

PREPARATION OF BLOOD SPOTS ON FILTER PAPER

MALARIA MICROSCOPY STANDARD OPERATING PROCEDURE – MM-SOP-10

1. PURPOSE AND SCOPE

To describe the procedure for preparing dried blood spots on filter paper suitable for DNA analysis.

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

Nucleic acid testing with the polymerase chain reaction (PCR) is more sensitive for malaria diagnosis than microscopy, particularly for mixed infections and low parasitaemia. Some samples in the laboratory might have to be analysed by PCR to confirm species and mixed infections.

Blood spots collected on filter paper can also be used for parasite strain genotyping to distinguish infections due to local transmission from imported cases in settings of malaria elimination.

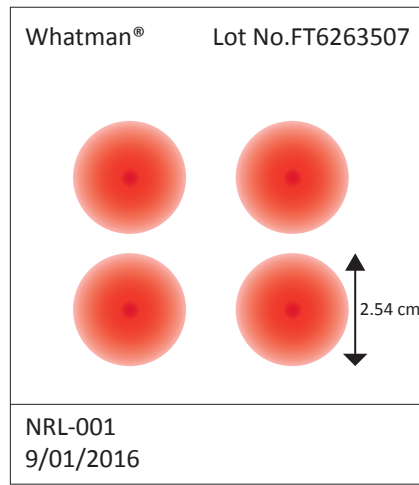
3. SUPPLIES, MATERIALS AND EQUIPMENT

- filter paper (Whatman “fast transient analysis” (FTA), Whatman 2 or 3 or Whatman 903) to allow both genotyping and serological analysis;
- a micropipette, volume capacity 20–200 µL, and fitted disposable tips;
- ziplock plastic bags, one for each patient;
- desiccant without cobalt chloride (those left-over from recently opened rapid diagnostic test pouches can be used, but there must be no colour change; otherwise, purchase desiccant commercially);
- fresh whole blood or blood with anticoagulant (EDTA, sodium citrate, citrate dextrose or heparin);
- a refrigerator at 4 °C (for samples to be examined within 4 weeks);
- a freezer at –20 °C (for samples to be examined within 3 months) and
- a deep-freezer at –80 °C (for samples to be examined in > 1 year).

4. PROCEDURE

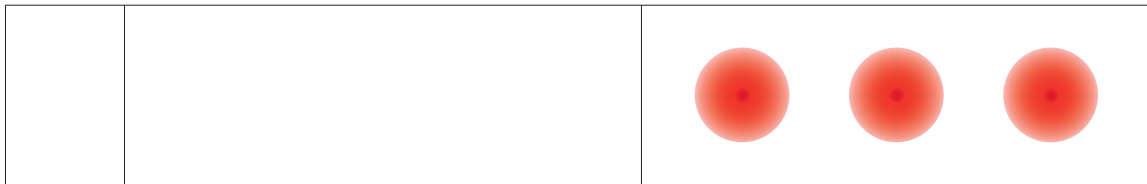
FLOW CHART	DESCRIPTION OF ACTIVITY
<pre> graph TD A([1. Label the filter paper. Refer to MM-SOP 06b: Labelling malaria blood films]) --> B[2. If you are using Whatman FTA card, pipette out 125 µL of blood, and drop it onto the centre of the circle.] B --> C[OR] C --> D[3. If using Whatman 2, 3 or 903, transfer two or three drops of 40–50 µL of a with the pipette onto the designated area.] D --> E[4. The samples applied to filter paper are ready for immediate storage at room temperature.] E --> F[5. Allow the sample to dry for at least 1 h at room temperature before placing it in a plastic ziplock bag.] F --> G[6. Add desiccant inside each ziplock bag containing filter paper samples.] G --> H[7. Ensure that the storage space is completely dry. Check desiccants regularly, and change if necessary.] H --> I([8. If samples are processed within 4 weeks, keep in the refrigerator at 4 °C. For longer storage, keep at –20 °C or –80 °C.]) </pre> <p>1. Label the filter paper. Refer to MM-SOP 06b: Labelling malaria blood films</p> <p>2. If you are using Whatman FTA card, pipette out 125 µL of blood, and drop it onto the centre of the circle.</p> <p>OR</p> <p>3. If using Whatman 2, 3 or 903, transfer two or three drops of 40–50 µL of a with the pipette onto the designated area.</p> <p>4. The samples applied to filter paper are ready for immediate storage at room temperature.</p> <p>5. Allow the sample to dry for at least 1 h at room temperature before placing it in a plastic ziplock bag.</p> <p>6. Add desiccant inside each ziplock bag containing filter paper samples.</p> <p>7. Ensure that the storage space is completely dry. Check desiccants regularly, and change if necessary.</p> <p>8. If samples are processed within 4 weeks, keep in the refrigerator at 4 °C. For longer storage, keep at –20 °C or –80 °C.</p>	<ol style="list-style-type: none"> 1. Label the filter paper with the laboratory code, patient identification or code and date of blood collection (refer to MM-SOP 06a: Labelling malaria blood films). Optionally, you may write the initials of the technician responsible for collection. See Fig. 1. 2. If you are using Whatman FTA cards, pipette out 125 µL of blood (per 2.54-cm circle) from the blood collecting tube, and drop it in a concentric circular motion onto the centre printed circle area on the card, with at least two circle areas for each patient. Avoid “puddling” the liquid sample, as it will overload chemicals onto the card. Also, do not rub or smear the blood onto the card. 3. If you are using Whatman 2, 3 or 903 filter paper, pipette out 40–50 µL of blood from the blood collecting tube, and drop it onto the area designated for the spots. Prepare two or three blood spots for each patient. See Figs 2 and 3. 4. The samples applied to filter paper are ready for immediate storage at room temperature. 5. If the sample is to be examined shortly after application to the filter paper, allow it to dry for at least 1 h at room temperature before placing it in a plastic ziplock bag. Do not apply heat to shorten the drying period. Dried blood spots will appear darker than freshly spotted ones. 6. Add desiccant inside each ziplock plastic bag containing filter paper samples. Store and transport the dried samples at room temperature, with the desiccant. 7. Ensure completely dry storage conditions (check the desiccants regularly for any colour change indicating exposure to moisture, and change if necessary). 8. If samples are examined over a 4-week period, keep them in a refrigerator at 4 °C. For longer storage, keep them at –20 °C or –80 °C.

