Anti - Malaria Campaign

SRI LANKA

Standard Operating Procedures

The following Standard operating procedures (SOPs) describe in detail the activities performed in laboratories providing malaria microscopy services. The SOPs aim to provide uniformity, consistency and reliability in each of the laboratory activities performed, to ensure high quality diagnosis (accuracy, sensitivity and specificity of smear reading results) and provides training and reference for old and new staff.

The SOPs were drafted by accredited expert malaria microscopists from ACTMalaria in consultation with AMC Central and Regional staff and finalized by the AMC Directorate.

Standard operating procedures prepared were for the following:

SOP No. **SOP TITLE**

1. Microscope use and Preventive Maintenance
2. Cleaning And Storing of Microscope Slides
3. Bio-safety in Handling Blood Specimens and Disposal of Infectious Waste Materials
4. Preparation of Thick and Thin Blood Smears for Diagnosis of Malaria
5. Preparation of Giemsa Stock Solution
6. Preparation of Working Solution of Giemsa stain and Staining of Blood Smears for diagnosis of malaria parasites
7. Reading of Malaria Blood Smear and Parasite Quantitation
8. Interpretation, Recording and Reporting of Results
9. Quality Assurance of Malaria Blood Smears
10. Feed backing of quality assurance of malaria microscopy
11. On-site Supervisory and Monitoring Visits in Microscopy Centers
12. Conduct of Proficiency Assessment on microscopical examination of malaria
13. Assessment of competency of trainee laboratory technician on microscopical examination of malaria
14. Conduct of Refresher Training on microscopical examination of malaria

Prepared by: Anti-Malaria Campaign Date: 01.12.2014

Approved by:  Date: 19.12.2014

	Anti-Malaria Campaign (AMC) **SOP for Malaria Microscopy**				
SOP title:	**Microscope Use and Preventive Maintenance**				
SOP No.	**01**	**Revision No.**	**0.0**	**Effective Date**	**01.01.2015**
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1. **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.

2. **PURPOSE:**

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

3. **SCOPE:**

   This procedure applies to all Laboratory Technicians doing Malaria Microscopy.

4. **RESPONSIBILITY:**

   4.1 It is the responsibility of the Parasitologist/RMO and QA/QC Laboratory Technician to ensure that the Laboratory Technicians concerned are conversant with the operations of the instrument and that they implement this SOP as written.

   4.2 Daily use and maintenance of the instrument according to this SOP and manufacturer's instructions.

5. **REQUIREMENTS:**

   The following materials, reagents, and equipment are the minimum requirements.

   5.1 Tools and apparatus:

   - 5.1.1 Lens tissue
   - 5.1.2 Aspirator Bulb / Rubber Bulb
   - 5.1.3 Camel Hair Brush / Soft Make-up Brush
   - 5.1.4 Soft lint- free cloth

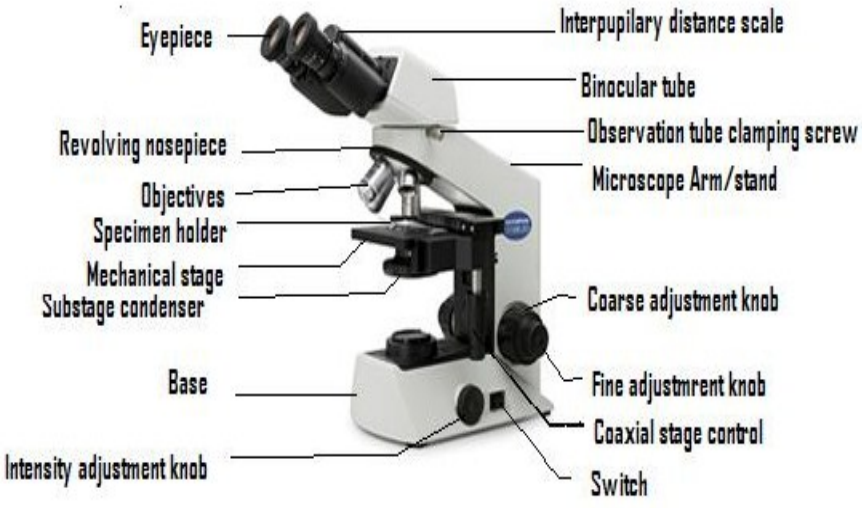
5.1.5 Applicator Stick
5.1.6 Spare Bulb
5.1.7 Concave mirror (in areas without electricity)
5.1.8 Equipment Maintenance Record (logbook)
5.1.9 Anti static cover for the microscope
5.1.10 Scissor (small)
5.1.11 Jewelry screw driver set (6pcs)
5.1.12 Allen key
5.1.13 Wrench (4pcs)

5.2 Solutions for cleaning

5.2.1 absolute ethanol

6. PROCEDURES:

6.1 Parts and Manipulation



6.2 General Care and Cleaning

6.2.1 The microscope is a precision instrument; avoid subjecting it to sudden or severe impact.
6.2.2 Do not use the microscope where it is subjected to direct sunlight, high temperature and humidity, dust or vibrations or near a water source.
6.2.3 Line up the low power objective with the ocular when microscope is not in use.
6.2.4 Install the microscope on a flat sturdy surface. The air vents on the underside of the base should never be blocked such as placing on a flexible surface e.g. carpet, table cloth.
6.2.5 Do not move the microscope by sliding it on the table. Otherwise, the rubber feet might be damaged or peeled off.
6.2.6 Never leave the microscope without the eyepiece (or eye piece dust cap).
6.2.7 Cover the microscope when not in use with the anti static cover provided (figure 2).

Figure 2 covering the microscope with anti static cover

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

6.2.9 Remove/install objectives using both hands. Loosely cup with one hand and twist the barrel with the other, being very careful not to touch the front lens with fingers. Take extreme caution not to drop the objective.

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

6.2.11 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.

6.2.12 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 (figure 3).

Figure 3 cleaning the body parts of the microscope

6.2.13 Take extreme care to never get immersion oil on a dry objective (this can happen if you rotate a 'dry' objective across an oil-coated slide). Since these lenses are not designed to be immersed in anything, the oil and cleaning agents can seriously damage them.

6.2.14 After an oil immersion objective is used, the excess oil should immediately be wiped away with lens tissue. Not only does this excess oil attract dust, it can drip down onto the stage or condenser top lens.

6.2.15 Cleaning the eye pieces

6.2.15.1 Blow off dust particles using the rubber bulb (figure 4)

Figure 4 blowing off the dust particles from eye pieces

6.2.15.2 Prepare a clean lens tissue swab using a clean lens tissue and an applicator stick (figure 5).

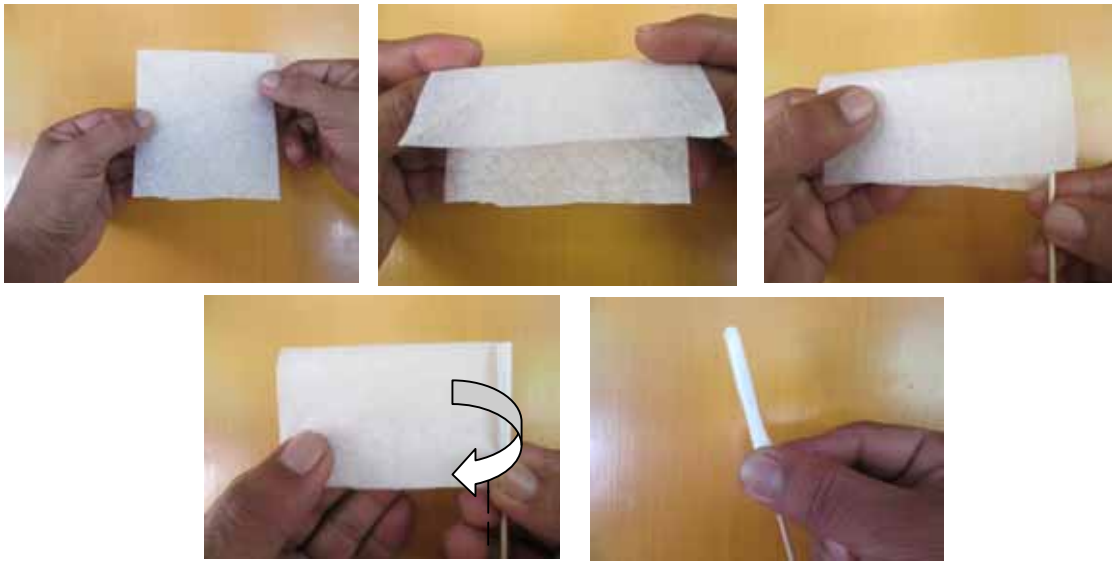


Figure 5: How to roll the lens tissue around a stick (lens tissue swab)

6.2.15.3 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 6).

6.2.15.4 Repeat the process using dry tissue swab.

Figure 6: Cleaning the eye pieces of the microscope

6.2.16 Cleaning the objectives

6.2.16.1 Never use ordinary paper or cotton wool to clean the lenses of the microscope.

6.2.16.2 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 so as

to drag the lens tissue across the objective lens (Figure 7). Repeat 3-4 times using a clean section of lens tissue for each pull.

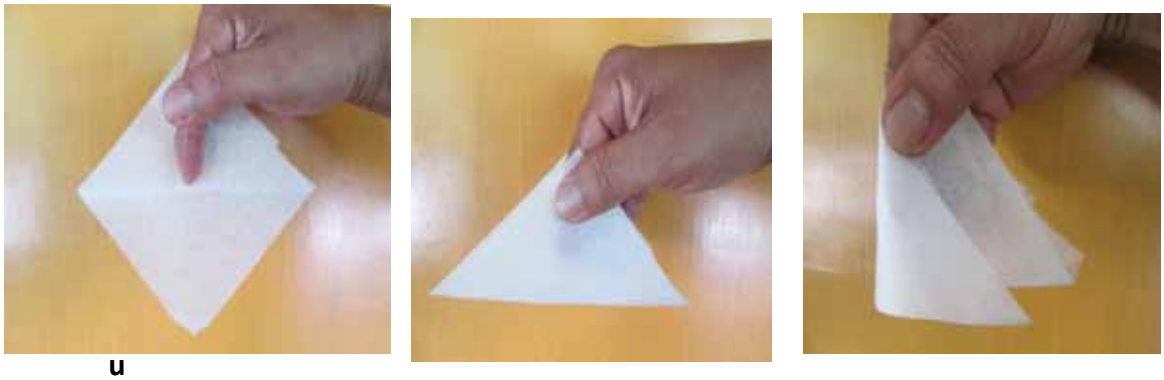
Figure 7: Cleaning the objectives of the microscope

6.2.16.3 Before cleaning the dry objectives (x4,x10,x40), with lens tissue, blow off dust particles using the rubber bulb.

