

East African Regional External Quality Assessment Scheme (EA-REQAS)

Learning Sheet Number Seven

GUIDANCE SHEET FOR MALARIA PARASITE AND TUBERCULOSIS BACILLI COUNTING



MALARIA PARASITE COUNTING (MALARIA PARASITE QUANTITATION)

Parasite counting in a thick blood film

1. Using the x100 oil immersion objective lens, count asexual forms of parasites (trophozoites and schizonts—not gametocytes) against white blood cells (WBC).
2. Start counting when the first parasite is detected. Use 2 tally counters, a double-key counter or write the numbers on a piece of paper.
3. Count both parasites and WBCs in each field (including fields with either no WBC or no parasites) until 200 WBCs have been counted. If 100 or more parasites have been counted at this point, you can proceed to perform the calculation below. If the parasite count is less than 100, continue counting asexual parasites and WBCs until 500 WBCs have been counted.

Parasite counting in a thin blood film

1. Thin blood films are used for counting when there is a very high parasite count (>100 parasites in each thin blood film field). At x1000 total magnification, each field in a thin blood film contains approximately 250 red blood cells (RBCs).
2. Using the x100 oil immersion objective lens, count infected RBCs against all the RBCs in at least 20 fields or until 5,000 total RBCs have been counted.

Calculating malaria parasite density per microlitre in a thick blood film

Calculate parasite density per microlitre using the formula below. The number, 8,000, is the "assumed" average number of WBCs in one microlitre (μL) of blood. The WBC count varies from patient to patient and if the true WBC count is known for that patient, the true figure should be used in the calculation.

$$\text{Parasite count} = \frac{\text{Number of parasites counted} \times 8,000}{\text{Number of WBCs counted}} \quad [\text{parasites}/\mu\text{L}]$$

Calculating malaria parasite density per microlitre in a thin blood film

Calculate parasite density per microlitre using the formula below. The number, 5,000,000 is the "assumed" average number of RBCs in one microlitre (μL) of blood. The RBC count varies from patient to patient and if the true RBC count is known for that patient, the true figure should be used in the calculation.

$$\text{Parasite count} = \frac{\text{Number of infected RBCs counted} \times 5,000,000}{\text{Total number of RBCs counted}} \quad [\text{parasites}/\mu\text{L}]$$

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COUNTING AND GRADING TUBERCULOSIS BACILLI IN A SMEAR

For Ziehl Neelsen staining

1. Using the $\times 100$ objective lens, examine the smear for thin red rods—acid fast bacilli (AFB)—singly, in a row, or in small clumps.
2. If AFB are seen, quickly scan 5—10 fields to establish whether each field has AFB, that is, heavy infection. Count AFB in 20 fields and find the average number of AFB per field.
3. In light infections, count the number of AFB in 100 fields. Use two tally counters, one to count the number of fields and one to count the number of AFB, or record the results on a piece of paper.
4. If no AFB are seen, systematically examine the whole smear.
5. Grade and record the findings as:

AFB negative.....if no AFB seen in at least 100 fields
Actual number of AFB seen.....1—9 AFB per 100 fields
AFB 1+.....10—99 AFB per 100 fields
AFB 2+.....1—10 AFB per field in at least 50 fields
AFB 3+.....>10 AFB per field in at least 20 fields

For fluorescent (auramine O) staining

1. Using the $\times 40$ objective lens, examine the smear for yellowish glowing bodies—*M. tuberculosis* bacilli or acid fast bacilli (AFB).
2. When AFB are seen, count the AFB in 40 fields and calculate the average number per field.
3. Grade and record the findings as:

AFB negative.....if no AFB seen in at least 40 fields
Actual number of bacilli seen.....1—19 AFB per 40 fields
AFB 1+.....20—199 AFB per 40 fields
AFB 2+.....550 AFB per 40 fields
AFB 3+.....>50 AFB per 40 fields

BIBLIOGRAPHY

1. World Health Organization. Implementing Tuberculosis Diagnostics; Policy Framework. 2015.
2. Jane Carter and Orgenes Lema. Practical Laboratory Manual for Health Centres in East Africa. AMREF Publication.