and broken glass must be discarded in a “sharps” container for safe disposal by incineration or autoclaving.

7.5. The thick film should be dried flat and be protected from dust and flies.

7.6. The thick film may auto fix if exposed to extreme heat and should therefore be stained immediately.

7.7. The thick film can be dried gently with a hair-dryer set at warm but care must be taken to avoid heat fixation, which can occur quickly.

7.8. Do not use a ballpoint or gel pen to label slides, as the ink will spread when the film is fixed.

8. Procedure

8.1. Collection of blood sample by venipuncture

8.1.1. Label an EDTA-containing tube with the patient’s name, date and time of collection.

8.1.2. Explain the procedure on blood collection to patient and make then sit comfortable.

8.1.3. Apply a tourniquet on the upper arm of the patient to enable the veins to be seen or felt. Ask the patient to make a tight fist so that the veins are more prominent.

8.1.4. With your index finger, feel for a sufficiently large, minimally moveable vein.

8.1.5. Disinfect the site with an alcohol swab or cotton dampened with 70% isopropyl or ethyl alcohol. Do not touch the cleansed area again.

8.1.6. Allow the venipuncture site to dry in air for 30 s to ensure that the blood sample collected is not contaminated with alcohol, which can lead to haemolysis.

8.1.7. Insert needle along the line of the vein, with the bevel of the needle facing directly upwards. Steadily draw > 2 mL to 4 mL of blood. Note: Anticoagulants may interfere with adhesion of blood to the slide and with Giemsa staining, especially if the ratio of blood to anticoagulant is not optimal. Hence, the volume of blood in a 5-mL EDTA tube should be > 2 mL.

8.1.8. When enough blood has been collected, release the tourniquet, and instruct the patient to open his or her fist. Remove the needle and press a piece of dry cotton firmly on the venipuncture site. Instruct the patient to continue pressing on the puncture site with the arm raised until any bleeding stops.

8.1.9. Transfer the blood to the EDTA-containing tube, and mix gently by inverting the tube six times. Do not shake the tube.

8.2. Preparation of thick and thin blood films

8.2.1. Gently mix collected venous blood in a vacuum tube containing EDTA before use.

8.2.2. Place a clean, labelled microscope slide on a standardized thick and thin blood film-slide preparation template

8.2.3. Transfer 6 µL of blood with a micropipette onto the larger circle of the slide. With the tip of the micropipette, swirl the blood, making a circle about 1 cm in diameter, and prepare the thick film; three to six quick strokes with the tip are sufficient.

8.2.4. Collect an additional 2 µL of blood with a micropipette and transfer the blood onto the small circle of the slide template.

8.2.5. To prepare the thin film, place the edge of a clean “spreader” slide at a 45* in front of the blood drop intended for the thin film.

8.2.6. Slowly pull the “spreader” back until it touches the drop of blood and the blood spreads along the edge of the spreader.

8.2.7. Rapidly push the spreader forwards (away from the centre) in a smooth, continuous motion, until the spreader leaves a “feathery” end for the thin film.

8.2.8. Dry the prepared slides horizontally. Poor adherence is a problem with EDTA-treated blood. If rapid drying is required, dry the films with low heat from a hair-dryer at some distance. Do not place the blood films too close, as the film might be fixed by heat.

9. Quality Control

9.1. Used WHO blood film preparation template to prepare good quality of thick and thin blood film

10. Maintenance

10.1. NA

11. Annexure

11.1. WHO template for preparation of thick and thin blood film

12. Reference

12.1. WHO Standard Operating Procedure for Malaria microscopy.

12.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

National Malaria Reference laboratory

SOP for Malaria Microscopy

SOP title:		**Labelling Malaria blood film**			
SOP No:	**SOP-NMRL-08**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This SOP describe the recommended procedure for labelling malaria blood films

2. Background

Correct labelling 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 unlabelled or incorrectly labelled blood films. Labelling is important even if only one slide is to be prepared.

3. Application

The SOP applies to all Laboratory Technicians doing Malaria Microscopy.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.2. Malaria register

6. Sample requirement

6.1. Lab consumables

- cleaned glass slides, 25 x 75 mm, with one frosted end for labelling
- a lead pencil or permanent marker pen.

7. Precautions

7.1. Labelling should be completed before taking blood from a patient. When labelling slides, avoid touching the blood film with writing instruments.

7.2. Do not use a ballpoint or gel pen to label slides, as the ink will spread when the film is fixed.

8. Procedure

8.1. Check the patient information on the test or request form and record it accurately in the register.

8.2. Write the following information on the frosted part of the slide: laboratory sample code, patient identification number and date of collection

Example:

01/001

19/01/2016

9. Quality Control

9.1. Used WHO blood film preparation template to prepare good quality of thick and thin blood film

10. Maintenance

10.1. NA

11. Annexure

11.1. WHO template for preparation of thick and thin blood film

12. Reference

12.1. WHO Standard Operating Procedure for Malaria microscopy.

12.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

National Malaria Reference laboratory

SOP for Malaria Microscopy

SOP title:		**Giemsa Staining of Malaria blood film**			
SOP No:	**SOP-NMRL-09**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This SOP describe the procedure for properly staining malaria blood films with Giemsa stain.

2. Background

A properly stained blood film is critical for malaria diagnosis, especially for precise identification of malaria species. 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 blue component stains the cytoplasm blue. The thin film is fixed with methanol. De-haemoglobinization of the thick film and staining take place at the same time. The ideal pH for demonstrating stippling of the parasites to allow proper species identification is 7.2.

Methods of staining

The two methods for staining with Giemsa stain are the rapid (10% stain working solution) and the slow (3% stain working solution) methods.

The rapid (10% stain working solution) method

This is the commonest method for staining 1–15 slides at a time. It is used in outpatient clinics and busy laboratories where a quick diagnosis is essential for patient care. The method is efficient but requires more stain.

The slow (3% stain working solution) method

The slow method is used for staining larger numbers of slides (≥ 20). It is ideal for staining blood films collected during cross-sectional or epidemiological surveys and field research and for preparing batches of slides for teaching. It is less appropriate when a quick result is needed. The slow method is less expensive than the rapid method because it requires much less stain (3% rather than 10% stain solution).

3. Application

The SOP applies to all Laboratory Technicians doing Malaria Microscopy.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. NA

6. Sample requirement

6.1. Regents / chemical

6.1.1. Giemsa stain

6.1.2. Absolute methanol, acetone free

6.1.3. Buffered water (pH 7.2) / Distilled water

6.2. Lab consumables

6.2.1. Container or beaker for Giemsa working stain;

6.2.2. Pasteur pipette

6.2.3. Container or beaker for methanol;

6.2.4. Staining rack- rapid method

6.2.5. Staining troughs- slow method

6.2.6. timer;

6.2.7. slide-drying rack

6.2.8. protective latex gloves

7. Precautions

7.1. Methanol (methyl alcohol) is inflammable and highly toxic if inhaled or swallowed; it can cause blindness and even death if swallowed in any quantity. Avoid contact and inhalation. When it is not in use, it should be stored in a locked cupboard.