6.2.16.4 To clean the oil Immersion objective, a lens tissue moistened with absolute alcohol can be used if needed.

6.2.17 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).



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

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

6.3 STORAGE

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

6.3.17 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).

6.3.18 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).

6.4 TRANSPORTING THE MICROSCOPE

6.4.1 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.

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

6.4.3 Put the transport band, whenever available.

6.4.4 Secure the stage and other movable parts.

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

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

6.5 SAFETY RULES

6.5.16 Never work with your hands, body or clothes wet.

6.5.17 Do not presume a circuit is off.

6.5.18 Do not remove equipment grounds.

6.5.19 Do not use defective plugs & cords.

6.5.20 Always use the correct replacement parts.

6.5.21 If the bulb burns out during observation, be certain to cool the defective bulb completely before replacement.

6.5.22 Keep the microscope out of reach of children.

6.6 INSPECTION AND PERFORMANCE CHECK

This part deals with the routine inspections and performance checks before the unit is put to use and/or being kept after using. A regular and thorough inspection of the microscope will certainly help maintain good operational results and increase its technical lifespan.

A weekly inspection of a microscope should include the following points:

- All outer surfaces of the instrument must be clean.
- All electrical plugs, sockets, cables, etc. must be cleaned and free of corrosion or other visible damage; screws must be tightened securely.
- The light bulb must be clean.
- The field iris diaphragm must move through its full range of opening smoothly and easily; all blades of the iris must move properly.
- The condenser height adjustment knob must run smoothly through its full range. Some types have an adjustable brake, while others are permanently set.
- The condenser centering screws must move the condenser smoothly and in all directions through its full range.
- The mechanical stage must move the specimen smoothly and without any play through its full range of adjustment in both directions. Check by observing at the highest magnification of the microscope.

- The slightest touch of the control knob must result in a corresponding movement of the specimen. Keep in mind the fact that one micron of stage movement observed at a magnification of 1000x looks like 1 mm viewed without a microscope and at a distance of 250 mm. Therefore, any disassembly, adjustment, re-greasing, etc. of mechanical stages should be performed only by a trained technician.
- The mounting shoulders of the objectives and the corresponding surfaces on the nosepiece must be absolutely clean. Otherwise, you will lose the proper positioning of the objectives regarding centering and focus. Be careful not to allow any dust or dirt to get into the objective during cleaning.
- The nosepiece must rotate smoothly through 360^0, reaching all click-stop positions properly from either side and without any play. Any disassembly at this position interferes with the optical alignment and requires special alignment aids for readjustment.
- The fine and coarse focusing knobs must run smoothly through their full range of adjustment. Some types have an adjustable brake (refer user manual), whereas others are permanently adjusted. It must especially be ensured that the stage does not lower itself automatically. Do not tighten the brake too much.
- The upper surface of the stand and the corresponding mounting surface of the observation tube must be clean and free of corrosion. The fastening screw for the tube must hold it securely in place.
- Adjustment of the interpupillary distance of the tube must run smoothly and without exerting undue force.
- All optical surfaces must be absolutely clean. Pay special attention to: light bulb, cover glass on field stop, condenser front lens, objective front lens, upper surface of eye lens.
- Report to the maintenance department/AMC HQ/RMO AMC of any malfunction observed.

6.7 EQUIPMENT MAINTENANCE RECORD

Records shall be maintained which include the following:

6.7.1 Name of equipment
6.7.2 Name of the Manufacturer, serial number or other unique identification
6.7.3 Origin (from what institution)
6.7.4 Date received
6.7.5 Date placed in service
6.7.6 Condition when received (new, used)
6.7.7 Details of maintenance carried out
6.7.8 Current location, where appropriate
6.7.9 Copy of manufacturer's operating instructions, where available
6.7.10 History of damage, malfunction or repair (date repaired)
6.7.11 Name and signature of technician

	Anti-Malaria Campaign (AMC) **SOP for Malaria Microscopy**				
SOP title:	**Cleaning and Storing of Microscope Slides**				
SOP No.	**02**	**Revision No.**	**0.0**	**Effective Date**	**01.01.2015**
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1. BACKGROUND:

These Standard Operating Procedures describe the procedure of cleaning and storing microscope slides. The availability of clean, good quality glass slides for the preparation of blood specimens for microscopic examination must be emphasized.

2. PURPOSE:

To ensure that microscope slides are properly cleaned, stored and readily accessible in all laboratories.

3. SCOPE:

This procedure applies to all Laboratory Technicians doing Malaria Microscopy.

4. REQUIREMENT:

4.1. Glass slides
4.2. Plastic bowls or basins
4.3. Good quality liquid detergents
4.4. Washing clothes or soft sponges
4.5. Clean, lint free cotton cloths (the kind used to dry glassware)
4.6. Good supply of clean water
4.7. Sheets of clean paper cut to 11 cm. x 15 cm.
4.8. Empty slide boxes
4.9. Clear adhesive tape
4.10. Desiccators
4.11. Gloves

5. PROCEDURE

5.1. Cleaning of slides

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.

*Wear gloves when washing microscope slides.

5.1.1 New slides

5.1.1.1 New slides are separated one from the other and placed individually to soak in a warm detergent solution for 2-3 hours, preferably overnight. After soaking, use the washing cloth or sponge to clean each slide on both sides by rubbing the two surfaces of the slide between the forefinger and thumb.

5.1.1.2 Slides are individually rinsed in clean water to remove all trace of detergent. This may require two bowls.

5.1.1.3 Handling slides by the edges, the excess water is drained from each slide before it is dried thoroughly using a clean, lint-free cotton cloth.

*Although the slides are new, chipped and scratched slides are unsuitable for hematology and must be discarded***.**

5.1.1.4 Using the cut paper pieces, wrap the dried slides in packs of ten; the ends of the wrapper being turned down and secured with clear adhesive tape; they are then placed in the cardboard boxes ready for use. Each box is kept secure with a rubber band.

5.1.2 Used slides:

5.1.2.1 Used slides are placed in a basin of a warm detergent solution for overnight.

5.1.2.2 Clean one by one with a washing cloth or soft sponge until all traces of the blood film and oil have been removed.

5.1.2.3 Transfer the slides to a fresh solution of good quality detergent and later to running water or several changes of clean water. Handle the slide by the edges to drain the excess water.

5.1.2.4 Dry the slides thoroughly with clean, dry and lint free cloth.

5.1.2.5 Using the cut paper pieces, wrap the dried slides in packs of ten; the ends of the wrapper being turned down and secured with clear adhesive tape; they are then placed in the cardboard boxes ready for use. Each box is kept secure with a rubber band.

5.2. Storing of slides:

5.2.1 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'.

5.2.2 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.

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

Procedural Note:

Reasons for discarding slides

- Scratches
- Chipped
- slides showing signs of frosting or devitrification

	Anti-Malaria Campaign (AMC) SOP for Malaria Microscopy				
SOP title:	**Bio-safety in Handling Blood Specimens and Disposal of Infectious Waste Materials**				
SOP No.	**03**	**Revision No.**	**0.0**	**Effective Date**	**01.01.2015**
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1. **BACKGROUND:**

These Standard Operating Procedures describe the biosafety guidelines to be followed when handling blood specimens for routine laboratory diagnosis of malaria

2. PURPOSE:

To ensure that proper biosafety guidelines are followed when handing blood for diagnosis of malaria (Special precautions/ procedures may be required when preparing blood smears for diagnosis of malaria from suspected cases of Hemorrhagic Fever Virus.)

3**. SCOPE:**

This procedure applies to all health workers working in the laboratories where blood tests for malaria are performed.

4. **REQUIREMENTS:**

4.1 Laboratory gown/ coat
4.2 Surgical/hand gloves
4.3 Soap
4.4 Puncture resistant container (sharps bin)
4.5 Hand towel
4.6 All-purpose disinfectant
4.7 Infectious and Non- Infectious bin
4.8 Hypochlorite solution

6. PROCEDURE:

5.1 All laboratory workers must be adequately trained, both in the duties they performed and in all aspects of laboratory work.
5.2 Wear a laboratory gown/coat when in the laboratory. Remove this protective clothing before leaving the laboratory.
5.3 Wear gloves when taking and handling blood specimens.
5.4 Do not touch your eyes, nose or other exposed membranes, or the skin with the gloved hands.
5.5 Do not leave the workplace or walk around the laboratory wearing gloves.
5.6 Discard gloves whenever they are thought to have become contaminated, wash your hands, and put on new gloves.
5.7 Wash your hands with soap and water immediately after any contamination and after work is completed. If gloves are worn, wash your hands with soap and water after removing the gloves.
5.8 Puncture wounds, cuts and skin contaminated by spills or splashes of blood should be thoroughly washed with soap and water. Bleeding the wound should be encouraged.
5.9 All spills, accidents and overt or potential exposure to infectious specimens should be reported immediately to the laboratory supervisor and appropriate action should be taken to prevent further occurrences.
5.10 Place used lancets in a puncture –resistant container immediately.
5.11 Discard cotton ball with blood and gloves in infectious container.
5.12 Disinfect work surfaces when procedures are completed before and after each working day. An effective all-purpose disinfectant is a hypochlorite solution with a concentration that provides 0.1% available chlorine (1g/L).
5.13 Do not eat or drink in the laboratory.
5.14 Access to the laboratory must be restricted to authorized personnel only.

