World Health Organization

COLLECTION OF BLOOD BY VENIPUNCTURE AND PREPARATION OF BLOOD FILMS FROM VENOUS BLOOD COLLECTED IN TUBES CONTAINING ANTICOAGULANT

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

1. PURPOSE AND SCOPE

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

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

In some health facilities, venous blood samples are collected for multiple analyses, which may include malaria diagnosis by microscopy. Larger volumes of venous blood are collected than in finger-prick samples, and they are stored in tubes containing anticoagulant (preferably ethylenediaminetetraacetic acid [EDTA]). Venous blood samples treated with EDTA can be used to prepare thin and thick blood films for malaria diagnosis with the procedures described in this document.

3. SUPPLIES, MATERIALS AND EQUIPMENT

- 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);
- a syringe with needle (gauge 21 or 23), one per patient;
- a vacuum tube containing an anticoagulant such as EDTA, 4–5-mL capacity, one per patient;
- 70% ethyl alcohol or alcohol swabs;
- dry cotton (cotton ball, swab or gauze);
- protective latex gloves (powder-free);
- a tourniquet;
- 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 micro-pipette;
- pipette tips;
- a smear preparation template;
- 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 might interfere with smear preparation. Wear gloves when handling blood, and remove them before leaving the work area or when writing notes.
- Always use a new needle and syringe for each patient.
- 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.

5. PROCEDURE

FLOW CHART	DESCRIPTION OF ACTIVITY
4.1. Collection of blood sample by venipuncture	**4.1. Collection of blood sample by venipuncture**
1. Label an EDTA-containing tube according to MM-SOP 6a: Labelling malaria blood films.	1. Label an EDTA-containing tube with the patient's name, date and time of collection. See MM-SOP 6a: Labelling malaria blood films.
2–3. Apply a tourniquet on the upper arm of the patient, and look for a large, minimally moveable vein.	2. Apply a tourniquet on the upper arm of the patient to enable the veins to be seen or felt. Ask the patient to make a tight fist so that the veins are more prominent. 3. With your index finger, feel for a sufficiently large, minimally moveable vein.
4–5. Disinfect the site with alcohol, and allow to dry in air.	4. Disinfect the site with an alcohol swab or cotton dampened with 70% isopropyl or ethyl alcohol. Do not touch the cleansed area again. 5. Allow the venipuncture site to dry in air for 30 s to ensure that the blood sample collected is not contaminated with alcohol, which can lead to haemolysis.
6. Insert the needle (attached to a syringe or vacutainer tube), and steadily draw blood.	6. Insert a sterile, non-reusable phlebotomy needle (attached to a syringe or vacutainer tube) along the line of the vein, with the bevel of the needle facing directly upwards. Steadily draw > 2 mL to 4 mL of blood. *Note: Anticoagulants may interfere with adhesion of blood to the slide and with Giemsa staining, especially if the ratio of blood to anticoagulant is not optimal. Hence, the volume of blood in a 5-mL EDTA tube should be > 2 mL.*
7. Release the tourniquet, remove needle and press firmly on the venipuncture site with a piece of dry cotton.	7. When enough blood has been collected, release the tourniquet, and instruct the patient to open his or her fist. Remove the needle, and press a piece of dry cotton firmly on the venipuncture site. Instruct the patient to continue pressing on the puncture site with the arm raised until any bleeding stops.
8. Transfer the blood to the EDTA-containing tube, and mix gently by inverting the tube six times.	8. Transfer the blood to the EDTA-containing tube, and mix gently by inverting the tube six times. Do not shake the tube.

FLOW CHART	DESCRIPTION OF ACTIVITY
4.2. Preparation of thick and thin blood films	**4.2. Preparation of thick and thin blood films**
1. Gently mix the blood in an EDTA-containing tube before use.	1. Gently mix collected venous blood in a vacuum tube containing EDTA before use.
2. Place a clean, labelled microscope slide on a malaria slide preparation template (see Fig. 1).	2. Place a clean, labelled microscope slide on a standardized thick (1.2 cm or 12 mm in diameter) and thin blood film-slide preparation template (see Fig. 1).
3. Use a micropipette with a fitted tip to transfer 6 µL of blood onto the larger circle of the slide, and prepare the thick film.	3. Transfer 6 µL of blood with a micropipette and fitted tip onto the larger circle of the slide. With the tip of the micropipette, swirl the blood, making a circle about 1 cm in diameter, and **prepare the thick film**; three to six quick strokes with the tip are sufficient.
4. Using a micropipette with a fitted tip, transfer an additional 2 µL of blood onto the smaller circle of the slide, and prepare the thin film.	4. Collect an additional 2 µL of blood with a micropipette and fitted tip, and transfer the blood onto the small circle of the slide template.
5–7. Using a clean "spreader" slide, make the thin film first by pushing the one drop of blood forward in a smooth, continuous motion.	5. **To prepare the thin film**, place the edge of a clean "spreader" slide at a 45^0 in front of the blood drop intended for the thin film. 6. Slowly pull the "spreader" back until it touches the drop of blood and the blood spreads along the edge of the spreader. 7. Rapidly push the spreader forwards (away from the centre) in a smooth, continuous motion, until the spreader leaves a "feathery" end for the thin film.
8. Air-dry in a horizontal position. A slide dryer may be used if rapid drying is required.	8. Dry the prepared slides horizontally. Poor adherence is a problem with EDTA-treated blood. If rapid drying is required, dry the films with low heat from a hair-dryer at some distance. Do not place the blood films too close, as the film might be fixed by heat.

6. PROCEDURE NOTES

- Blood collected in EDTA-containing tubes should not be kept for more than 4 h before preparing thick and thin blood films, because the anticoagulant will affect the morphology of the parasite
- If venous blood is collected elsewhere than in the laboratory where the thick and thin blood films will be prepared, it must be transported immediately (ideally within 1 h), without cooling or refrigeration, to the laboratory.
- Thick films should be dried flat and be protected from dust and flies.
- Thick films may be autofixated if exposed to extreme heat; they should therefore be stained immediately.
- A thick film can be gently dried with a hair-dryer set at warm or by other methods, but care must be taken to avoid heat fixation, which can occur quickly. Issue a hair-dryer to technicians with demonstrated competence in use of 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, 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.

7. RELATED SOPs

MM-SOP-01: Cleaning and storing microscope slides

MM-SOP-06a: Labelling malaria blood films

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

8. REFERENCES

WHO. Malaria microscopy quality assurance manual. Version 2. Geneva: 2015.

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