7.2. Universal precautions – including use of relevant personal protective equipment such as gloves, safety glasses and a laboratory coat or gown – must be practised.

8. Procedure

8.1. For rapid- 10% method

8.1.1. Calculate the amount of 10% Giemsa working solution required for the number of slides to be stained. Each slide requires approximately 3 mL of stain to cover it.

8.1.2. To fix the thin film, preferably use a Pasteur pipette or dip the thin film for 2 s into a small container or beaker containing methanol. Avoid contact between the thick film with methanol as its vapours will quickly fix the thick film and interfere with haemolysis of the thick film.

8.1.3. Place the slides on a tray or drying rack. Allow the methanol-fixed thin smear to dry completely in air (approximately 2 min) by placing the slides on a flat surface. Never let the slide dry in a vertical position with the thin film down, as this may lead to fixing of the thick film by methanol vapour.

8.1.4. Place slides on staining rack.

8.1.5. Gently pour the stain onto the top of slides

8.1.6. Set the timer to 8–10 min (the exposure time should be determined previously by testing the batch of stock staining solution used), and allow the blood films to stain.

8.1.7. At the end of the staining time, gently flush the stain from the slide by adding drops of buffered water until all the stain has been washed away. Do not pour the stain directly off the slides, as the metallic green surface scum will stick to the film, spoiling it for microscopy.

8.1.8. When the stain has been washed away, place the slide in the drying rack in a vertical position with the thick film down to drain and dry. Ensure that thick films are not scraped against the edge of the rack.

8.1.9. Discard the remaining 10% Giemsa solution.

8.2. For slow -3 % method

8.2.1. Calculate the amount of 3% Giemsa stain working solution needed for the number of slides to be stained.

8.2.2. Fix each thin film, preferably using a Pasteur pipette or by dipping the thin film for 2 s into a small container or beaker containing methanol. Avoid contact between the thick film with methanol as its vapours quickly fix thick films and interfere with the haemolysis of the thick film.

8.2.3. Place the slides back-to-back in a staining trough, making sure that the thick films are together at one end of the tray.

8.2.4. Pour the stain gently into the staining tray. Do not pour it directly onto the thick films, as they may float off the slides.

8.2.5. Set the timer for 45–60 min (the exposure time should be determined previously by testing the batch of stock staining solution), and stain the blood films.

8.2.6. Take out the staining trough and place it into the new troughs containing buffer water / distilled water to rinse the stain.

8.2.7. Final rinse with buffered water if possible

8.2.8. Carefully remove the slides, one by one, and place them film side down in the drying rack to dry. Make sure that the thick films do not touch the edge of the rack.

8.2.9. Discard the remaining 3% Giemsa solution.

9. Quality Control

9.1. Always run the quality control on every new batch of the giemsa stain stock is used or whenever required in between.

10. Maintenance

10.1. NA

11. Annexure

11.1. WHO template for preparation of thick and thin blood film

12. Reference

12.1. WHO Standard Operating Procedure for Malaria microscopy.

12.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

		National Malaria Reference laboratory **SOP for Malaria Microscopy**			
SOP title:		**Microscopic examination of thick and thin blood film for identification of malaria parasite**			
SOP No:	**SOP-NMRL-09**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This SOP describe the procedure for correct detection and identification of malaria parasites in Giemsa-stained blood films by light microscopy

2. Background

Identification of the species and stages of malaria parasites and determination of their density is crucial in clinical management of malaria patients, drug efficacy trials, malaria epidemiological surveys and control programmes. Therefore, malaria diagnoses based on examination of blood films must be correct, with an accurate parasite count. Examination of blood films also allows detection of several blood pathogens, morphological diagnosis of anemia and identification of several hematological disorders, which must be reported by the microscopist.

3. Application

The SOP applies to all Laboratory Technicians doing Malaria Microscopy.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. Malaria register

5.2. WHO Bench aids for malaria diagnosis

5.3. WHO Basic malaria microscopy- part I learner's guide. Second edition, Geneva 2010

6. Sample requirement

6.1. Equipment

6.1.1. Compound microscope

6.2. Sample

6.2.1. Giemsa-stained slides to be examined

6.3. Lab consumables

6.3.1. Immersion oil

6.3.2. Lens cleaning paper

6.3.3. Tissue paper

6.3.4. Pen

7. Precautions

7.1. Methanol (methyl alcohol) is inflammable and highly toxic if inhaled or swallowed; it can cause blindness and even death if swallowed in any quantity. Avoid contact and inhalation. When it is not in use, it should be stored in a locked cupboard.

7.2. Universal precautions – including use of relevant personal protective equipment such as gloves, safety glasses and a laboratory coat or gown – must be practised.

8. Procedure

8.1. Examining the thick film

8.1.1. Place the Giemsa-stained blood film to be examined on the microscope stage, with the label to the left. Position the thick film in line with the 10x objective lens.

8.1.2. Switch on the microscope, adjust the light source optimally and find the focus by looking through the ocular and the 10x objective.

8.1.3. 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.

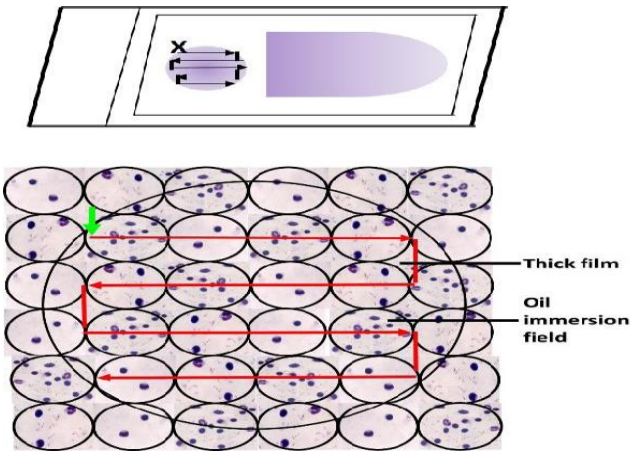
8.1.4. Place a small drop of immersion oil on the thick film. Do not allow the 40x objective to touch the oil.

8.1.5. Switch the 100x oil immersion objective over the selected portion of the thick film. Use the fine focus adjustment to see the image clearly.

8.1.6. Using the fine adjustment, focus on the cell elements, and confirm that 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.

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

8.1.8. When the other end of the film is reached, move the slide slightly downwards, then to the left, field by field, and so forth (see below). For efficient examination, continuously focus and refocus with the fine adjustment throughout examination of each field.



Examining a thick blood film

8.2. Determining whether a thick film contains malaria parasite and identifying the species

8.2.1. A minimum of 100 high-power fields must be examined before a thick film can be declared as having “no malaria parasites seen”. If possible, the whole thick film should be scanned.

8.2.2. If parasites are observed, a further 100 fields must be examined before final identification of the species, ensuring that a mixed infection is not overlooked.

8.2.3. The thin blood film should always be examined to identify parasite species definitively. The thin film allows visualization of parasite and red cell morphology, unlike the thick film. Perform an examination at the feathery end or edge of the thin film, as described in procedure 4.3 below.