	Anti- Malaria Campaign (AMC) **SOP for Malaria Microscopy**				
SOP title:	**Preparation of Thick and Thin Blood Smears for Diagnosis of Malaria**				
SOP No.	**04**	**Revision No.**	**0.0**	**Effective Date**	**01.01.2015**
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1. BACKGROUND:

Examination of malaria blood smears by microscopy is a basic technique, which is considered as the gold standard for the diagnosis of the different species of malaria parasite.

2. PURPOSE:

To provide guidelines for proper collection, preparation, labeling, drying and fixing of malaria thick and thin blood smears to produce high quality, standardized specimens for microscopy in all microscopy centers.

3. SCOPE:

This procedure applies to preparation of blood smears for diagnosis of malaria under normal laboratory conditions by all Laboratory Technicians doing Malaria Microscopy.

4. MATERIALS AND REAGENTS:

4.1. Materials and reagent

4.1.1 Clean Glass slides
4.1.2 Lancet
4.1.3 70% isopropyl or ethyl alcohol
4.1.4 Cotton or alcohol swab
4.1.5 Pencil
4.1.6 Latex gloves
4.1.7 Biohazard container (for used lancets)
4.1.8 Slide folders/trays
4.1.9 Slide boxes
4.1.10 Micropipette
4.1.11 H/AMC/P1 form
4.1.12 Request form

4.2. Specimen

Capillary blood or venous blood (if from venipuncture)

5. PROCEDURE:

5.1 Record information of the patient data in a H/AMC/P 1 form *(Serial no., date, name, age, sex, address, history of fever)*

5.2 Holding patient's left hand palm upwards, select the third finger from the thumb. (The big toe can be used with infants).

5.3 Clean the finger with a piece of cotton soaked with 70% alcohol, using firm strokes to remove grease and dirt from the ball of the finger. Puncture the ball of the finger with a sterile lancet, using a quick rolling action.

5.4 Apply gentle pressure to the finger to express the first drop of blood and wipe it away with a dry piece of cotton wool. (Make sure that no strands of cotton remain on the finger to be later mixed with the blood.)

5.5 Holding the slide by the edges and keeping the hand palm upwards, collect blood to the lower surface of slide as follows:

5.5.1 One small drop of blood to the centre of the slide for the thin smear

5.5.2 Three drops of blood at a distance of 1 cm away from the centre of the slide for the thick smear.

5.6 If venous blood (anticoagulated blood) is used, with the use of a micropipette, place 2 µl for the thin smear and 6µl to prepare thick smear

5.7 To prepare the thin smear:

5.7.1 Get another clean slide to be used as a spreader. With the slide resting on a flat, firm surface, place the spreader in front of the single drop of blood intended for the thin smear, at an angle of 45° pull back the spreader and hold it until the blood is evenly spread along the edge of the slide. Do not delay between applying and spreading the drop.

5.7.2 Rapidly push the slide forward in a single, smooth, continuous motion. Avoid hesitation or jerky motion when spreading the slide. A properly prepared thin smear is thick at the beginning end and thin or feathered on the other end. The feathered end of the smear should not reach the end of the glass slide and should have areas optimal for microscopy having red blood cells that are in a single distinctive layer.

5.8 To prepare the thick smear.

5.8.1 With one corner of the spreader slide, spread the blood out in a circular motion (3-6 circles) to make a circle with a diameter of 1 cm finishing off at the center.

5.8.2 The ideal thickness of the smear should allow for printed text to be readable when it is placed on it, while blood is still fresh (figure 9).

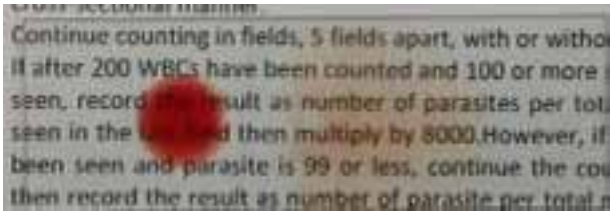

Figure 9.

5.9. Let the blood smear air dry. Using lead pencil, label the upper 1/3, thick portion of the thin smear with the code number, serial number and the date of collection (should correspond to the entry in the H/AMC/P1 form).

PROCEDURE NOTES:

1 Always use new, pre-cleaned and grease free glass slides. If not available, clean the slides before use.
2 Always use new lancet for each patient. Dispose used lancet to a sharps bin. NEVER re-use lancets.
3 Always wear gloves when collecting blood for malaria blood smears.
4 Collect blood for PCR at the same time, if required.
5 May have to perform a RDT, if requested.
6 Whenever possible blood smears should be prepared immediately using finger prick blood.
7 If immediate blood smear preparation is not possible, blood may be collected to an anticoagulated tube. Acceptable anticoagulant agents are K_2EDTA or K_3EDTA, sodium citrate, and acid citrate-dextrose (ACD). Heparin is not recommended for collecting blood for diagnosis of malaria. It has an inhibitory effect on PCR assays and may also causes the development of a purple/blue hue on stained blood smears.
8 Blood smears from anti-coagulated blood should be prepared as quickly as possible, ideally within 1 hour of collection. Parasite morphology and red blood cell morphology may be distorted and stippling of the infected red cells may not be visible if a smear is made from the blood specimen that has been kept for too long.
9 Anti-coagulants may interfere with adhesion of blood to the slide and with Giemsa Staining especially if the ratio of blood to anti-coagulant is not optimal.
10 Slides must always be held by the edges to avoid any trace of oil from the finger on its surface.
11 Special precautions/ procedures may be required when preparing blood smears for diagnosis of malaria from suspected cases of Hemorrhagic Fever Virus.

	Anti-Malaria Campaign (AMC) **SOP for Malaria Microscopy**				
SOP title:	**Preparation of Giemsa Stock Solution**				
SOP No.	**05**	**Revision No.**	**0.0**	**Effective Date**	**01.01.2015**
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1. PURPOSE:

For the production of good quality Giemsa stock solution.

2. SCOPE:

The procedures described herein apply to laboratory technicians preparing stock solutions of Giemsa stain from Giemsa powder.

3. REQUIREMENTS:

3.1 Reagents:

3.1.1 Giemsa powder, 3.8 grams
3.1.2 Glycerol, 250 ml
3.1.3 Methanol, 250 ml

3.2 Materials and equipment:

3.2.1 Weighing boat
3.2.2 Spatula
3.2.3 Magnetic stir bar
3.2.4 Amber colored bottle, 500 mL
3.2.5 Paraffin film
3.2.6 Erlenmeyer flask, 500 mL.
3.2.7 2 nos. Graduated cylinder, 250 mL.
3.2.8 Electronic magnetic stirrer
3.2.9 Electronic balance
3.2.10 Glass beads

4. PROCEDURE:

4.1 Using an electronic balance weigh 3.8 grams of Giemsa powder in a weighing boat.
Refer to manufacturer's operational manual for the proper preparation/warm up, calibration and usage of electronic balance.

4.2 Measure 250 ml of methanol using a graduated cylinder.
4.3 Measure 250 ml of glycerol in a separate graduated cylinder.
4.4 Put the magnetic stir bar inside the flask and pour in the measured amount of methanol.
4.5 While turning the stirrer on at 500 RPM (rounds per minutes), slowly add the glycerol to the methanol.
4.6 Gradually add the Giemsa powder to the methanol-glycerol solution, using small quantity at a time, stirring constantly. Continue stirring for at least 4 hours increasing the speed to 1000 RPM.
4.7 Leave the bottle for 4-5 days, stirring the solution for at least 4 hours continuously in a day until the stain is thoroughly mixed. This is the stock solution. Keep the stock solution in a small bottle for routine use to avoid contamination of the stock solution.

Each newly prepared batch of stock solution should be properly labeled, including date of preparation, and should be tested for optimum stain dilution and staining time. A log of the same, including brand or type of reagents used, time of preparation, batch number, minimum time for harvest or maturation and remarks, should be kept for records.

Important reminders:

- Always wear laboratory gown, masks and gloves, when working in the laboratory.
- During the stirring process, make sure that the Erlenmeyer flask is covered with aluminum foil to prevent exposure to light and with its mouth tightly stoppered or covered with paraffin film to prevent evaporation of the solvent.
- The laboratory must be equipped with exhaust system and fire extinguisher.
- Follow regulations on proper disposal and recycling of reagent bottles as waste materials.

5. QUALITY CONTROL:

To ensure good quality of the Giemsa stain produced, the quality of Giemsa should be evaluated at least a month after it has been prepared. In order to do this you had to have at least two sets of three freshly prepared thick and thin blood smears and evaluated as follows:

- 3 slides for 10% Staining Method, - staining the slides at 5, 10, and 15 minutes;
- 3 slides for 3% Staining Method, staining the slides at 30, 45, and 60 minutes.
- Slides should be kept for archiving and a log of each staining evaluation for each slide should be kept for records.

	Anti – Malaria Campaign (AMC) **SOP for Malaria Microscopy**				
SOP title:	**Preparation of Working Solution of Giemsa stain and Staining of Blood Smears for diagnosis of malaria parasites**				
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Approved:				**Date**	**19.12.2014**

1. BACKGROUND:

The use of Giemsa stain is the most reliable procedure to stain malaria blood smears, which is composed of eosin and methylene blue. The eosin component stains the parasite nucleus red, while the methylene component stains the cytoplasm blue.

2. PURPOSE:

To provide guidelines for proper preparation of working solution of Giemsa staining and staining of thick and thin blood smears for diagnosis of malaria in all laboratory facilities.

3. SCOPE:

This procedure applies to all Laboratory Technicians doing malaria microscopy.

4. MATERIALS, REAGENTS & EQUIPMENTS:

4.1 Giemsa Stock solution
4.2 Buffered distilled water (pH 7.2)
4.3 Methanol
4.4 Pasteur pipettes
4.5 Graduated cylinder
4.6 Drying Rack
4.7 Staining trough
4.8 Clean tap water
4.9 Timing clock
4.10 Wash bottle

5. PROCEDURE:

Two methods of staining with Giemsa stain:

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

5.1 Rapid Staining Method (10% Giemsa working solution)

This rapid method is used where a quick diagnosis is essential for patient care like in outpatient clinics and busy laboratories.

