

MALARIA PARASITE COUNTING

MALARIA MICROSCOPY STANDARD OPERATING PROCEDURE – MM-SOP-09

1. PURPOSE AND SCOPE

To describe the procedure for counting malaria parasites on thick and thin blood films.

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

The parasite density provides information on the severity of infection and on the response to treatment. Parasite counts are performed for *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* asexual stages. Unless the protocol dictates otherwise, gametocytes are not counted, but their presence is always reported. All identified parasite species should be reported, even if they are counted together.

Most parasite counts are performed on thick blood films. If there is a no thick film or it is damaged, a thin film count is performed. A thin film count is also performed when there are > 100 parasites in each field of the thick film, which corresponds to > 80 000 parasites/ μ L.

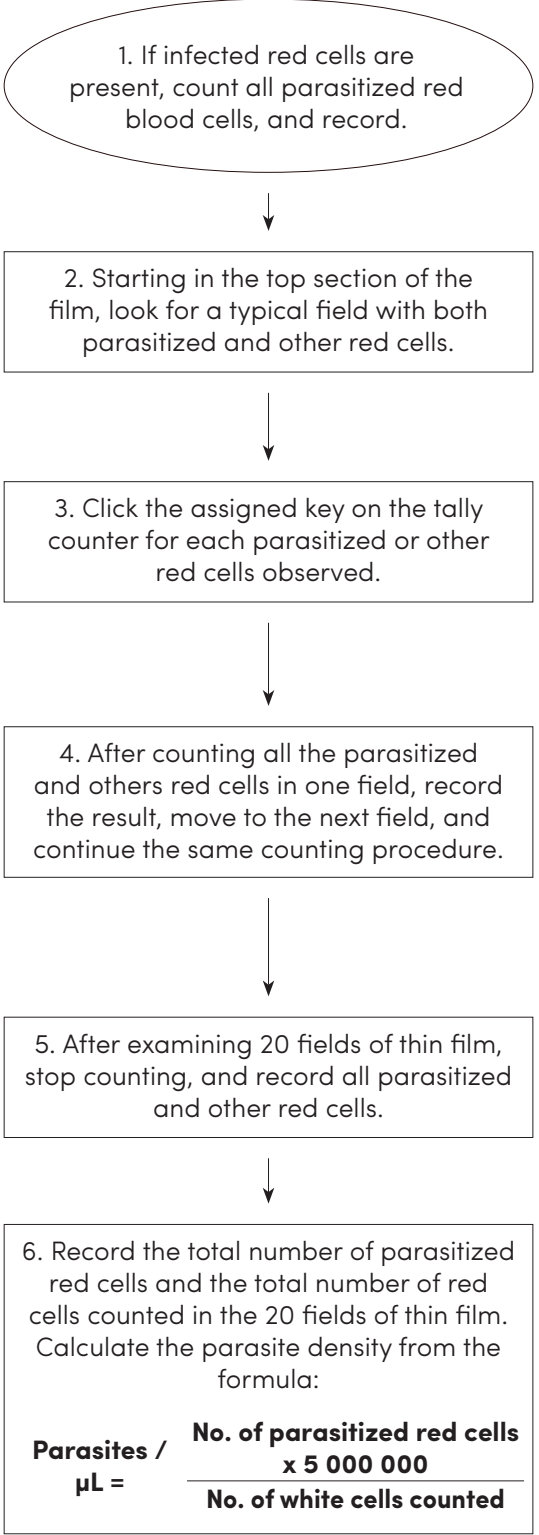
3. SUPPLIES, MATERIALS AND EQUIPMENT

- 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);
- a multiple tally counter or two-key tally counters, one to count malaria parasites and one to count white blood cells;
- Giemsa-stained blood slides to be examined;
- immersion oil, type A, high quality;
- lens paper;
- a pen and pencil and
- a handheld calculator.

4. PROCEDURE

FLOW CHART	DESCRIPTION OF ACTIVITY
<p>4.1. Performing a parasite count on a thick film and calculating parasite density</p> <pre> graph TD A([1. Place the glass slide on the microscope, with the label to the left.]) --> B[2. Determine the presence of malaria parasites and their species and stages, and record (see MM-SOPs 6b and 08).] B --> C[3. Starting at the top left of the smear, look for a typical field with both parasites and white cells. Start counting.] C --> D[4. Click the assigned key on the tally counter for each parasite or white cell observed.] D --> E[5. After counting all the parasites and white cells in one field, move to the next, and repeat the counting procedure, and so on.] </pre>	<p>4.1. Performing a parasite count on a thick film and calculating parasite density <i>Note: Before starting counting, examine 100 fields of the thick film to detect the presence of malaria parasites at 100x oil immersion.</i></p> <ol style="list-style-type: none"> Place the glass slide on the microscope stage with the label to the left. This allows a standardized approach for the start point for counting and also to record parasite locations using the marked divisions on the slide holder. If malaria parasites are present (see MM-SOP 08: Microscopy examination of thick and thin blood films for identification of malaria parasites), count asexual forms (in either single or mixed species infections) without sexual (gametocyte) forms, which are not counted but just reported. In mixed infections, all asexual parasites are counted together and the presence of multiple species is reported (see MM-SOP 6a: Recording and reporting microscopy results). Starting at the top most left part of the film, look for a field with a good number of white cells and parasites are observed together and start counting. Using a multiple type tally counter, count parasites and white cells simultaneously by clicking on the assigned key as parasites or white cells are observed. If two tally counters are being used use one for the WBCs and the other for parasites. After counting all the parasites and white cells in one field, move to the next field following the pattern of movement shown in Figure 1 and repeat the same counting procedure and so on. Be careful not to overlap fields. <p>Fig. 1. Pattern of movement for counting parasites and white blood cells</p>