8.2.4. Identify and record all species and stages observed in the malaria microscopy blood register.

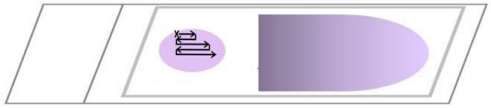
8.3. Examining the thin film to confirm species and mixed infections

8.3.1. To confirm the parasite species or mixed infections after examining the thick film, examine the thin film.

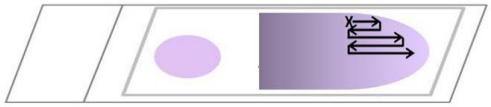
8.3.2. Place a drop of immersion oil on the feathered edge of the thin film.

8.3.3. Move from the 10x lens to the 100x oil immersion lens.

8.3.4. Examine the feathery end or edge of the thin film where the red cells lay side by side and there is minimal overlap. Follow the pattern of movement shown in Fig. 2. Move along the edge of the film, then move the slide outwards by one field, inwards by one field, returning in a lateral movement and so on.



a. Diagram for examining the thick film



b. Diagram for examining the thin film

Examining a thin film

8.3.5. Continue examining the thin film until the presence and species of malaria parasites have been confirmed. Identify and record all species and stages observed in the malaria microscopy blood register.

9. Quality Control

9.1. Always run the quality control on every new batch of the giemsa stain stock is used or whenever required in between.

10. Maintenance

10.1. NA

11. Annexure

11.1. WHO template for preparation of thick and thin blood film

12. Reference

12.1. WHO Standard Operating Procedure for Malaria microscopy.

12.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

National Malaria Reference laboratory

SOP for Malaria Microscopy

SOP title:		**Malaria Parasite Counting**			
SOP No:	**SOP-NMRL-11**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This SOP describe the procedure for counting malaria parasites on thick and thin blood films.

2. Background

The parasite density provides information on the severity of infection and on the response to treatment. Parasite counts are performed for P. falciparum, P. vivax, P. malariae and P. ovale asexual stages. Unless the protocol dictates otherwise, gametocytes are not counted, but their presence is always reported. All identified parasite species should be reported, even if they are counted together. Most parasite counts are performed on thick blood films. If there is a no thick film or it is damaged, a thin film count is performed. A thin film count is also performed when there are > 100 parasites in each field of the thick film, which corresponds to > 80 000 parasites/ μ L.

3. Application

The SOP applies to all Laboratory Technicians doing Malaria Microscopy.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. Malaria register

5.2. WHO Bench aids for malaria diagnosis

5.3. WHO Basic malaria microscopy- part I learner's guide. Second edition, Geneva 2010

6. Sample requirement

6.1. Equipment

6.1.1. Compound microscope

6.1.2. 2 keys tally counter

6.1.3. Calculator

6.2. Sample

6.2.1. Giemsa-stained slides to be examined

6.3. Lab consumables

6.3.1. Immersion oil

6.3.5. Lens cleaning paper

6.3.6. Tissue paper

6.3.7. Pen

7. Precautions

7.1. NA

8. Procedure

8.1. Performing a parasite count on a thick film and determining parasite density

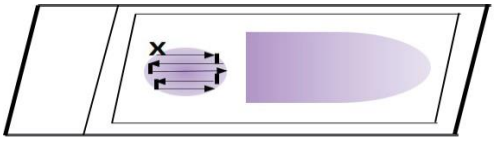
8.1.1. Place the glass slide on the microscope stage with the label to the left. This allows a standardized approach for the start point for counting.

8.1.2. If malaria parasites are present, count asexual forms (in either single or mixed species infections) without sexual (gametocyte) forms, which are not counted but just reported. In mixed infections, all asexual parasites are counted together, and the presence of multiple species is reported

8.1.3. Starting at the top most left part of the film, look for a field with a good number of white cells and parasites are observed together and start counting.

8.1.4. Using tally counter, count parasites and white cells simultaneously by clicking on the assigned key as parasites or white cells are observed. If two tally counters are being used use one for the WBCs and the other for parasites.

8.1.5. After counting all the parasites and white cells in one fi eld, move to the next fi eld following the pattern of movement shown in Figure 1 and repeat the same counting procedure and so on. Be careful not to overlap fields.



Pattern of movement for counting parasite and white blood cells

8.1.6. Decide when to stop counting

- If you have counted **≥ 100 parasites in 200 white cells**, stop counting, and record the results as the number of parasites per 200 white cells.
- If you have counted **≤ 99 parasites in 200 white cells**, further count parasite in **500 white cells** and record the results as the number of parasites per 500 white cells.

8.1.7. Count all parasites and white cells in the final fi eld, even if the white cell count exceeds 200 or 500.

8.1.8. Record the actual numbers of parasites and white cells counted on an appropriate worksheet.

8.1.9. When counting is completed, calculate the parasite density on the basis of the patient's actual white cell count. If this is not available, use an estimated average white cell count of 8000/µL.

8.1.10. Use the following formula for the calculation:

$$\text{Parasites / µL blood} = \frac{\text{Number of parasites counted} \times \text{8000 white cells/µL}}{\text{No. of white cells counted}}$$

8.2. Performing a parasite count on the thin film and determining parasite density

8.2.1. If infected red cells are present, count all parasitized red cells.

8.2.2. In the top section of the thin film, locate a field with about 250 red cells. Count the total number of red cells in that field and the number of parasitized red cells. A typical field (at 100x magnification) should contain approximately 250 red cells.

8.2.3. Using a tally counter, count parasitized and other red cells by clicking the assigned keys for parasitized and non-parasitized red cells.

8.2.4. After counting all the parasites and red blood cells in one field, move to the next field, following the pattern of movement shown in Fig. 1, and repeat the counting procedure in each field.

8.2.5. Stop counting when about 20 fields with about 250 red cells (about 5000 red cells) have been counted. Record the actual numbers of parasitized and other red cells counted on an appropriate worksheet. Use these figures to calculate the total parasite count per µL of blood.

8.2.6. When counting is completed, calculate the parasite density from the patient's actual red cell count. If this is not available, use an estimated average red cell count of

5,000,000/µL and the following formula. Note that the final result is rounded to the nearest whole number.

8.2.7. Use the following formula for the calculation:

$$\text{Parasites / µL blood} = \frac{\text{Number of parasitized red cells} \times 5{,}000{,}000}{\text{No. of non-parasitized red cells counted}}$$

9. Quality Control

9.1. Always run the quality control on every new batch of the giemsa stain stock is used or whenever required in between.

10. Maintenance

10.1. NA

11. Annexure

11.1. WHO template for preparation of thick and thin blood film

12. Reference

12.1. WHO Standard Operating Procedure for Malaria microscopy.

12.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

National Malaria Reference laboratory

SOP for Malaria Microscopy

SOP title:		**General Safety procedure in Malaria Microscopy**			
SOP No:	**SOP-NMRL-12**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This SOP describe the procedure for proper recording and reporting of the results of microscopic examination of blood films for malaria diagnosis

2. Background

Proper recording and reporting of the results of microscopy examination of blood films is very important for the clinical management of malaria patients and for the reliability of malaria surveillance data, which are the basis for monitoring, evaluating and planning programme interventions.