5.1.1 Preparation of 10% Giemsa stain solution for mass staining

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

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

5.1.1.3 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 tapping the base of the cylinder with the mixture against the palm of the hand.

5.1.1.4 Giemsa working solution must be freshly prepared.

5.1.2 Preparation of 10% Giemsa stain solution for individual slide staining

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

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

5.1.3 Procedure of rapid staining of Malaria blood smears

5.1.3.1 Allow the thick smear to dry completely, if really rapid results are required, drying maybe hastened by using a wooden small cupboard (2' X 1.5') with 60w bulb. Care should be taken to avoid overheating; otherwise the thick smear will be heat-fixed.

5.1.3.2 Fix the thin smear by dipping it briefly in a container with methanol for a few seconds. To permit dehemoglobinization, the thick smear should not be fixed; thus avoid methanol or its fumes touch the thick smear.

5.1.3.3 Place the slide on a staining rack and gently pour the working solution of Giemsa stain on to the slide; a pipette can be used for this purpose. Alternatively, slides can be placed faced down on a concave staining plate and the stain introduced underneath the slide.

5.1.3.4 Stain for 10 minutes (ideally according to the duration specified for the stock of Giemsa stain).

5.1.3. 5 Gently flush the stain off the slide with clean tap water; do not tip off the stain on the slide and wash, as this will leave a deposit of scum over the smear.

5.1.3.6 Place the slide on the rack, smear side downwards, to drain and dry ensuring that the blood smears do not touch the slide rack.

5.2 Regular Staining Method (3% Giemsa stain working solution)

This regular method is used for staining larger number of slides such as those collected during surveys or research studies.

5.2.1 **Preparation of 3% Giemsa stain working solution**

5.2.1.1 Pour 97 mL of buffered distilled water (pH 7.2) into a 100 mL graduated cylinder.
5.2.1.2 Using Pasteur pipette, draw 3 mL of Giemsa stain. Add the stain to the buffered water in the graduated cylinder.
5.2.1.3 Cover the top of the graduated cylinder with Para film or gently invert the cylinder several times until completely mixed.
5.2.1.4 Working solution of Giemsa stain must be prepared fresh

5.2.2 **Staining Procedure.**

5.2.2.1 Fix the thin blood smear by briefly dipping it in a container with methanol for a few seconds. Prolong fixation make it difficult to demonstrate Schüffner's dots and Maurer's spots. To permit dehaemoglobinization, the thick smear should not be fixed, therefore avoid methanol or its vapor touching the thick smear.
5.2.2.2 Place malaria blood smears on a staining rack and gently pour the 3% working solution of Giemsa stain on to the slide. To avoid cross-contamination, staining slides individually is preferred. Batch staining is advisable only when several malaria blood smears are from a single patient.
5.2.2.3 Stain films for 30-45 minutes out of the sunlight.
5.2.2.4 Gently flush the stain off the slide with clean tap water; do not tip off the stain on the slide and wash, as this will leave a deposit of scum over the smear. If slides are stained in batches, pour clean tap water gently into the trough to float off the iridescent scum on the surface of the stain or gently immerse the whole trough in a vessel filled with clean tap water.
5.2.2.5 Rinse slides briefly and gently under running tap water or by a gentle flow of clean water from a beaker.
5.2.2.6 Place the stained slides smear side downwards, in a drying rack to drain and dry, making sure that the film does not touch the slide rack.
5.2.2.7 At all times during preparation and storage, slides should be protected from exposure to dust and insects.

6. EVALUATION OF STAINED SMEAR

6.1 Macroscopic appearance of blood smears:

6.1.1 A malaria blood smear that is too pinkish suggests low pH or over staining.

6.1.2 A malaria blood smear that is too bluish or purplish suggests high pH or under staining.

6.2 Evaluation of a well-stained thick blood smear:

6.2.1 Thick smear is >90% intact and red cells should be dehemoglobinized

6.2.2 The background should be clean and free from debris, with a pale mottled-grey color derived from the lysed erythrocytes.

6.2.3 Leukocytes nuclei are a deep-rich purple.

6.2.4 Malaria parasites are well defined with deep-red chromatin pale purplish blue cytoplasm. In *P. vivax* and *P. ovale* infections, the presence of stippling in the "ghost" of host erythrocytes can be seen especially at the edge of the film.

6.2.5 Grades of staining

Grade I- Lysis incomplete. Leukocyte nuclei and eosinophilic granules beginning to stain; malaria parasites not yet visible but pigment clearly shown; background pale.

Grade II- Lysis complete; Cytoplasm and nuclear chromatin of parasites visible; background pale.

Grade III- Leukocyte nuclei deep purple, vague granulation in cytoplasm; eosinophilic granules and cytoplasm and chromatin of malaria parasites well defined; background mottled grey; colour contrast optimum.

Grade IV- Malaria parasites deeply stained but background blue grey and colour contrast lessened: Schüffner's dots visible.

Grade V- All cellular elements deeply stained: background dark blue-grey: colour contrast poor.

6.3 Evaluation of a well-stained thin blood smear:

6.3.1 The background should be clean and free from debris; the color of erythrocyte is pale grayish pink.

6.3.2 Thin smear has RBCs that are in one single, distinctive layer

6.3.3 Leukocytes have deep purple nuclei and well defined granules

6.3.4 The chromatin of malaria parasite is a deep purplish red and clear purplish blue cytoplasm.

6.3.5 Schüffner's stippling are visible in erythrocytes containing *P. vivax* or *P. ovale.* Maurer's dots may be seen in erythrocytes containing the larger ring forms of *P. falciparum*.

6.3.6 Grades of staining

Grade I- Erythrocytes are pale pink and the nuclei of the Leukocyte pale blue: The cytoplasm of the Leukocytes and the chromatation and cytoplasm of the malaria parasites are unstained.

Grade II- Brings out the granules of the Leukocytes, with the parasites just visible.

Grade III- Differentiates the blue cytoplasm and the red chromatin of the parasites and shows Schüffner's stippling in vivax-infected cells.

Grade IV- Intensifies all colours and emphasizes the features of immature Erythrocyte.
Grade V- Demonstrates the stippling of cells infected with *Plasmodium falciparum*.

	Anti-Malaria Campaign (AMC) **SOP for Malaria Microscopy**				
SOP title:	**Reading of Malaria Blood Smear and Parasite Quantitation**				
SOP No.	**07**	**Revision No.**	**0.0**	**Effective Date**	**01.01.2015**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**01.12.2014**
Approved:				**Date**	**19.12.2014**

1. BACKGROUND:

In a thick blood smear, the red blood cells (RBCs) are lysed and dehemoglobinized while the malaria parasites are left intact and concentrated. This allows detection and identification of the parasites. The thin blood smear, when fixed with the absolute methanol, enables the RBCs to retain their original morphology with malaria parasites, if present, visible inside the cells.

2. PURPOSE:

To provide guidelines for the proper detection, identification and quantitation of malaria parasites in Giemsa stained thick and thin blood smears in all microscopy centers.

3. SCOPE:

This procedure applies to all Laboratory Technicians doing malaria microscopy.

4. EQUIPMENTS, REAGENTS AND MATERIALS:

4.1 Materials

- 4.1.1 Reporting Form
- 4.1.2 Pen
- 4.1.3 Lens paper
- 4.1.4 Slide for examination
- 4.1.5 Slide boxes
- 4.1.6 H/AMC/P1

4.2 Reagents

- 4.2.1 Immersion Oil

4.3 Equipment

- 4.3.1 Binocular microscope

5 Procedures (routine & QC slide reading):

5.1 Examining thick blood smear

Routinely the thick blood smear is examined for malaria parasites provided that it has been well made and correctly stained, there should be no problems in identifying any stages of malaria parasite present.

5.1.1 Place the slide to be examined on the stage, positioning the thick blood smear in line with the objective lens.

5.1.2 Using paired x10 or x5 oculars and a x40 objective, briefly scan the blood smear for microfilariae and any larger parasites or obvious debris. While doing this, select a part of a film that is well stained, free of debris, and well-populated with white blood cells (WBC).

5.1.3 Place a drop of oil immersion on the thick blood smear then shift to the oil immersion objective (100x). Using the fine adjustment, focus on the cell elements confirming the portion of the smear is acceptable for a routine examination. (About 15-20 WBC's per thick smear field, using the x10 or x5 oculars and x100 objective, will give a satisfactory blood smear thickness for examination. Poorer quality blood smears will need a more extensive search.)

5.1.4 Examine carefully the entire blood smear, field by field, moving each field continuously following the pattern shown in the figure 10 below, starting at the x mark. For an efficient examination remember to continuously refocus with the fine adjustment throughout examination of each new field.

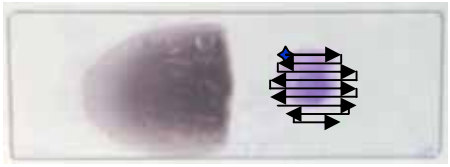

Figure 10

5.1.5 If parasites are found, extend more time to examine the whole smear, this allows for possible detection of a mixed infection. Make a tentative species determination and then examine the thin smear to confirm the species present.

5.1.6 Get a tentative parasite count according to the plus system based on 100 thick smear fields.

+ system

+	1-10 parasites / 100 fields
++	11-100 parasites / 100 fields
+++	1-10 parasites / field
++++	more than 10 parasites / field

5.2.3 Then get the actual parasite count as parasite density per microliter examining the thick smear.

5.2.3.1 Start the count in the top left hand section of the blood smear, move in a cross-sectional manner.

5.2.3.2 Continue counting in fields, 5 fields apart, with or without WBC.

5.2.3.3 If after 200 WBCs have been counted and 100 or more parasites have been seen, record the result as number of parasites per total number of WBC seen in the last field then multiply by 8000. However, if after 200 WBC has been seen and parasite is 99 or less, continue the count up to 500 WBC then record the result as number of parasite per total no. of WBC seen in the last field then multiply by 8000. Record results with the number of parasite per micro liter of blood.

$$\text{parasite density} = \frac{\text{number of parasites counted} \times 8000}{\text{number of WBC counted}}$$

= no. of parasites/µl.