FLOW CHART	DESCRIPTION OF ACTIVITY
<p>6. Depending on the number of parasites observed, stop counting after you have examined 200 or 500 white cells.</p> <ul style="list-style-type: none"> • If you have counted ≥ 100 parasites in 200 white cells, stop counting, and record the results as the number of parasites per 200 white cells. • If you have counted ≤ 99 parasites in 500 white cells, stop counting, and record the results as the number of parasites per 500 white cells. <p style="text-align: center;">↓</p> <p>7. Count all parasites and white cells in the final field.</p> <p style="text-align: center;">↓</p> <p>8. Record the actual numbers of parasites and white cells counted.</p> <p style="text-align: center;">↓</p> <p>9. Calculate the parasite density from:</p> $\text{Parasites / } \mu\text{L blood} = \frac{\text{Number of parasites counted} \times 8000 \text{ white cells/}\mu\text{L}}{\text{No. of white cells counted}}$	<p>6. Decide when to stop counting by following these rules:</p> <ul style="list-style-type: none"> • If you have counted ≥ 100 parasites in 200 white cells, stop counting, and record the results as the number of parasites per 200 white cells. • If you have counted ≤ 99 parasites in 500 white cells, stop counting, and record the results as the number of parasites per 500 white cells. <p>7. Count all parasites and white cells in the final field, even if the white cell count exceeds 200 or 500.</p> <p>8. Record the actual numbers of parasites and white cells counted on an appropriate worksheet.</p> <p>9. When counting is completed, calculate the parasite density on the basis of the patient's actual white cell count. If this is not available, use an estimated average white cell count of 8000/μL.</p> <p>Use the following formula for the calculation:</p> $\text{Parasites / } \mu\text{L blood} = \frac{\text{Number of parasites counted} \times 8000 \text{ white cells/}\mu\text{L}}{\text{No. of white cells counted}}$

FLOW CHART	DESCRIPTION OF ACTIVITY
<p data-bbox="229 300 770 360">4.2. Performing a parasite count on the thin film and calculating parasite density</p>  <p data-bbox="229 488 770 663">1. If infected red cells are present, count all parasitized red blood cells, and record.</p> <p data-bbox="229 734 770 860">2. Starting in the top section of the film, look for a typical field with both parasitized and other red cells.</p> <p data-bbox="229 965 770 1090">3. Click the assigned key on the tally counter for each parasitized or other red cells observed.</p> <p data-bbox="229 1211 770 1361">4. After counting all the parasitized and others red cells in one field, record the result, move to the next field, and continue the same counting procedure.</p> <p data-bbox="229 1496 770 1621">5. After examining 20 fields of thin film, stop counting, and record all parasitized and other red cells.</p> <p data-bbox="229 1704 770 1877">6. Record the total number of parasitized red cells and the total number of red cells counted in the 20 fields of thin film. Calculate the parasite density from the formula:</p> $ \text{Parasites / } \mu\text{L} = \frac{\text{No. of parasitized red cells} \times 5\,000\,000}{\text{No. of white cells counted}} $	<p data-bbox="876 300 1417 360">4.2. Performing a parasite count on the thin film and calculating parasite density</p> <p data-bbox="876 365 1417 456">Note: <i>If ≥ 100 parasites are present in each field of a thick film under the 100x objective, calculate the parasite count on the thin film.</i></p> <ol data-bbox="818 472 1463 1995" style="list-style-type: none"> <li data-bbox="818 472 1463 667">1. If infected red cells are present, count all parasitized red cells. If sexual forms (gametocyte) are seen, do not count them, but report them. In mixed infections, all parasitized red cells are counted together, and the presence of multiple parasite species is reported. <li data-bbox="818 696 1463 891">2. In the top section of the thin film, locate a field with about 250 red cells. Count the total number of red cells in that field and the number of parasitized red cells. A typical field (at 100x magnification) should contain approximately 250 red cells. <li data-bbox="818 920 1463 1122">3. Using a multiple type tally counter, count parasitized and other red cells by clicking the assigned keys for parasitized and non-parasitized red cells. If you have two tally counters, use one for parasitized red cells and the other for non-parasitized red cells. <li data-bbox="818 1144 1463 1473">4. After counting all the parasites and red blood cells in one field, move to the next field, following the pattern of movement shown in Fig. 1, and repeat the counting procedure in each field. Be careful not to overlap fields. Continue in a longitudinal manner, moving stepwise across the film as required. Count all parasitized and other red cell in each field, even if the total red cell count per field exceeds 250. <li data-bbox="818 1503 1463 1727">5. Stop counting when about 20 fields with about 250 red cells (about 5000 red cells) have been counted. Record the actual numbers of parasitized and other red cells counted on an appropriate worksheet. Use these figures to calculate the total parasite count per μL of blood. <li data-bbox="818 1749 1463 1995">6. When counting is completed, calculate the parasite density from the patient's actual red cell count. If this is not available, use an estimated average red cell count of 5 000 000/μL and the following formula. Note that the final result is rounded to the nearest whole number. Number of parasites per μL blood: $ \text{Parasites / } \mu\text{L} = \frac{\text{No. of parasitized red cells} \times 5\,000\,000}{\text{No. of white cells counted}} $

5. RELATED SOPs

MM-SOP 6b: Recording and reporting microscopy results

MM-SOP 08: Microscopy examination of thick and thin blood films for identification of malaria parasites

6. REFERENCES

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

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).

7. 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