3. Application

The SOP applies to all Laboratory Technicians doing Malaria Microscopy.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. Malaria register

6. Sample requirement

6.1. Lab consumables & others

6.1.1. 70% isopropyl alcohol

6.1.2. Disposable latex gloves

6.1.3. Soap

6.1.4. Sharp container

6.1.5. Non-infectious waste container

7. Precautions

7.2. NA

8. Procedure

8.1. Laboratory area

8.1.1. Laboratory should be designated and provide area label such as

- Smear preparation area
- Drying area
- Staining area
- Mounting area
- Microscope area
- Clean area – for paperwork

8.1.2. Provide a clean, tidy, dust-free area with enough space for at least two laboratory technicians or staff working side-by-side.

8.1.3. Clean or decontaminate work surfaces with 10% bleach (sodium hypochlorite), isopropyl alcohol or 70% ethyl alcohol immediately after a spill and in any case at the end of the working day.

8.1.4. Label all reagents and stains to be used properly, with the name, date prepared or opened and expiry date, if applicable.

8.1.5. Clean all glassware and other materials for re-use, such as staining containers, with detergent, rinse with water.

8.1.6. Keep all supplies and materials in designated drawers or boxes, properly labelled on the outside, in areas free from dust, dirt and insects.

8.1.7. Store hazardous chemicals such as methanol in a locked cupboard when not in use.

8.2. Personal protection

8.2.1. All employees who handle blood samples should be vaccinated according to national policy (e.g. against hepatitis B).

8.2.2. Use disposable latex gloves when handling human blood, whether in the laboratory or in the field.

8.2.3. Wear gloves for all procedures that may involve accidental, direct contact with blood or infectious material. Discard contaminated or perforated gloves.

8.2.4. Wear a laboratory gown when working inside the laboratory and remove it before leaving and when outside the laboratory.

8.2.5. Wear safety glasses.

8.2.6. Cover skin lesions such as cuts, abrasions, ulcers and dermatitis with a waterproof dressing or band aid before putting on gloves.

8.2.7. Do not store medicines and food items in the laboratory, and do not eat or drink in laboratory work areas.

8.2.8. Use of mobile phone should not be allowed in the laboratory- infectious area

8.2.9. Remove gloves when using a personal computer or a phone in the laboratory.

8.2.10. Practice proper hand-washing under running water and use a skin disinfectant, antibacterial liquid soap or 70% alcohol before and after work and before leaving the laboratory.

8.2.11. Read the "material safety data sheet" for each reagent used.

8.3. Safety during blood collection and handling

8.3.1. Use only disposable lancets, hypodermic needles and syringes. Never re-use them.

8.3.2. Use micropipettes properly, i.e. never pipette by mouth; use mechanical pipetting devices.

8.3.3. Open hypodermic needles just before use and handle them carefully. After use, do not recap, clip or remove needles from disposable syringes. Dispose of the complete assembly in a dedicated sharps waste container.

8.3.4. While handling blood samples, be careful to avoid producing bubbles and aerosols.

8.4. Management of spills and accidental exposure to potentially infectious blood specimens

8.4.1. All first aid procedures should be displayed in the laboratory for ready reference and should provide clear instructions for the management of spills and accidental exposure.

8.4.2. Report any of the injuries and accidents listed below as soon as possible to the laboratory's designated infection control officer or to the laboratory manager, head or person in charge:

- Spills or accidents involving potentially infectious specimens (e.g. blood, serum, plasma);
- any blood spill in the eye or on broken skin, cuts with broken glass or punctures with needles or syringes; and
- any pathological symptoms occurring after an accident.

8.4.3. Any blood spill should be immediately covered with a paper towel to absorb the spill, and then 5 g/L hypochlorite solution or 70% ethanol should be poured carefully over the towel

8.4.4. When intact skin is broken, cut, damaged or punctured by a contaminated needle, remove contaminated clothing, and immediately wash the area vigorously for 15 min with soap and water.

8.4.5. When the eyes are splashed with blood, immediately flush the eyes thoroughly with water for at least 15 min.

8.4.6. Refer to the standard safety procedures for immediate advice.

8.4.7. Seek medical advice if necessary.

9. Quality Control

9.1. NA

10. Maintenance

10.1. NA

11. Annexure

11.1. WHO template for preparation of thick and thin blood film

12. Reference

12.1. WHO Standard Operating Procedure for Malaria microscopy.

12.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

	National Malaria Reference laboratory **SOP for Malaria Microscopy**				
SOP title:		**Recording and Reporting Malaria Microscopy**			
SOP No:	**SOP-NMRL-13**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This SOP describe the procedure for proper recording and reporting of the results of microscopic examination of blood films for malaria diagnosis

2. Background

Proper recording and reporting of the results of microscopy examination of blood films is very important for the clinical management of malaria patients and for the reliability of malaria surveillance data, which are the basis for monitoring, evaluating and planning programme interventions.

3. Application

The SOP applies to all Laboratory Technicians doing Malaria Microscopy.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. Malaria register

5.2. Patient result form

6. Sample requirement

6.1. Equipment

6.1.1. Compound microscope

6.2. Sample

6.2.1. Giemsa stained slide

6.3. Lab consumables & others

6.3.1. 70% isopropyl alcohol

6.3.2. Lens cleaning paper

6.3.3. Tissue paper

6.3.4. Oil immersion

6.3.5. Pen or pencil

6.3.6. Calculator

7. Precautions

7.1. NA

8. Procedure

8.1. After microscopic examination according to MM-SOPs 08 and 09, the results should be recorded and reported.

8.2. Record all malaria species and stages observed during microscopic examination of the blood films as follows

8.2.1. P. falciparum- Pf

8.2.2. P. vivax - Pv

8.2.3. P malariae - Pm

8.2.4. P ovale - Po

8.2.5. P. falciparum + P. vivax (Mixed) – FV

8.2.6. Trophozoite – T

8.2.7. Schizont – S

8.2.8. Gametocyte – G

8.2.9. No malaria parasite seen – Nmps

8.3. When counting on the thick film is completed, if the patient's actual white cell count is not available, calculate the parasite density from an estimated white cell count of 8000/µL, as follows:

$$\textbf{Parasites / µL blood} = \frac{\textbf{Number of parasites counted x 8000 white cells/µL}}{\textbf{No. of white cells counted}}$$

Example 1:

Plasmodium falciparum trophozoites counted = 155 White cells counted relative to parasites = 208

Parasite count: $\frac{155 \times 8000}{208}$ = 5962 parasites/µL blood

Report as: *P. falciparum* trophozoites = 5962 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: $\frac{88 \times 6500}{505}$ = 1133 parasites/µL blood

Report as: *P. vivax* trophozoites = 1133 parasites/µL blood

8.4. In mixed infections or infections by more than one species, count all the species together (sexual and asexual stages), and express the results as in example 3.