5.2.4 It is important to screen the entire thick blood smear before declaring a slide negative. Upon examination of the whole thick smear and no parasite found, examine also the thin smear.

5.3 Examining thin blood smear

5.2.1 Place a drop of immersion oil on the edge of the middle of the blood smear.

5.2.3 Examine the distal third of the blood film following the pattern of movement shown in the figure 11; that is, by moving along the edge of the thin film, then moving the slide inwards by one field, returning in a lateral movement and so on.

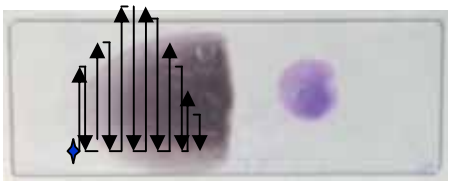

Figure no. 11

5.2.4 If necessary % parasitaemia from the thin smear as follows.

$$\frac{\text{No. of infected red cells}}{\text{RBC in a field x no. of fields examined}} \times 100$$

(*approximately 50 fields examined)

Infected red cells are counted. (“multiple infection” counted as one)

5.3 Finish the examination by recording the result in appropriate record form(s) and log books

5.4 Keep the examined slides in a covered slide box for 3-6 months until the slides are selected for quality control and for possible later reference.

NOTES:

It is important to screen the entire thick smear before declaring a blood smear negative.

In following instances, it is important to examine at least 800 thin smear fields in addition to screening the entire thick smear before declaring the slide negative.

a. in clinically suspected malaria patients
b. if the thick smear is not properly prepared /washed off

Shift the mechanical stage downwards before removing the slide after examining under high power objective.

	Anti- Malaria Campaign **SOP for Malaria Microscopy**				
SOP title:	**Interpretation, Recording and Reporting of Results**				
SOP No.	**08**	**Revision No.**	**0.0**	**Effective Date**	**01.01.2015**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**AMC**			**Date**	**01.12.2014**
Approved:				**Date**	**19.12.2014**

1. PURPOSE:

To provide guidelines for the interpretation, recording and reporting of results of malaria blood smear examination in all laboratory facilities.

2. SCOPE:

This procedure applies to all Laboratory Technicians doing malaria microscopy.

3. MATERIALS AND SUPPLIES:

3.1 H/AMC/P1 form
3.2 H/AMC/P 15 and P17
3.3 Pen

4. PROCEDURE:

4.1 After examining the blood smear, record the results as follows:

P. falciparum:

Early Trophozoite	**Pf (R)**
Late Trophozoite	**Pf (T)**
Schizonts	**Pf (Sch)**
Gametocytes only	**Pf (G)**

Early Trophozoites, Late Trophozoite, schizonts & gametocytes **Pf (R/T/Sch/G)**

P. vivax

Early Trophozoite only	**Pv(R)**
Late Trophozoite only	**Pv (T)**
Schizonts	**Pv (Sch)**

Gametocytes only	**Pv(G)**
Trophozoite & Schizonts only	**Pv (T/Sch)**
Trophozoites & gametocytes	**Pv(R/G)**
Early Trophozoites Trophozoites, schizonts & gametocytes	**Pv (T/Sch/G)**

P. malariae

Early Trophozoite only	**Pm (R)**
Trophozoite only	**Pm (T)**
Gametocytes only	**Pm (G)**
Trophozoite & Schizonts only	**Pm (T/Sch)**
Trophozoites & gametocytes	**Pm (T/G)**
Trophozoites, schizonts & gametocytes	**Pm (T/Sch/G)**

P. ovale

Late Trophozoite only	**Po (T)**
Gametocytes only	**Po (G)**
Trophozoite & Schizonts only	**Po (T/Sch)**
Trophozoites & gametocytes	**Po (T/G)**
Trophozoites, schizonts & gametocytes	**Po (T/Sch/G)**

Mixed Infection:

Plasmodium falciparum trophozoite and gametocyte and Plasmodium vivax trophozoite	**Pf (T/G)Pv(T)**

No Malaria Parasite Seen **MP Negative**

4.2 Record the parasite density based on the plus system and parasite/microliter. If requested indicate the % parasitaemia.

	Anti-Malaria Campaign (AMC) **SOP for Malaria Microscopy**				
SOP title:	**Quality Assurance of Malaria Blood Smears**				
SOP No.	**09**	**Revision No.**	**0.0**	**Effective Date**	**01.01.2015**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**01.12.2014**
Approved:				**Date**	**19.12.2014**

1. PURPOSE:

Provide methodology for standard assessment of malaria microscopy service making use of blinded evaluation of sample blood smears.

2. SCOPE:

All Laboratory Technicians doing Malaria Microscopy.

3. MATERIALS:

3.1 Examined slides
3.2 Relevant H/AMC/P1 forms
3.3 Pen
3.4 Form 1

4. PROCEDURE:

4.1 Validation of slides will be done initially at regional level. RMO/AMC and Laboratory Technician doing QA/QC must determine schedule and integrate this with the monitoring plan, and microscopy centers to be assessed must be informed of the quality assurance scheme.
4.2 Once the slides are validated at the regional level, the Laboratory Technician doing QA/QC should submit the slides to the AMC HQ for final validation.
4.3 In districts where there is no Regional Malaria Officer or QA/QC Laboratories, the slides should be either submitted to the AMC HQ directly or to a designated QA/QC Laboratory in another region.
4.4 Two options can be considered for quality assurance based on the number of slides examined in a particular microscopy centre during the previous year (Table 1).

Table 1 Options and Frequency for Quality Assurance

Option	Option 1	Option 2
No. of slides examined during the previous year	> 240 slides	< 240 slides
Frequency	monthly	monthly
Mechanism of Quality Assurance	Submit slides monthly regardless of the result, all positive and at least 10 slides read as negative to be submitted to the Regional Malaria Office. Quality Assurance will be carried out initially by the Laboratory Technician doing QA/QC at regional level and later at AMC HQ. In addition, slide panel for proficiency assessment will be sent by the AMC HQ once a year.	Submit all slides examined monthly for quality assurance regardless of the result to the Regional Malaria Office. Quality Assurance will be carried out initially by the Laboratory Technician doing QA/QC at regional level and later at AMC HQ. In addition, slide panel for proficiency assessment will be sent by the AMC HQ twice a year.

4.5 Preparation and submission of blood smears for quality assurance

4.5.1 All slides examined and reported by the laboratory technician must be stored sequentially according to the serial numbers in a secure slide boxes with relevant copies of H/ AMC/P1 forms, protected from excessive heat and/or humidity until the slides for Quality Assurance have been selected.

4.5.2 All slides must be submitted monthly regardless of the results. Routine slides should not be washed or discarded until the QA/QC slides have been selected.

4.5.3 Laboratory Technician must prepare appropriate number of slides (of the previous month) based on selected option within the first week of the current month.

4.5.3.1 In option 1, slides will be selected randomly by getting every 10^{th} slides examined until 10 slides are selected. The first slide will be based on the number of the month (e.g. for the month of February – first slide is no. 2).

4.5.3.2 In option 2 and when less than 10 slides have been examined in a particular month, all slides have to be submitted.

4.5.4 Laboratory Technician must transfer the result of microscopy on form 1-Malaria blood smear submission form for validation following the instruction provided.

4.5.5 Laboratory Technician must ensure that the information provided in Form 1 regarding the serial number, code number and date is consistent with the information written on the slides.

4.5.6 Laboratory Technician should submit the accomplished Form 1 and copies of H/AMC/P1 forms in a sealed envelope together with the slides in the slide box to the Regional Malaria Officer /Parasitologist AMC.

4.6 Validation of blood smears

4.6.1 The Regional Malaria Officer/ Parasitologist AMC should keep the sealed envelope with him/her and handover the slide to the Laboratory Technician doing QA/QC for validation.

4.6.2 The Laboratory Technician/s doing QA/QC should validate the submitted blood smears and record the results on Form 2B according to the instructions given within 1 week and submit the results to the Regional Malaria Officer/ Parasitologist AMC.

4.6.3 Laboratory Technician/s doing QA/QC should also assess the quality of smears and stains and categorize the result as follows:

4.6.3.1 A good smear is determined by the quality of the thin and thick smears prepared in a given slide. A good quality smear requires that both thin and thick smears are properly prepared according to the relevant SOPs.

4.6.3.2 A good stained smear is determined by the quality of staining of the thin and thick smears in a given slide. A good stained smear requires that both thin and thick smears are properly stained according to the relevant SOPs.

4.6.3.3 Proper labelling should include the serial no, date and the code number.

4.6.4 Once the QA/QC technician submits the results, the Regional Malaria Officer/ Parasitologist AMC should open the sealed envelopes and copy the result from Columns12 – 14 of form 1 and transfer to column 12-14 of form 2B.

4.6.5 Regional Malaria Officer/ Parasitologist together with the Laboratory Technician/s doing QA/QC should compare the validated results with initial results and record any inconsistency.

4.6.6 The regional malaria officer should submit the validated slides together with regional QA/QC report to the AMC Central Laboratory by the 3rd week of the month.

4.7 Feed backing and Reporting of the results of validation to the microscopy centres

4.7.1 Feedback of the results of validation must be sent within one month from the receipt of the slides. The report must be endorsed by the head

or supervisor of the QA/QC laboratory before it is sent to relevant recipients.

4.7.2 The accomplished Form 2a must be sent back to the head of the facility and the laboratory technician concerned, together with the slide box for information and appropriate action. However, the discrepant slide should be saved for discussion with the laboratory technician.

4.7.3 Parasitologist/RMO AMC should provide a feedback report to the microscopy centers specifying the level of accuracy, percentage of false positive and false negative, stain quality, smear size and labeling including recommendations.

4.7.4 Parasitologist/Regional Malaria Officer and Laboratory Technician/s doing QA/QC must retain hard and electronic copies of the report must be maintained at central and regional QA/QC laboratories.

