World Health Organization

MICROSCOPY EXAMINATION OF THICK AND THIN BLOOD FILMS FOR IDENTIFICATION OF MALARIA PARASITES

MALARIA MICROSCOPY STANDARD OPERATING PROCEDURE – MM-SOP-08

1. PURPOSE AND SCOPE

To describe the procedure for correct detection and identification of malaria parasites in Giemsa-stained blood films 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

Identification of the species and stages of malaria parasites and determination of their density is crucial in clinical management of malaria patients, drug efficacy trials, malaria epidemiological surveys and control programmes. Therefore, malaria diagnoses based on examination of blood films must be correct, with an accurate parasite count.

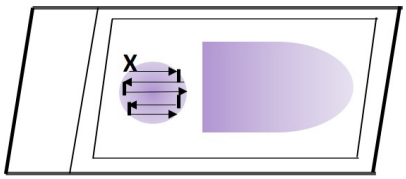
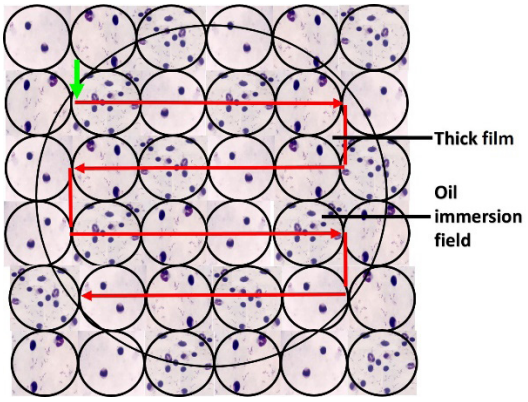
Examination of blood films allows also detection of several blood pathogens, morphological diagnosis of anaemia and identification of several haematological disorders, which must be reported by the microscopist.

3. SUPPLIES, MATERIALS AND EQUIPMENT

- a compound microscope, fitted with paired 10x oculars (eyepieces); 10x, 40x and 100x objectives; and a mechanical stage (An objective marker and a 60x objective may also be fitted);
- Giemsa-stained blood films to be examined;
- immersion oil, type A, high quality;
- lens paper;
- a pen and pencil and
- a malaria registry or log-book.

4. PROCEDURE

FLOW CHART	DESCRIPTION OF ACTIVITY
4.1. Examining the thick film	**4.1. Examining the thick film**
1. Place the Giemsa-stained blood film on the microscope stage with the label to the left and the thick film under the 10x objective lens.	1. Place the Giemsa-stained blood film to be examined on the microscope stage, with the label to the left. Position the thick film in line with the 10x objective lens.
↓	
2. Switch on the microscope, and adjust the light optimally.	2. Switch on the microscope, adjust the light source optimally and find the focus by looking through the ocular and the 10x objective.
↓	
3. Place a drop of immersion oil on the thick film.	3. Scan the blood film for parasites and blood elements. Select part of the film that is well stained and has evenly distributed white blood cells.
↓	
4. Scan and select a well-stained, even portion of the blood film.	4. Place a small drop of immersion oil on the thick film. To avoid cross-contamination, ensure that the immersion oil applicator never touches the slide. Do not allow the 40x objective to touch the oil.
↓	
5. Switch to the 100x oil immersion objective, and allow the lens to touch the oil.	5. Switch the 100x oil immersion objective over the selected portion of the thick film. Use the fine focus adjustment to see the image clearly. Raise the mechanical stage to avoid damaging the slide.
↓	
6. Using the fine adjustment, focus on the blood film.	6. Using the fine adjustment, focus on the cell elements, and confirm that the film is acceptable for routine examination: 15–20 white blood cells per thick film field will give a satisfactory film thickness. Films with fewer white blood cells per field will require more extensive examination.
↓	

FLOW CHART	DESCRIPTION OF ACTIVITY
7. Start with the field on the top left part of the film, and then move the slide to the right, field by field. ↓ 8. When the other end of the film is reached, move the slide downwards, then to the left, field by field, and so forth.	7. Examine the slide in a systematic manner. Start at the top left of the film (marked with a vertical green arrow on **Fig. 1**) and begin at the periphery of the field, then move horizontally to the right, field by field. 8. When the other end of the film is reached, move the slide slightly downwards, then to the left, field by field, and so forth (see below). For efficient examination, continuously focus and refocus with the fine adjustment throughout examination of each field. **Fig. 1. Examining a thick blood film**  