Example 3:

P. falciparum gametocytes + *P. vivax* trophozoites = 360 parasites (all stages) counted in 202 white cells

Report as: *P. falciparum* gametocytes + *P. vivax* trophozoites = 14 257 parasites/µL blood

8.5. Also report the presence of:

8.5.1. Gametocytes. Gametocytes of P. falciparum are counted separately, but they are still included in the general parasite count. It is rarely possible to separate the gametocytes of P. vivax or P. malariae from asexual parasites with sufficient accuracy to justify a gametocyte count.

8.5.2. Schizonts, as they might be an indication of disease severity.

8.6. Record in the microscopy section of the laboratory register the patient identification number, the date and time of examination and parasite species, stages and count if performed. The reporting should be uniform. For example: P. vivax trophozoites seen.

8.6.1. P. falciparum trophozoites seen; count, 42 000 parasites/µL.

8.6.2. P. falciparum gametocytes seen.

8.7. No malaria parasites seen. This phrase should be used rather than “Negative”.

9. Quality Control

9.1. NA

10. Maintenance

10.1. NA

11. Annexure

11.1. NA

12. Reference

12.1. WHO Standard Operating Procedure for Malaria microscopy.

12.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

National Malaria Reference laboratory

SOP for Malaria Microscopy

SOP title:		**Corrective Action**			
SOP No:	**SOP-NMRL-14**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

To provide guidance in the process of identifying, evaluating, recording, investigation, correcting the cause of and determining the disposition of nonconforming process, services and work products. The corrective action involves written and retrievable record of action taken and follow up monitoring to determine that corrective action have been performed, documented and found to be effective.

2. Background

Nonconformance can occur at various places within the quality system and technical operations. Example include customer complaints, unacceptable quality control samples, instrument and sample problems, environment problems that affect results, purchased material for laboratory use, staff observation, management review and audits. roper contents of reagent ingredient in the solution and standard for their preparation is must to achieve reliable and accurate results.

Processes, services and product considered to be nonconforming may be identified in the following ways:

- Incoming product from suppliers
- Services provided by external sources
- Processes producing unacceptable results or products
- Internal or external quality audits

3. Application

The SOP applies to concerned staff of NMRL work product and processes. This procedure directly concerns the laboratory's quality assurance program.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

4.1. Laboratory unit incharge / Focal:

- Take action to control and correct nonconformance when they occur.

- Ensure that corrective actions are performed, implemented and communicated
- Review corrective actions that have been taken and approve or recommend if further corrective actions are needed
- Complete appropriate sections of nonconformance and corrective action record in the quality management information system.
- Monitor and facilitates the corrective action process

4.2. Staff:

- Initiate or participate in the completion of corrective actions
- Complete appropriate sections of the nonconformance and corrective action records in system

5. Associated Documents

5.1. Worksheet for reagent preparation and to record their QC findings

5.2. Reagent inventory

5.3. Equipment inventory

6. Precautions

6.1. NA

7. Procedure

7.1. Review of nonconformity

- When a nonconformity occurs, the laboratory must take action to control and correct it with actions appropriate to the effects of the nonconformities encountered.
- A review of the consequences of the nonconformity is performed to determine if a corrective action is warranted.

7.2. Correction:

- If minor nonconformance is detected where a product was not affected nut absolute compliance to a statement of intent or clause of a standard was not met on basis of objective evidence, it can be recorded as a correction only with rectification actions recorded and closed.
- An obvious trend in a repeated minor nonconformance can escalate it to a corrective action.

7.3. Root cause & corrective action:

- Once a nonconformance that impacts a lab's product, processes, or service is detected an evaluation of the need for action to eliminate the cause(s) will be performed.
- The laboratory determines if similar nonconformities exist or could potentially occur.
- An investigation to determine the root cause(s) of the problem will be initiated to determine an effective corrective action.
- Often the root cause is not obvious, therefore careful analysis of all potential causes of the problem is required. Areas to investigate can include:
    - Customer requirements
    - The samples or sample specifications
    - Methods and/or procedures
    - Staff skills and training
    - Consumables and/or vendors used
    - Equipment and its calibration
- Once the root cause has been determined potential corrective actions shall be identified.
    - Decide what can be done to prevent the problem from recurring.
    - Determine how the solution will be implemented.
    - Define who will be responsible for implementation.
    - Evaluate the risks of implementing the solution.
- The corrective action(s) most likely to eliminate the problem and to prevent a recurrence shall be selected for implementation.
- Changes required as a result of the investigation shall be recorded and implemented (i.e. procedure revisions, training, resumption of work where it was stopped due to the nonconformance, etc.).
- The QSM closes the corrective action when there is objective evidence that the actions are completed and effective.

7.4. Monitoring for effectiveness

- Corrective actions that are implemented must be monitored to determine if they are and/or continue to be effective.
- The QSM can keep a completed action report open for a specified time to monitor effectiveness, and then close the issue once it has been determined to be effective.

- Once an action report has been closed its effectiveness can still be determined with an audit in the area affected by the original nonconformance.
- In the event a corrective action is found to be ineffective a new nonconformance report will be initiated with a different root cause investigation to determine why the first corrective action was not effective, if the true root cause was determined, and to evaluate and identify the best corrective action to implement and record.
- This additional corrective action must also undergo monitoring to determine its effectiveness

7.5. **Recording corrective actions**

- Record nonconformance, investigation, correction, corrective action information in Lab QMS and according to local laboratory procedures.

8. Quality control

8.1. Record the incident findings in the incidence form

8.2. Record the corrective action into CAPA form

9. Limitation

NA

10. Annexure

10.1. Incident form

10.2. Corrective action form

11. Reference

11.1. WHO Standard Operating Procedure for Malaria microscopy.

11.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

National Malaria Reference laboratory

SOP for Malaria Microscopy

SOP title:		**QC for Giemsa stain and Buffered Water**			
SOP No:	**SOP-NMRL-15**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

To describe the procedure for quality control (QC) assessment of stock solutions of Giemsa stain and of buffered water (pH 7.2) for routine staining of malaria blood films. This SOP is applicable to both in-house preparations and commercially available reagents

2. Background

Monitoring of the performance of reagents is known as “quality control” (QC). In order to make an accurate diagnosis of malaria, it is essential that blood films be stained with good-quality preparations of Giemsa stock solution and buffered water at pH 7.2. These solutions should be tested before use, and, as good practice, a QC check must be performed:

- for every new batch or lot of stock solution prepared.
- before sending a stock solution to laboratories for use;
- In the field, after receiving a stock solution from a national reference laboratory.
- before using a stock solution to prepare a working solution of Giemsa stain and
- for every new batch of water buffered to pH 7.2 prepared.

The quality of the stain and buffered water should be checked on a thin blood film known to be positive for malaria. Blood films with Plasmodium vivax parasites are preferable to demonstrate the characteristic Schüffner dots. If these are not available, use a P. falciparum-positive blood film and examine it for Maurer clefts (if mature trophozoites are present). If a positive malaria blood film is not available, a negative blood film could be used to examine the colour and staining of red and white blood cells.