4.7.5 Laboratory Technician must file the validation report for future reference.

4.8 Follow up action based on the validation

4.8.1 If the number of slides with error is more than 50%, an immediate on-site supervisory visit must be conducted. For those with less than 5% error in any month, they must ensure to visit the facility at least once a year.

FORM 1

MALARIA BLOOD SMEAR SUBMISSION FORM FOR VALIDATION

(1) Month: _____ (2) Year: _____ (3) RMO Region/District: _____

(4) MOH area : _____ (5) Microscopy center/Facility Name: _____

(6) Type of facility:

(7) Total No. of slides submitted: /___/___/___/ (8)Total No. of Positive for the month: _____

(9) Total No. of slides examined for the month: _____

	(10) Slide ID No.	(11) Date Examined (dd/mm/yyyy)	Malaria Smear Result (12) Negative/Positive (If positive species and stages)	(13) Parasites/ µl blood (asexual stages)	(14) Parasit blood (sexu
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

Examined by: _____

(Signature over printed Name of Laboratory Technician

Date of Submission: _____

Noted by: _____

Head of the Facility

Guidelines for Laboratory Technician in accomplishing Form 1

1. Month – Write the month (Jan, Feb) of slide submission in the blank.
2. Year – Write the year (i.e., 2014) in the blank.
3. RMO region/District – Write the complete name of the RMO region/district
4. MOH area – Write the complete name of the MOH area.
5. Microscopy center / Facility Name – Write the complete name of the Microscopy center /Health Facility
6. Facility Type – Tick the appropriate description of the facility.
7. Total number of slides submitted- Write the exact number of slides submitted to the validator in the boxes (e.g. 25)
8. Total No. positive slides for the month
9. Total No. of slides examined
10. Slide ID Number – Write the ID number of the slide.

11. Date examined - Write the actual date the slide was examined by the laboratory technician
12. Species – Write the Plasmodium species seen. According to the SOP 8.
13. & 14 Parasite counts – should be done on the thick smear and indicated as no. of parasites/micorlitre
15. Remarks – Source of smears and any other comment

FORM 2b

MALARIA BLOOD SMEAR QUALITY ASSURANCE REPORT

(1) Month: _____ **(2)** Year: _____**(3)** District: _____
(4) MOH area: _____ **(5)** Microscopy Center: _____
(6) Facility Name: _____

(7) Type of the facility: _____
(8) Total No. of slides received: /___/__/___/ (9) No. of Positive slides received_____

	Initial Malaria Smear Result					SMEAR ASSESSMENT by QAQC Laboratory Technician								
	(10) Slide ID No.	(11) Date Examined (mm/dd/yyyy)	(12) Smear Result	(13) Parasite density (Asexual stages)	(14) Gametocyte density	(12a) Smear Result	(13a) Parasite density (Asexual stages)	(14a) Gametocyte density	(15) Smear Quality		(16) Quality of Staining		(17) labelling	(18) Remarks
									Thick	Thin	Thick	Thin		
1														
2														
3														
4														
5														
6														
7														
8														

9														
10														
Quality %														

Date slides received: _____

Date report sent to Head of facility/Laboratory Technician: _____

Report submitted by: _____
(Name and Signature)

(Note: Validator should accomplish Form 2b in duplicates – laboratory technician and file copy.)

Form 2b

1. Slide ID Number – Write the ID number as it appears on the slide.
2. Date examined - Write the actual date the slide was examined by the cross-checker.
3. Parasite counts –parasite counting has to be done on the thick smear.
4. Smear quality – Categorize as good, average, not accurate (refer SOP no.4).
5. Quality of staining - Categorize as grade 1,2,3,4, and 5 for thin and thin separately according to SOP no. 6.
6. Labelling - Categorize as good, average, not accurate (refer SOP no.4).
7. Remarks - Write down any relevant comments or observations regarding the smear, e.g., smear too thick or too small, blood film not fit for diagnosis, etc.

Anti Malaria Campaign (AMC) SOP for Malaria Microscopy					
SOP title:	**Feed backing of quality assurance of malaria microscopy**				
SOP No.	**10**	**Revision No.**	**0.0**	**Effective Date**	**01.01.2015**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**01.12.2014**
Approved:				**Date**	**19.12.2014**

1. **BACKGROUND:**
   Examination of malaria blood smears by microscopy remains the gold standard for the diagnosis of Malaria parasites. A high level of quality of smear preparation and staining will aid in providing accurate results. Quality assurance of smears and timely feedback of results are important components of Quality Assurance to evaluate the performance of laboratory technicians doing malaria microscopy, thus, ensuring quality services in the laboratory.

2. **PURPOSE:**
   To provide guidelines on feed back system for quality assurance programme of malaria microscopy.
3. **SCOPE:**
   This Standard Operating Procedure applies to all the Laboratory Technician involved in quality assurance of malaria microscopy.

4. **REQUIREMENTS:**
   4.1 Reporting Form
   4.2 Pen

FORM 2a

VALIDATION SUMMARY REPORT

Report prepared by:	
for the attention of: *(name of laboratory technician and head of laboratory/facility)*	
Date report prepared:	

SUMMARY OF RESULTS:

No. of slides received:			
Date received:			
Date of validation:			
Correct Diagnosis:	**No. of slides correctly diagnosed**	**Total No. of slides validated**	**% accuracy**
Wrong Diagnosis:	**No. of slides wrongly diagnosed**	**Total No. of slides validated**	**% error**

Interpretation of Results:

A Laboratory Technician with >20% error shall be visited by the QA/QC Laboratory Technician and supervisory officers for technical assistance and supervision.

Comments and recommendations of the QA/QC Laboratory Technician **/RMO/Parasitologist:**

_____

_____

_____

Schedule and slide selection scheme for next quality assurance:

_____

_____

____

Schedule of on-site visit (if applicable only):

_____

Name and Signature of the Validator

Name _____

Signature _____

Laboratory Technician doing quality assurance:		
Endorsement of the Director/Parasitologist/RMO:	_____	_____
Date:	_____	_____

Copies of report to: Head of Laboratory/Facility

(Note: Hard and electronic copies to be retained by the QA/QC Laboratory Technician.)

Guidelines for validators in accomplishing Forms 2a

1. QA/QC Laboratory Technician should prepare Form 2a in triplicate and original and a copy should be sent to the AMC HQ/ RMO within 1 month from the time the slides are received by the. The manner of sending reports must be decided upon during the orientation workshop (i.e., personal delivery, mail, etc.) together with the quality assured slides.
2. The comments may include a general assessment on the quality of smears and staining and suggestions on how to improve them, if necessary.
3. The Parasitologist/RMO should endorse the report including Form 2a before sending the feed back to the Laboratory Technician concerned together with the slides and slide box.
4. The schedule of slide submission and selection must be determined beforehand by the RMO and QA/QC Laboratory Technician and included in the report.
5. The report must be endorsed by the DAMC/supervisor of the QA/QC Laboratory / RMO before it is sent out to the proper recipients.
6. Hard and electronic copies of the reports have to be maintained at the QA/QC laboratories.

	Anti Malaria Campaign (AMC) **SOP for Malaria Microscopy**				
SOP title:	**On-site Supervisory and Monitoring Visits to Microscopy Centers**				
SOP No.	**11**	**Revision No.**	**0.0**	**Effective Date**	**01.01.2015**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**AMC**			**Date**	**01.12.2014**
Approved:				**Date**	**19.12.2014**

1. **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.

2. **PURPOSE:**
   To provide guidelines for technical supervision of Laboratory Technicians doing malaria microscopy.

3. **SCOPE:**
   This procedure applies to all officers doing technical supervision of laboratory technicians on malaria microscopy.

4. **MATERIALS, REAGENTS AND EQUIPMENTS:**
   4.1 Microscopy QA Form 3, On-site Supervisory Checklist
   4.2 Pen/pencil
   4.3 Reference slides for EQAS
   4.4 Notebook
   4.5 Calculator

5. **PROCEDURE:**
   5.1 Planning
   5.1.1 Set schedule (date, site/area to visit, person responsible)
   5.1.2 Send communication

   5.2 Conduct visit
   5.2.1 Courtesy call to head of facility
   Accomplish checklist (*QA Form 3)*
   5.2.2 **Interview** Laboratory Technician
   5.2.3 Conduct **inspection** of laboratory set-up, laboratory supplies/materials, reagents, equipment, documentation/logbook

5.2.4 **Evaluate performance** of laboratory technician in malaria microscopy

5.2.5 **Write** findings and recommendations

5.2.6 **Discuss** findings, comments and recommendations with the laboratory technician and head of facility.

5.3 Consolidate report(s) of monitoring/supervisory visit conducted

5.4 Submission of copies of all onsite supervisory check lists (Form 3a) and consolidated reports (Form 3b) to Director/AMC and RMO/AMC

5.5 Update database on QAQC

FORM 3a

On-site Supervisory Checklist

I. General Information

Name of the laboratory/facility		Date of supervision	
Type of facility			
Address of the laboratory/facility			
Tel/mobile		Fax	
Email			
Name of the facility head/director			
Name of the head of laboratory			
No. of technicians in the laboratory			
Name of the Laboratory Technician (supervised)			
Name of the PHFO			
Date of last training on malaria			
District, RMO Region & MOH area			

2.Workload

		OPD	Inward	ANC	Blood Bank	MMC	Other
Average per year	Number of slides received						
	Number examined						
	No. of slides assessed						
Previous month	Number of slides received						
	Number examined						
	No. of slides assessed						
Current month	Number of slides received						
	Number examined						
	No. of slides assessed						

On the day of supervision	Number of slides received						
	Number examined						
	No. of slides assessed						

3. Blood smear preparation

	No. of blood smears reviewed	Quality			
		good	average	not accurate	% of good smears
thick smear preparation					
thin smear preparation					
Labelling					
procedure of fixing					

4 a. Staining of blood smears - general procedures

Amount of working solution prepared	
working solution dilution ratio	
water source	
measuring method	
duration	
washing method	

4 b. Staining of blood smears -macroscopic appearance of stained blood smears

Type of blood smear	Thick blood smears	Thin blood smears
No. of smears reviewed		
macroscopic appearance of smear good		
Over stained		
Under stained		
% of good smears		