Fig. 1. Dried blood spots on Whatman FTA card filter paper

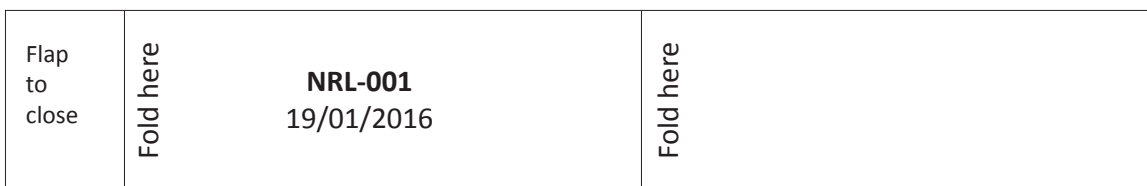


NRL-001
9/01/2016

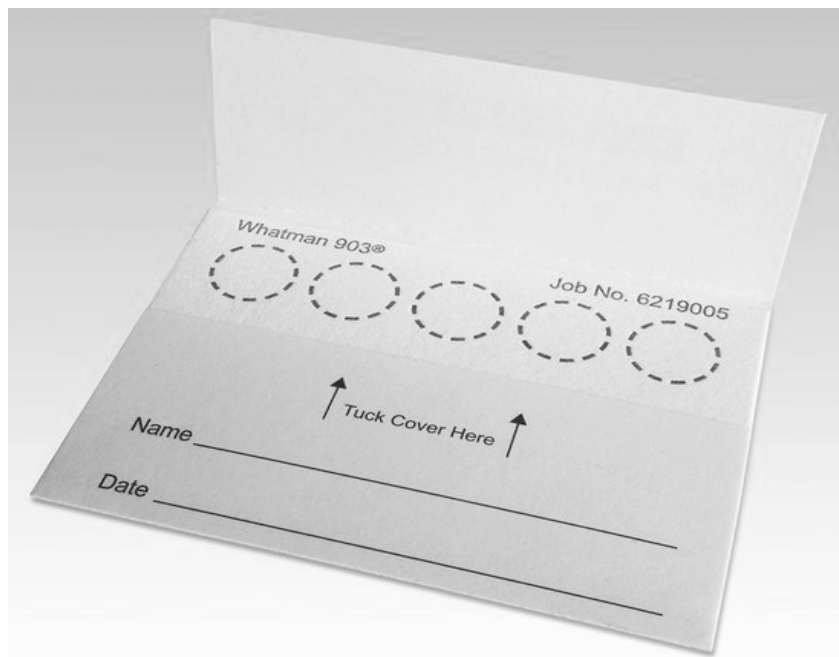
Fig. 2. Dried blood spots on Whatman 2 or 3 filter paper cut into a piece measuring 2.54 x 6.35 cm and stapled or attached to a 2.54 x 6.35 cm piece of stiff paper



Filter paper, with two or three blood spots (inside view)



Stiff paper (front view, serves as cover and label for the filter paper with blood)

Fig. 3. Dried blood spots on Whatman 903 filter paper

5. PROCEDURE NOTES

- Wear gloves to prevent contamination of filter paper.
- Universal precautions, including relevant personal protective equipment such as laboratory coats or gowns, must be used.
- Store samples in a refrigerator at 4 °C when examination (e.g. genotyping and serology) is not to be performed immediately after collection of the sample but within a 4-week period.
- Store samples in a freezer at –20 °C when they are to be examined within 3 months.
- Store samples in a deep freezer at –80 °C when they are to be examined after a longer period, e.g. >1 year.

6. RELATED SOPs

MM-SOP-6a: Labelling malaria blood films

7. REFERENCE

WHO. National malaria slide bank standard operating procedures. Geneva; 2015 (in preparation).

8. 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