A supply of thin blood films could be prepared when fresh blood is available and stored at –20 °C or colder. The procedure is described in this SOP. Unstained films stored at room temperature will deteriorate over time.

3. Application

The SOP applies to all laboratory personnel of National Malaria Reference Laboratory (NMRL) performing malaria microscopy.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. IQC worksheet for quality control

6. Sample requirement

6.1. Equipment

- Analytical balance
- Distillation plant
- Electromagnetic stirrer

6.2. Reagent & chemical

- Giemsa powder- preferably biological stain commission grade
- Absolute methanol, pure, high- grade, acetone free
- Glycerol, high grade, pure

6.3. Lab Consumables

- Filter paper, 11 cm in diameter
- Conical flask, 1 L capacity
- Glass beaker, 250 ml capacity
- Measuring cylinder, 1000 ml capacity
- Spatula
- Container, 5 L capacity- to store distilled water
- Glass bottle, 250 ml capacity- to store Giemsa stock solution- amber color preferably
- Funnel
- Labeling paper
- Filter paper
- Slide box
- Desiccant / Silica gel.
- Plastic Ziplock bags

7. Precautions

7.1. Clean and dry glassware should be used while preparing the regent.

7.2. Used sterile distilled water to prepared quality reagent.

7.3. Always wash your hand before and after completing work.

7.4. Process sample with minimum PPEs: wear lab coat, powder free disposable gloves and protective goggle where necessary.

7.5. Store the Giemsa stock and buffered water in tightly stoppered, dark bottles in a cool place away from direct sunlight.

7.6. Filter the small amount of Giemsa stain that will be used as a working solution and not the whole bottle.

7.7. Do not return unused stain to the stock bottle or to the bottle used in daily routine. Once stain is out of the bottle, it must be used quickly or discarded.

7.8. If buffered water tablets are used and the expected result is not obtained, the pH should be checked and adjusted, if there is the facility to do so. Otherwise, the buffer should be discarded and a fresh solution made. If the second buffered water solution fails the QC check, contact the national reference laboratory for further investigation. Use a different batch of tablets, if available.

7.9. Check the pH of the buffered water at regular intervals.

7.10. If you notice deterioration in staining, check the stocks of Giemsa stain and buffered water.

7.11. Macroscopically, the blood film should look bluish–grey. A pinkish red colour is a sign of poor staining (too acid).

7.12. When staining time is being optimized, a number of slides could be stained simultaneously but for different times. For example, with 10% stain, three slides could be stained together for 8, 9 and 10 min, and the staining time that gives the best results should be chosen for that batch of Giemsa stain stock.

8. Procedure

8.1. **Quality control of giemsa stock / 7.2 Buffered water:**

- Prepare a blood film (Thick and thin smears) of blood known to be positive for malaria, ideally containing P. vivax or P. ovale – if available or prepare blood film from normal blood sample.
- Prepare 3% and 10% working solution with the new buffered water stock, as described in SOP- Preparation of giemsa working solution at pH 7.2.

- Fix the slides with methanol and allow them to dry. Stain the slides according to SOP-Giemsa staining of malaria blood film for both rapid and slow stain method.
- Examine slides to determine the quality of staining at different time interval such as 8,9,10,11,12 minutes. (Show fig below)
- Record the results, observations and actions in the QC log book. Record the name of the staff who performed QC.

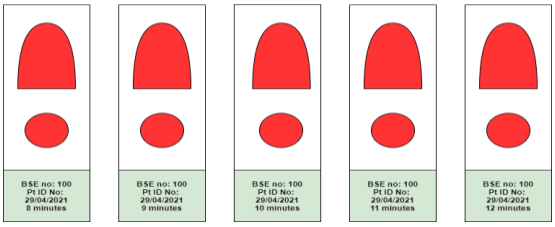


Fig 1: IQC panel to check QC of giemsa / buffered water

8.2. **Expected Results:**

- Nucleus: Red to pink
- Cytoplasm: blue
- Schüffner dots (P. vivax): Even carpet of pink dots in the cytoplasm of the rbcs
- Maurer clefts (P. falciparum): unevenly distributed, course bodies in red cell cytoplasm.
- Red blood cell: Pinkish grey
- Platelets: Deep pink
- White blood cell:
- Lymphocyte, neutrophils and monocytes: Purple-blue nucleus and pale cytoplasm.
- Eosinophils: Course, bright purple-red granules in the cytoplasm
- Neutrophils: finer, purple granules
- Basophilic stippling: blue
- Blood film too dark or too pale: corrected by adjusting the staining time. Pale: longer time required.

8.3. **Preparation of malaria -positive blood films for QC:**

- Using clean, washed slides, prepare thick and thin blood film (See SOP-Preparation of blood film) from positive sample whenever positive detected. Allow to dry in air
- Fix the thin smear with methanol and allow to dry in air.

- Pack the slides tightly in a slides box front to back and label with the species and stages of parasites, the number of slides, date of preparation and the name of the staff who prepared them.
- Place the box of slides into a plastic box or Ziplock bag containing silica gel.
- Store at -20*C or colder, ideally at -70*C. Record the results in the QC log book.
- Remove slides as needed and allow them to thaw or come to room temperature.

8.4. **Checking and adjusting the pH of buffered water:**

- Check the pH of prepared buffered water is 7.2 or not.
- Pour some of the buffer water to be tested into beaker.
- Place pH meter into the solution, check the reading of pH value of the given solution.
- To adjust the solution of too alkaline – means pH is above 7.2, add small quantity of 2% KH_2PO_4 correcting fluid and check the pH of the solution.
- To adjust the solution of too acidic – means pH is below 7.2, add small quantity of 2% Na_2HPO_4 correcting fluid and check the pH of the solution.

9. Quality control

10.1. Perform a quality control check:

- For every new batch or lot of stock solution prepared
- Before using it as a working solution during staining
- Before sending it to laboratories for use and
- In the field, after receiving it form a national reference laboratory

10.2. Record the findings in the worksheet.

10. Limitation

NA

11. Annexure

11.1. Worksheet for IQC record

12. Reference

12.1. WHO Standard Operating Procedure for Malaria microscopy.

12.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

12.3. WHO Basic malaria microscopy. Part I. Learner's guide. Second Edition. Geneva; 2010.

National Malaria Reference laboratory

SOP for Malaria Microscopy

SOP title:		**Malaria Blinded Rechecking**			
SOP No:	**SOP-NMRL-16**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This Standard Operating Procedure (SOP) describes the procedure for regular blinded rechecking of malaria slides receive form participating laboratory (PL) for assessing the competency of malaria microscopists regarding malaria parasite speciation, stage identification, parasite counting, quality of blood film prepared and quality of giemsa stain.

2. Background

Blinded rechecking of malaria slides plays a vital role in monitoring the accuracy of diagnosis to ensure that high quality microscopy services are provided and maintained by the PL in their health centers. It is very important to pre-qualify the microscopists both in qualitative assessment (Species identification) and quantitative assessment (parasite density) in order to ensure accurate microscopy, include multiple blinded readings and averaging of counts between readers.