4 c. Staining of blood smears -microscopic appearance of stained blood smears

Type of blood smear	Thick smears	Thin blood smears
No. of smears reviewed		
Staining quality-grade 1		
Staining quality-grade 2		
Staining quality-grade 3		
Staining quality-grade 4		
Staining quality-grade 5		
Quality % (Grade 3&4/total)		

5.a. Examination of blood smears-validation

	No reviewed	accuracy
positive samples		
negative samples		
species identification		
no. of fields examined		

5.b. Examination of blood smears - Reference slides (EQAS)

No. of slides examined	
Sensitivity (%)	
Specificity (%)	

6. Performance of Rapid diagnostic test kits

Competency in performing RDT	
Competency in interpreting the result	
monthly requirement	
amount available	

7. Maintenance of returns and registers

	by PHLT	by PHFO	monthly requirement of forms	amount available	accuracy in maintenance
H/AMC/P1					
H/AMC/P4					
H/AMC/P5					
H/AMC/P15					
H/AMC/P16					

H/AMC/P17					
H/AMC/P18					
H/AMC/P19					
H/AMC/P20					
H/AMC/P21					
H/AMC/P22					
village register					
positive case register					

8. Maintenance/availability of stocks of laboratory items, chemicals and reagents

	monthly requirement	amount available	date received	date of expiry	storage condition	quality of reagents/goods
Giemsa stock solution						
Methanol						
anisole/immersion oil						
70% ethanol						
blood lancets						
glass slides used and cleaned						
glass slides new						
lens tissue						
buffered distilled water						
cotton wool						
tally counters						
slide box for storage						
chemicals for buffer solution						

9. availability of relevant documents

SOP	
lab manuals	
bench aids	
other	
stocks inventory book	

10.microscope

	model	year	eye pieces (magnification)	objective	wooden box	Anti static cover	mirror	power supply	maintenance
Microscope									
monocular									

11.biosafety measures

	monthly requirement	amount available	date received	storage condition	proper usage
Gloves					
lab coats					
sharps bins					
Containers for infectious materials					
Containers for dry waste/trash					

12. General Findings and Recommendations

_____
_____
_____
_____
_____
_____
_____
_____
_____

13. Comments of the Supervisor:

_____
_____
_____
_____
_____

Accomplished by:_____ _____
Name of Supervisor Signature of Supervisor

Date: _____

Form 3b

CONSOLIDATED REPORT OF ON-SITE SUPERVISORY AND MONITORING VISIT
District of _____

Facility	Date of Visit	General Findings	Actions Taken	Recommendations

Submitted by: **Noted by:**

Date:_____

	Anti Malaria Campaign (AMC) **SOP for Malaria Microscopy**				
SOP title:	**Conduct of Proficiency Assessment on microscopical examination of malaria**				
SOP No.	**12**	**Revision No.**	**0.0**	**Effective Date**	**01.01.2015**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**01.12.2014**
Approved:				**Date**	**19.12.2014**

1. PURPOSE:

To describe the procedure of conducting the proficiency assessment on microscopical examination of malaria

2. SCOPE:

This document applies to the proficiency assessment on microscopical examination of malaria conducted by the Anti-Malaria Campaign**.**

3. POLICY/PRINCIPLE:

3.1 POLICY

3.1.1 This activity shall be performed in accordance with quality assurance procedure of malaria microscopy in Sri-Lanka. National Core Group of Trainer in Malaria Microscopy will be involved in training.

3.1.2 The following are the intended participants of this assessment:

3.1.2.1. Laboratory Technicians who have obtained grading under Class B and C classification at the previous proficiency assessment.

3.1.2.2 Laboratory Technicians who have received panel testing slides prior to the attendance to the course

3.1.2.3 Laboratory Technicians who have attended refresher training course

3.1.3. This procedure shall be reviewed annually; any deviation from the procedures outlined shall be recorded and reported.

3.2 PRINCIPLE:

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

4. MATERIALS, EQUIPMENT AND REAGENTS:

4.1 Materials

4.1.1 Timetable
4.1.2 Assessment kit (Bag or folder, notebook, Pen and Pencil)
4.1.3 Syllabus
4.1.4 Answer sheet/reporting form (¼ sheet of papers, one each per participant per slide)
4.1.5 Profile of participant
4.1.6 Pretest exam
4.1.7 Evaluation form
4.1.8 Assessment slides
4.1.9 Practice slides
4.1.10 Tissue paper/ Lens paper
4.1.11 Tally counters
4.1.12 Calculator
4.1.13 Bench Aids and Learners Guide

4.2 Reagents

4.2.1 Immersion Oil

4.3 Equipment

4.3.1 Binocular microscope

5. PROCEDURES:

5.1 Prepare training materials and equipment for the training.

5.2 Actual conduct of the course

5.2.1 Discuss the mechanics of the assessment before the assessment proper.

5.2.2 The assessment will be conducted over a five-day period from 8:00AM to 5:00PM.

5.2.3 The assessment will consist of six microscopy test series which will start on the 2nd day until the 5th day. It will be conducted under "examination" condition; therefore, no conferring of results among the participants will be allowed. Furthermore, no consultation with the facilitators will be entertained while the test series is going on.

5.2.4 The participants will read a total of 50 slides for species identification and 15 slides for parasite counting.

5.2.5 Slides are color coded as to whether for species identification or for parasite counting. Slides for parasite counting are all P. *falciparum* with varied densities.

5.2.6 Participants shall be given 10 minutes to examine a slide.

5.2.7 The participants will be writing the answers in ¼ sheet of papers which will be collected by the facilitator after ten minutes.

5.2.8 Results and feedback will be given to the participants after every test series examination.

5.3 Conduct and follow activities as per timetable.

5.4 Slide composition for the assessment:

P. falciparum	High	>5,000	5
	Medium	>500-4,900	10
	Low	<500	5
P. vivax	High	>5,000	5
	Medium	>500-4,900	5
	Low	<500	5
negative slides			11
P.malariae		Count depends on the Availability of the slide	2
P. ovale		Count depends on the Availability of the slide	1
Mixed		Count depends on the Availability of the slide	1
Total Species identification 50			
P. falciparum	High	>5,000	5
	Medium	>500-4,900	5
	Low	<500	5
Total Parasite counting 15			

5.5 Grading system

The following shall be used as basis for grading the results:

Species score:

Correct species/negative	2 points
Only 1 correct species in the mixed infection	1 point
Wrong species	0 point
Positive to Negative / Negative to positive	0 point

Parasite count: 1 point (+/- 25%)

5.5 Certification

5.5.1 Certificate of Proficiency (Grade A)
ID score ≥ 90%
Counting ≥ 40%

5.5.2 Certificate of Proficiency (Grade B)
ID score 80-89%
Counting 20-39%

5.5.3 Certificate of Proficiency (Grade C)
ID score < 80%
Counting <20%

5.6 Recommendations

5.6.1 Participants will be classified as Grade **A** with accuracy ≥ 90% for species identification and ≥ 40% in parasite counting. Recommended for Training as Trainer /Facilitator or Quality Assurance/validator or Reference Microscopists. This participant will receive panel testing using unknown slides once a year and regular proficiency assessment (at least every 3 years).

5.6.2 Participants will be classified as Grade **B with** accuracy of 80%-89% in species identification and 20-39% in parasite counting. Recommended for continuing provision of malaria microscopy services and may serve as an Interim Cross checker with portion of validated slides to be cross checked by national/regional cross checker (Grade A). Refresher training for cross checker or mentoring to be provided by Grade A Microscopist; Panel testing using unknown slides at least twice (or more) a year; .

5.6.3 Participants will be classified as Grade C with results of <80% in species identification and less than 20% in parasite counting. This participant will be recommended to take refresher course in Malaria Microscopy.

5.7 At the end of the assessment report card will be given to the participants, duplicate copy of report card will be sent to the AMC director attention to RMO (Regional Malaria Officer).

5.8 Validity period for the certification would be 3 years after the proficiency assessment

5.9 evaluation of the assessment

At the end of the assessment, participants' evaluation will be administered and analyzed for further improvement of the assessment.

Computation for participant's assessment evaluation of the assessment.

Simple and easy method in computing the responses to the questionnaire:

Example: Overall, the teaching methods used in this training course were effective.

1	2	4	5
	II	IIII	IIII
		IIII	IIII
			IIII
			III

Multiply the number of answer by the corresponding coefficient:

(2x2)+ (10x4) + (18x5) =134

Calculate the satisfactory index as percentage. For the above example, the number 134 is multiplied by 20 divide by the number of responded.

Inform the participants on the result of the final evaluation on the last day of assessment.

Anti Malaria Campaign (AMC) SOP for Malaria Microscopy					
SOP title:	**Assessment of competency of trainee laboratory technician on microscopical examination of malaria**				
SOP No.	**13**	**Revision No.**	**0.0**	**Effective Date**	**01.01.2015**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**01.12.2014**
Approved:				**Date**	**19.12.2014**

1. BACKGROUND:

Microscopy, being the 'gold standard' for malaria laboratory diagnosis requires satisfactory skills and knowledge for accuracy. Competency based training ensures that trainees reach a satisfactory level of competence in each of the designated skills.

2. PURPOSE:

Assessment of competency of trainee laboratory technicians on microscopical examination of malaria

3. SCOPE:

This guideline applies to all Trainee Laboratory Technicians undergoing training of at least 2-month duration on malaria microscopy organized by the Anti Malaria Campaign/any recognized university or organization.