FLOW CHART	DESCRIPTION OF ACTIVITY
4.2. Determining whether a thick film contains malaria parasites and identifying the species	**4.2. Determining whether a thick film contains malaria parasites and identifying the species**
1. Examine the thick film under the oil immersion objective, field by field, horizontally or vertically.	1. Continue to examine the slide for 100 high-power or oil immersion fields. Move the blood film by one high-power field each time, following the pattern shown in Fig. 1. Use the fine adjustment to focus.
↓	
2. Read a minimum of 100 fields before declaring that no malaria parasites were seen.	2. A minimum of 100 high-power fields must be examined before a thick film can be declared as having "no malaria parasites seen". If possible, the whole thick film should be scanned.
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3. If parasites are found, scan additional 100 fields to increase the chance of identifying mixed infections.	3. If parasites are observed, a further 100 fields must be examined before final identification of the species, ensuring that a mixed infection is not overlooked.
↓	
4. The thin blood film should always be examined to confirm the same malaria parasite.	4. The thin blood film should always be examined to identify parasite species definitively. The thin film allows visualization of parasite and red cell morphology, unlike the thick film. Perform an examination at the feathery end or edge of the thin film, as described in procedure 4.3 below.
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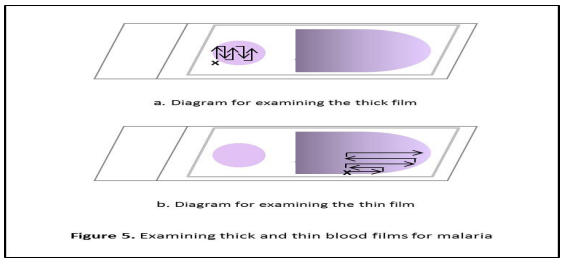
5. Determine all species and stages observed, and record them.	5. Identify and record all species and stages observed in the malaria microscopy blood register. See MM-SOP 6b: Recording and reporting microscopy results. *Note: Refer to the WHO bench aids for the diagnosis of malaria for identification of each species.*

FLOW CHART	DESCRIPTION OF ACTIVITY
4.3. Examining the thin film to confirm species and mixed infections	**4.3. Examining the thin film to confirm species and mixed infections**
1. The thin blood film must be examined to confirm species and mixed infections.	1. To confirm the parasite species or mixed infections after examining the thick film, examine the thin film.
2. Place a drop of oil on the feathery edge of the film.	2. Place a drop of immersion oil on the feathered edge of the thin film.
3. Move from the 10x lens to the 100x oil immersion lens, and focus on the thin film.	3. Move from the 10x lens to the 100x oil immersion lens.
4. Read the thin or feathery edge of the film, moving from one field to the next, horizontally or vertically	4. Examine the feathery end or edge of the thin film where the red cells lay side by side and there is minimal overlap. Follow the pattern of movement shown in Fig. 2. Move along the edge of the film, then move the slide outwards by one field, inwards by one field, returning in a lateral movement and so on. **Fig. 2. Examining a thin film**  **Figure 5.** Examining thick and thin blood films for malaria
5. Scan the film until all the species have been confirmed.	5. Continue examining the thin film until the presence and species of malaria parasites have been confirmed. Identify and record all species and stages observed in the malaria microscopy blood register. See MM-SOP 6b: Recording and reporting microscopy results. *Note: Refer to the WHO bench aids for the diagnosis of malaria for morphological confirmation of each species.*

5. PROCEDURE NOTES

A count may be performed on a thin film if there is no thick film or if the thick film is poorly stained or damaged. Additionally, when the parasite count is > 100 parasites each oil immersion field on the thick film, the thin film should be used to calculate parasitaemia. (See MM-SOP-09: Counting malaria parasites).

6. FORMS AND DOCUMENTS

- Results reporting form (could vary from every laboratory)
- Malaria microscopy blood register book (could vary from every laboratory)

7. RELATED SOPs

MM-SOP-06: Recording and reporting microscopy results

8. REFERENCES

WHO. Bench aids for malaria diagnosis. Geneva; 2009.

WHO. Bench aids for the diagnosis of filarial infections. Geneva; 1997.

WHO. Bench aids for the morphological diagnosis of anaemia. Geneva; 2001.

WHO. QA manual for malaria microscopy, 2nd Edition. Geneva; 2015.

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

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

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