3. Application

The SOP applies to all laboratory personnel of National Malaria Reference Laboratory (NMRL) performing blinded rechecking of malaria slides received from PL

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. Worksheet to record the finding while cross checking the slides

5.2. Daily microscope maintenance log

6. Sample requirement

6.1. **Sample:**

- 10% of negative malaria slides

- All positive slides

6.2. **Equipment**

- Microscope
- Double key Talley counter

6.3. **Lab Consumables**

- Immersion oil
- Lens cleaning paper
- Tissue paper
- Slide box

7. Precautions

7.1. Selection of slides for blinded rechecking should be done from register not directly select from the slide box.

7.2. All the positive slides must be sent for blinded rechecking for concordance agreements.

7.3. All the slides to be sent for blinded rechecking should be free of oil immersion- clean the oil from the slide before shipping the slide to NMRL

8. Procedure

8.1. **Slide registration**

- Slide received form PL should registered in the sample reception form. (See Annexure No:)
- Check whether the total slides volume received is matching in the requesting QA form A. (See Annexure no:)

8.2. **Blinded rechecking of slide by Controller 1:**

- Controller 1 must examine the slides within 2 weeks after receiving the slide from the health centers
- Record all the finding in the cross-checking worksheet (See Annexure no:)
- If positive slide detected, controller 1 must perform his/ her examination including parasite counting independently and should handover to controller 2 for further validation.
- Controller 2 also examined the slide maintaining the confidential and get cross check with the result of controller 1 and parasite count ranged is determined (% discrepancy should be less than 20 %) to check concordance with the district microscopist.
- Archive positive slide if detected in the designated slide box.

- Clean the microscope optic and maintained records in the daily record sheet on their usage.

8.3. **Validation and preparation of feedback report:**

- The controller 2 must validation and analyzed the results and determines the % agreement between the microscopist and the validator using the analysis tables and tool (Excel?).
- 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 (Annexure no). The validator must provide feedback to the microscopy center specifying the level of accuracy, smear and staining quality including recommendations.

8.4. **Sending of report to the health centers**

- For the health centers, 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 3 weeks after receiving the slides.
- All validation reports must be signed by the validator and head of the NMRL.
- All validation reports must be summarized every quarter and submitted to the relevant agencies.

8.5. **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 for rapid correction. A supervisory visit must be scheduled immediately.

9. Quality control

9.1. Record the findings in the cross-checking worksheet form.

9.2. Filing of form in their respective file.

10. Limitation

10.1. NA

11. Annexure

11.1. Malaria slide reception and registration form

11.2. Malaria blinded rechecking form A (Confidential)

11.3. Malaria blinded rechecking form B

11.4. Worksheet for blinded rechecking

11.5. Daily microscope maintenance log

12. Reference

12.1. WHO Standard Operating Procedure for Malaria microscopy.

12.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

		National Malaria Reference laboratory **SOP for Malaria Microscopy**			
SOP title:		**On-site Monitoring and Supervisory visit to microscopy centers**			
SOP No:	**SOP-NMRL-17**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This Standard Operating Procedure (SOP) describes the procedure on how provide guidelines for technical supervision of Laboratory Technicians doing malaria microscopy.

5. Background

Supervisory visits are likely to be the most effective form of supervision of a programme and provide an opportunity to correct poor performance identified by proficiency testing and quality assurance activities.

6. Application

The SOP applies to all laboratory personnel of National Malaria Reference Laboratory (NMRL) doing technical supervision of laboratory technicians on malaria microscopy and QA/QC

7. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

8. Associated Documents

8.1. On-site supervisory checklist on lab performance

8.2. On-site supervisory checklist on lab staff performance

9. Sample requirement

9.1. **Sample:**

- Reference slides consists of positive and negative malarial slides

9.2. **Equipment**

- Microscope
- Double key Talley counter
- Calculator

9.3. **Lab Consumables**

- Immersion oil
- Lens cleaning paper
- Tissue paper
- Slide box

10.Precautions

10.1. NA

11.Procedure

11.1. **Planning**

- Set schedule (date, site/area to visit, person responsible)
- Send communication

11.2. **Conduct visit**

- Courtesy call to head of facility
- Accomplish checklist (QA Form 3)
- Interview concern Laboratory technician / malaria technician
- Conduct inspection of laboratory set-up, laboratory supplies/materials, reagents, equipment, documentation/logbook
- Evaluate performance of laboratory technician / malaria technician in malaria microscopy
- Write findings and recommendations
- Discuss findings, comments and recommendations with the laboratory technician and head of facility.
- Consolidate report(s) of monitoring/supervisory visit conducted
- Submission of copies of all onsite supervisory check lists (Form 3a) and consolidated reports (Form 3b) to Concern health centers head and related agencies
- Update database on QAQC

11.3. **On-site examination of reference slides**

- Participant should examine thick and thin blood smears
- Different parasite species in their different stages were asked to identified.

12.Quality control

12.1. Record the findings in the cross-checking worksheet form.

12.2. Filing of form in their respective file.

13. Limitation

13.1. NA

14. Annexure

14.1. Malaria slide reception and registration form

14.2. Malaria blinded rechecking form A (Confidential)

14.3. Malaria blinded rechecking form B

14.4. Worksheet for blinded rechecking

14.5. Daily microscope maintenance log

15. Reference

15.1. WHO Standard Operating Procedure for Malaria microscopy.

15.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

National Malaria Reference laboratory

SOP for Malaria Microscopy

SOP title:		**Malaria Panel Testing**			
SOP No:	**SOP-NMRL-18**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This Standard Operating Procedure (SOP) describe the procedure of conducting the proficiency testing to participating laboratories performing malaria diagnosis.

2. Background

The main objective of the assessment is to assess/confirm the proficiency of laboratory technicians / malaria technician on microscopical examination of malaria (specifically in species identification, stages identification and parasite counting).

3. Application

The SOP applies to proficiency assessment on microscopical examination of malaria conducted by the National Malaria reference Laboratory (NMRL).

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. MP Panel Testing reporting form

5.2. MP Panel testing feedback form

6. Sample requirement

8.1. **Sample:**

- Reference slides consists of positive and negative malarial slides

8.2. **Equipment**

- Microscope
- Double key Talley counter
- Calculator

8.3. **Lab Consumables**

- Immersion oil
- Lens cleaning paper
- Tissue paper
- Slide box

9. Precautions

9.1. NA

10.Procedure

10.1. **Slides selection**

- Total of 5 giemsa stained slides consists of both positive and negatives slides
- MP panel testing reporting form is also sent along with slides

10.2. **Slides examination**

- Every microscopist must examine the slides by maintaining proficiency by not disclosing their results within the staff.
- Send their examine results within the time frame- 1 month upon reception of slides by participating laboratory.
- After examining all slides by staff, participating laboratory must sent their panel slide back to reference laboratory.