4. MATERIALS, EQUIPMENT AND REAGENTS

4.1 Materials:

4.1.1 Training kit (Bag, notebook, Pen and Pencil)
4.1.2 Timetable
4.1.3 Syllabus
4.1.4 Answer sheet/reporting form
4.1.5 Profile of participant
4.1.6 Pretest exam and posttest (Theoretical exam)
4.1.7 Evaluation form
4.1.8 Training slides
4.1.9 Practice slides
4.1.10 Tissue paper/ Lens paper
4.1.11 Bench Aids and Learners Guide
4.1.12 Cotton Balls
4.1.13 Lancet

4.2 General Equipment

4.2.1 Binocular microscope
4.2.2 Multi-viewer microscope (optional)
4.2.3 Tally counters
4.2.4 Timer
4.2.5 Calculator

4.3 Plastic and glassware

4.3.1 Plastic basin for washing glassware (graduated cylinder and coplin jar)
4.3.2 Plastic slide box
4.3.3 Graduated Plastic Pipettes (1ml)
4.3.4 Graduated Cylinder (10ml and 100ml)
4.3.5 Staining jar
4.3.6 Staining trough
4.3.7 Glass slides

4.4 Reagents

4.4.1 Giemsa stock solution
4.4.2 Buffered distilled water (pH 7.2)
4.4.3 Immersion Oil
4.4.4 Methanol
4.4.5 70% isopropyl Alcohol
4.4.6 Detergent

5. PROCEDURES:

5.1 Prepare training materials and equipment for the training.
5.2 Conduct activity as per timetable
5.3 Evaluation of the responses of the participants at the end of the training

6. GUIDELINES:

6.1 Slide composition for microscopy series (%):

35 negative slides
30 *Pf*
25 *Pv*
5 Pm /Po / other species
5 Mixed

6.2 Grading system

The following shall be used as basis for grading the results:

6.2.1 Theoretical Examination (5%) Written theoretical examination of 20 items shall be given before the lectures are presented. This will show the trainers which area in the training will be given enough time and focus

Another written theoretical examination shall be given at the middle of the course.

The same written theoretical examinations that were given at the start will be given at the end of the training. This will show if the participants gained knowledge in malaria microscopy. Twenty minutes is given to answer the multiple- choice questions. This weighs 5% in the final grade of the participants.

6.2.2 Slide Spot test (15%)

This is a 15 slide, move system practical examination.

i. Thick and thin blood smears are focused in each microscope. Different parasite species in their different stages either in thick or thin smears will be focused.

ii. Participants should be able to diagnose the smear in 90 seconds before moving to the next station.

iii. If the parasite and its stage is identified correctly it will be given a score of two points. One point if only correct parasite species but wrong parasite stage.

iv. Participants should not move the Coarse adjustment knob, only the fine adjustment and the eyepiece adjustment can be used.

6.2.3 Microscopy Test Series (80%)

Sets of slides in 10's and 15's shall be given in a series of seven days.

Each set of slides is composed of 30% *P. falciparum*, 35% Negative, 25% *P. vivax*, and 5% *P. malariae & and P. ovale* and 5% mixed infection. Results of these slides should be standardized by 3 members of the national Core group of microscopists and by PCR.

After each set, there will be a period for feedback and evaluation. Participants will have the chance to defend their answers and re-examines their slides which they diagnosed inaccurately.

Scoring:

Correct diagnosis	2 points
False negative/ positive	0
1 correct species in mixed infection	1 point
Misidentification	0

Participant's accuracy shall be monitored in each set.

This test series will weigh 50% in the final grade.

b. Computation for participant's assessment evaluation.

Simple and easy method in computing the responses to the questionnaire:

Example: Overall, the teaching methods used in this training course were effective.

1	2	4	5
	II	IIII	IIII
		IIII	IIII
			IIII
			III

Multiply the number of answer by the corresponding coefficient:

(2x2)+ (10x4) + (18x5) =134

Calculate the satisfactory index as percentage. For the above example, the number 134 is multiplied by 20 divide by the number of responded.

Inform the participants on the result of the final evaluation on the last day of assessment.

	Anti Malaria Campaign **SOP for Malaria Microscopy**				
SOP title:	**Conduct of Refresher Training on microscopical examination of malaria**				
SOP No.	**14**	**Revision No.**	**0.0**	**Effective Date**	**01.01.2015**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**01.12.2014**
Approved:				**Date**	**19.12.2014**

1. PURPOSE:

The purpose of this procedure is to describe the procedure for conducting refresher training for trained laboratory technicians in malaria microscopy.

2. **SCOPE:**

This document applies to refresher training conducted by the Anti Malaria Campaign on malaria microscopy.

3. **POLICY/PRINCIPLE:**

3.1 POLICY

3.1.1 This activity shall be performed in accordance with the procedure for quality assurance of malaria microscopy in Sri Lanka.

3.1.2 Members of the National Core Group of Trainers and Validators shall be involved in conducting this activity.

3.1.3 Intended participants for the training will be laboratory technicians who have had a basic training on malaria microscopy. Priority should be given to laboratory technicians showing poor performances in validation and panel testing conducted by the Anti Malaria Campaign and those who have not received an in-service training during the past 3 years or more.

3.1.4. This procedure shall be reviewed annually; any deviation from the procedures outlined shall be recorded and reported.

3.1.5. Duration of the training – ideally 5 days

3.1.6. Number of participants per training would ideally be 15 to 20.

3.2 PRINCIPLE

The main objective of the refresher training is to maintain a high level of proficiency among Laboratory technicians in malaria microscopy and update their knowledge on malaria microscopy

4. MATERIALS, EQUIPMENT AND REAGENTS

4.1 Materials

4.1.1 Training kit (Bag, notebook, Pen and Pencil)
4.1.2 Timetable
4.1.3 Syllabus
4.1.4 Answer sheet/reporting form
4.1.5 Profile of participant
4.1.6 Pretest exam and posttest (Theoretical exam)
4.1.7 Evaluation form
4.1.8 Training slides
4.1.9 Practice slides
4.1.10 Tissue paper/ Lens paper
4.1.11 Bench Aids and Learners Guide
4.1.12 tally counters
4.1.13 Timer
4.1.14 Calculator

4.2 General Equipment

4.2.1 Binocular microscope

4.3 Plastic and glassware

4.3.1 Plastic basin for washing glassware (graduated cylinder and coplin jar)
4.3.2 Plastic slide box
4.3.3 Graduated Plastic Pipettes (1ml)
4.3.4 Graduated Cylinder (10ml and 100ml)
4.3.5 Staining jar
4.3.6 Staining trough
4.3.7 Glass slides

4.4 Reagents and other items

4.4.1 Giemsa stock solution
4.4.2 Buffered distilled water
4.4.3 Immersion oil
4.4.4 Methanol
4.4.5 70% isopropyl Alcohol

4.4.6 Powdered detergent
4.4.7 Lancet
4.4.8 Cotton balls

5. PROCEDURES:

5.1 Prepare training materials and equipment for the training.
5.2 Conduct activity as per timetable
5.3 At the end of the training, participant's evaluation will be analyzed for further improvement of the training.

6. GUIDELINES:

6.1 Percentage slide composition for microscopy series:
30 *Pf*
12 Pv
2 Pm
2 Mixed
5 negatives
Po and other spp. depending on availability

6.2 Grading system
The following shall be used as basis for grading the results:

6.2.1 Theoretical Examination (5%)

6.2.1.1 Written theoretical examination of 10 items (duration of 20 minutes) shall be given on the first day before the lectures are presented. This will show the trainers which area in the training will be given enough time and focus.
6.2.1.2 The same written theoretical examinations that were given at the start will be given at the end of the training. This will show if the participants gained knowledge in malaria microscopy. Twenty minutes is given to answer the multiple- choice questions. This weighs 5% in the final grade of the participants.

6.2.2 Slide Spot Test (5%)

This is a 15 slide, move system practical examination.
6.2.2.1 Thick and thin blood smears are focused in each microscope. Different parasite species in their different stages either in thick or thin smears will be focused.
6.2.2.2 Participants should be able to diagnose the smear in 90 seconds before moving to the next station.

6.2.2.3 If the parasite and its stage are identified correctly it will be given a score of two points. One point if only correct parasite species but wrong parasite stage.

6.2.2.4 Participants should not move the feed knob, only the fine adjustment and the eyepiece adjustment.

6.2.3 Microscopy Test Series (90%)

6.2.3.1 The microscopy series will consist of 10 slides per test which will start on the 2nd day until the 4th day. It will be conducted under "examination" condition; therefore, no conferring of results among the participants will be allowed. Furthermore, no consultation with the facilitators will be entertained while the test series is going on.

6.2.3.2 The participants will read a total of 50 slides for species identification. Parasite counting will be done on the 4th and 5th series for all positive slides

6.2.3.3 Participants shall be given a 10 minute per slide to make a species diagnosis and parasite count.

6.2.3.4 The participants have to write answers on a ¼ sheet of paper which will be collected by the facilitator after ten minutes.

6.2.3.5 Results and feedback will be given to the participants after every test series examination.

6.2.3.6 The following will be used in grading the result

Correct species/negative	2 points
Only 1 correct species in the mixed infection	1 point
Wrong species	0 point
Positive to Negative / Negative to positive	0 point

6.3 Certification upon completion of the training will be based on the overall rating of a particular participant and will be of 2 types.

6.4.1 If overall rating is $\geq$ 80% a Certificate of Completion will be given
If overall rating is < 80% a Certificate of Participation will be given

6.4 Recommendations

6.4.1 Laboratory technicians with $\geq$80% overall rating will perform malaria microscopy and should submit slides for validation. They should participate/ continue to participate in EQA programs for malaria

6.4.2 Laboratory technicians with <80% overall rating can perform malaria microscopy and should submit slides for validation. They will be reassessed through panel testing within 3 months, and will be subjected to refresher training based on the results. In addition,

they should participate/ continue to participate in EQA programs for malaria.

6.5 Report card

At the end of the training, a report card will be given to the participants; duplicate copy will be sent to the Head of Institution.

6.6 Computation for evaluation of participant's assessment of the training.

Simple and easy method in computing the responses to the questionnaire:

Example: Overall, the teaching methods used in this training course were effective.

1	2	4	5
	II	IIII	IIII
		IIII	IIII
			IIII
			III

Multiply the number of answer by the corresponding coefficient:

(2x2) + (10x4) + (18x5) =134

Calculate the satisfactory index as percentage. For the above example, the number 134 is multi plied by 20 divide by the number of responded.

Inform the participants on the result of the final evaluation on the last day of assessment.