World Health Organization

COLLECTION OF FINGER-PRICK BLOOD AND PREPARATION OF THICK AND THIN BLOOD FILMS

MALARIA MICROSCOPY STANDARD OPERATING PROCEDURE – MM-SOP-05A

1. PURPOSE AND SCOPE

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

This procedure is to be modified only with the approval of the national coordinator for quality assurance of malaria microscopy. All procedures specified herein are mandatory for all malaria microscopists working in national reference laboratories, in hospital laboratories or in basic health laboratories in health facilities performing malaria 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. SUPPLIES AND MATERIALS

- cleaned glass slides, 25 x 75 mm, with one frosted end for labelling, preferably with ground edges, and of good quality (See MM-SOP-01: Cleaning and storing microscope slides);
- 70% ethyl alcohol or alcohol swabs;
- 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;
- record forms (i.e. malaria register) and
- a lead pencil or permanent marker pen.

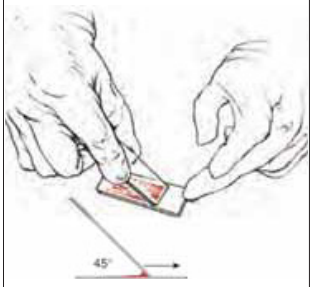
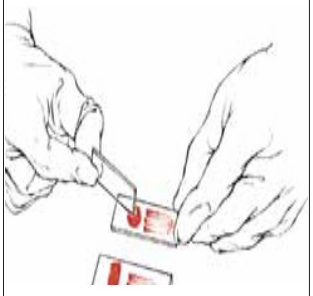
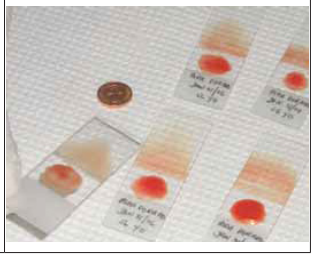
4. SAFETY PRECAUTIONS

- 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. Wear gloves when handling blood, and remove them before leaving the work area or when writing notes.
- Always use a new lancet for each patient. Do not recap the lancets. **Never** re-use lancets.
- Avoid getting blood, wet or dry, on your fingers or hands.
- Cover cuts or abrasions on your hands with a waterproof dressing.
- Avoid accidentally pricking yourself when handling sharp instruments that have been in contact with blood.

- Thoroughly wash your hands with soap and water as soon as you finish a job.
- If you get blood on your skin, quickly wipe it off with a cotton swab dampened with alcohol; then wash the affected area with soap and water as soon as possible.
- Sharps such as lancets and broken glass must be discarded in a "sharps" container for safe disposal by incineration or autoclaving. Materials that are not sharp but are contaminated with blood must be discarded in a covered pan or autoclave bag for safe disposal by incineration or autoclaving.
- Collect blood in an area where there is proper lighting.

5. PROCEDURE

FLOW CHART	DESCRIPTION OF ACTIVITY	ILLUSTRATION
1. Label the slide with the patient's details and record in the register.	1. Label the frosted end of the glass slide with the patient's details, and document in record form or a malaria register. See SOP 06: Labelling malaria blood films.	
2–3. Wearing latex gloves, clean the third finger from the thumb with 70% ethanol or an alcohol swab. Let the finger dry in air.	2. Wearing protective latex gloves, select the third finger from the thumb of the non-dominant hand (or big toe for infants, not the heel). Do not use the thumb for either children or adults. 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.	
4. Prick the finger with a new, sterile lancet.	4. Using a new, sterile lancet and a quick rolling action, puncture the centre of the ball of the finger or toe.	
5–6. Express the first drop of blood and wipe it off with dry cotton.	5. Apply gentle pressure to the finger (or toe), and express the first drop of blood. 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.	
7. Express and touch the blood with the slide to collect a small drop of blood, and use it to make the thin film.	7. Working quickly and handling the slides only by the edges, 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.	

FLOW CHART	DESCRIPTION OF ACTIVITY	ILLUSTRATION
8. Collect two or three more small drops of blood, and use them to make the thick film. ↓ 9. Wipe the remaining blood from the finger. ↓ 10. Place the slide with the blood facing up on a flat surface. ↓ 11–13. Using a clean "spreader" slide, make the thin film by pushing forwards the one drop of blood in a smooth, continuous motion. ↓ 14. With the corner of the "spreader", make the thick film by swirling the three drops of blood together to form a circle. ↓ 15. Air-dry in a horizontal position. A slide dryer may be used if rapid drying is required.	8. Apply further gentle pressure to express more blood, and collect two or three drops on the slide about 1 cm from the drop intended for the thin film. 9. Wipe the remaining blood from the finger with clean, dry cotton. 10. Do not pause between applying and spreading the drops. Prepare the blood films with the slide lying on a flat surface. 11. **To prepare the thin film**, place the edge of a clean "spreader" slide at 45° in front of the blood drop intended for the thin film. 12. Slowly pull the "spreader" back until it touches the drop of blood and the blood spreads along the edge of the "spreader". 13. 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. 14. 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. Do not stir the blood. A circular or rectangular film can be made by three to six quick strokes with the corner of the spreader. 15. After preparing the thin and thick blood films, allow them to dry in air in a horizontal position on a slide tray. If rapid drying is required, dry the films with low heat from a hair-dryer for 5 s, at a distance of 30 cm. Do not place the slides too close to the dryer, as the films might become heat fixed.	   

6. PROCEDURE NOTES

- The thick film should be dried flat and be protected from dust and flies.
- The thick film may autofixate if exposed to extreme heat and should therefore be stained immediately.
- The thick film can be dried gently with a hair-dryer set at warm or another drying method, but care must be taken to avoid heat fixation, which can occur quickly. Issue a hair-dryer only to technicians who have demonstrated competence with this method.
- Do not use a ballpoint or gel pen to label slides, as the ink will spread when the film is fixed.
- Correctly made slides leave little blood on the spreader slide, which can be used for making thick and thin slides from the next patient, while another, clean slide from the package is used as a fresh spreader. Do not use a slide as a spreader more than once.

7. RELATED SOPs

MM-SOP-01: Cleaning and storing microscope slides

MM-SOP-06: Labelling malaria blood films

MM-SOP-11: General safety procedures in the malaria microscopy laboratory

MM-SOP-13 Management of wastes generated from malaria diagnostic tests

8. REFERENCE

WHO. Basic malaria microscopy. Part I. Learner's guide. Second edition. Geneva: 2010.

9. DOCUMENT HISTORY

Date (mmm/yyyy)	Version	Comments	Responsible person (First name, last name)
Jan 2016	1	Reviewed and finalized by experts, edited and formatted	Glenda Gonzales, Technical Officer, WPRO