10.3. **Slides examination**

- Evaluate performance of laboratory technician / malaria technician microscopy report
- Write findings and recommendations and sent provisional report to each health centers
- Submission of copies panel testing reports to Concern health centers head and related agencies
- Update database on QAQC

11. Quality control

11.1. Record the findings in the database

11.2. Filing of report form in their respective file- common file

12. Limitation

12.1. NA

13. Annexure

13.1. Malaria panel testing reporting form

14. Reference

14.1. WHO Standard Operating Procedure for Malaria microscopy.

14.2. VDCP Standard Operating Procedure for malaria microscopy- first edition

National Malaria Reference laboratory SOP for Malaria Molecular Testing (nPCR)					
SOP title:		**Preparation of Dried Blood Spot (DBS) Sample**			
SOP No:	**SOP-NMRL- 19**	**Revision No:**		**Effective date**	
Prepared by:				**Date:**	
Approved by:				**Date:**	

1. Purpose

This SOP describes procedure on how to prepare and transport of prepared (Dried Blood Spot) for molecular analysis.

2. Background

The collection and transportation of a high-quality sample is critical in assuring accurate test results. DBS can be prepared either directly from capillary blood collected from the finger of a patient or can be prepared from blood collected in EDTA tube. The blood is eluted in the appropriate filter paper and when stored correctly it can be preserved malaria DNA for longer period of time for PCR analysis for parasite spices identification and genetic diversity.

3. Application

The SOP applies to all laboratory personnel while collection and preparation of malaria DBS sample to be used for molecular purposes.

4. Responsibilities

It is the responsibility of authorized lab personnel to read and perform the procedure as per the SOPs.

5. Associated Documents

5.1. DBS requisition form

5.2. DBS line listing form

6. Sample requirement

6.1. **Sample:**

6.1.1. Whole blood collected in EDTA or capillary blood

6.2. **Reagent & Chemical**

6.2.1. Hand rub / 70% alcohol

6.3. **Lab Consumables**

6.3.1. FTA card or Whatman filter paper no 3

6.3.2. Zip-lock plastic bags

6.3.3. Disposable gloves

6.3.4. Sharp container

6.3.5. Desiccant / Silica gel

6.3.6. Disposable pasture pipette or micro pipette

6.3.7. Bio-hazard bag- Red

7. Precautions

7.1. Always collect DBS sample wearing gloves to avoid contamination of FTA card

8. Procedure

8.1. **(Collection of DBS by Finger Prick Method)**

8.1.1. Label the FTA Card / Whatman filter paper provided with patient name, age, sex and identification number.

8.1.2. Place FTA card / whatman filter paper on the clean area

8.1.3. Examine the patient's fingers to identify the best location for the finger stick:

8.1.4. Position the patient (lying down or sitting) and hyperextend the patient's arm.

NB. Ensure the patient's arm is kept to low in order to maintain blood flow.

8.1.5. Massage the finger to increase blood flow by gently squeezing from hand to fingertip 5-6 times.

NB. Excessive squeezing can cause concentration of tissue fluids which can adversely affect test results.

8.1.6. Clean the fingertip using an 70% alcohol and allow to air dry.

8.1.7. Remove the sterile lancet from its packaging, grasping it between thumb and forefinger.

8.1.8. Firmly press the lancet to make a skin puncture just off the centre of the finger pad

8.1.9. Wipe away the first drop of blood with a sterile gauze or cotton ball.

NB. The first drop of blood tends to contain excess tissue fluid which can adversely affect sample quality.

8.1.10. When a full hanging drop of blood is formed, gently touch the drop to the filter paper or inside the first circle.

8.1.11. Blood should only be applied from one side of the paper and appear as an even, uniform layer.

8.1.12. Do not layer blood drops on top of one another.

8.1.13. Absorb the blood directly from the collection site onto the paper while watching the circle to ensure that it completely fills.

8.1.14. If needed, continue to apply blood drops onto open areas of the first circle until it is completely covered.

8.1.15. Fill the first circle completely before going onto the second circle and so on.

8.1.16. Once there is an adequate amount of blood on the filter paper, apply clean cotton ball to the puncture site until the bleeding stops.

8.1.17. Air dry the samples for 3-4 hours on a flat, nonabsorbent surface

NB. Do not air-dry samples for less than 3 hours. Do not expose samples to direct sunlight or extreme temperature or humidity. Do not heat-assist the samples to dry.

8.1.18. Place the filter papers in individual plastic bags with at least 5 individual desiccant pouches.

NB. If samples are to be stored prior to transportation, they should be kept at +4 *C. For long term storage (over 90 days), samples must be stored at -20*C / -80*c

8.2. **Preparation of DBS sample form EDTA tube**

8.2.1. Mix the EDTA tube 5 times by inverting up and down

8.2.2. Pipette out 50 ul blood for 3 time (total blood volume 150 ul/per each circle) or 3 drop if using sterile pasture pipette from the blood collecting tube.

8.2.3. Drop it in a concentric circular motion onto the printed circle area on the card.

8.2.4. Avoid pudding the liquid sample, as it will overload chemical onto the card and do not rub or smear the blood onto the card.

8.2.5. Prepare at least of 4 blood spots for patient.

8.2.6. Air dry the samples for 3-4 hours on a flat, nonabsorbent surface – make sure all the spots were completely dried.

NB. Do not air-dry samples for less than 3 hours. Do not expose samples to direct sunlight or extreme temperature or humidity. Do not heat-assist the samples to dry.

8.2.7. Place the filter papers in individual zip lock plastic bags with at least 5 individual desiccant pouches.

NB. If samples are to be stored prior to transportation, they should be kept at +4 *C. For long term storage (over 90 days), samples must be stored at -20*C / -80*c

9. Sample Transportation

9.1. Enclose the completed requisition form with each batch of samples (retain one copy for receiving results)

9.2. Samples should be transported to the laboratory by the fastest means possible. It is recommended that samples be transported in a cooler (with ice packs) to protect against high temperatures and sunlight.

NB. When samples have been stored at +4 ºC or -20 ºC, they must be removed from cold storage, allowed to reach room temperature (in the closed bag) and the old desiccant pouches discarded and replaced prior to transportation.

10. Quality control

10.1. It is critical to ensure the DBS sample is of the highest quality possible.

10.2. Always verify patient and sample ID on the specimen with that of the requisition form before sending to the testing laboratory.

• Specimens of insufficient quantity for testing.	
• Specimens of insufficient quantity for testing.	
• Specimen not dried before mailing;	
• Specimen appears supersaturated;	
• Specimen appears diluted, discolored or contaminated;	
• Specimen exhibits serum rings;	
• Specimen appears clotted or layered;	
• There is no blood;	
• A high quality DBS sample should look like this	

11. Limitation

11.1. It can be difficult to obtain an adequate volume of blood from some patients. Keeping the finger at a level below the elbow and massaging the hand/finger should help.

12. Annexure

12.1. Worksheet for DNA extraction

12.2. DBS line listing form

13. Reference

13.1. WHO Standard Operating Procedure for Malaria microscopy.

13.2. VDCP Standard Operating Procedure for malaria microscopy- first edition