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Short Communication

Methods of Estimating and Counting Malaria Parasites Density

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Introduction

Counting the parasite and estimating the density of parasitemia will help the clinician to monitor the malarial case if its response for treatment as quality or as a dose. The clinician will request many tests for parasite counting whereas the first test is the basic one which will used as a slandered indicator and a reference test for comparison with the following requested counting tests.

Parasite count is very important in Plasmodium falciparum infection cases which are always considered potentially dangerous.

Parasitemia density for positive malaria blood film must be known because the clinician needs to:

- Know the severity of the infection.
- Know case response for treatment.

Methods

The level of parasitemia may be expressed either as the number of parasites per microliter of blood or as a percentage of parasitized erythrocytes.

1. Parasites/µL.
2. Determining the percentage of parasitemia (Infected RBCs %).
3. The 'plus system'.

Parasite/µL (use thick blood film)

- Count parasites in relation to a predetermined number of WBCs and an average of 8000/µl is taken as standard.
- 200 leucocytes are counted.
- All parasite species and forms including both sexual and asexual forms are counted together.
- If >10 parasites are counted/100 field, then the formulae below can be applied: (No. of Parasites/No. of WBCs counted) x 8000 = No. of parasites/µl) or if 200 leukocytes are counted the formulae below can be applied: No. of parasites counted x 40 = No. of parasites/µl
- If the parasites are < 9/100 field, then 500 WBCs should be counted, and the formula will be: No. of parasites counted x 16 = No. of parasites/µl.

Determining the percentage of parasitized erythrocytes (Thin Blood Film)

- The number of infected red cells (not number of parasites) in 1000 RBCs is converted to percentage.
- This method estimates the percentage of red blood cells infected with malarial parasites.
- The smear is scanned carefully, one 'row' at a time.
- The total number of red cells and the number of parasitized red cells are tabulated separately.

- If 1000 red cells are counted, then divide the number of parasitized red cells by 10 to get the percentage (i.e. if 30 out of 1000 cells are parasitized, then the parasitized red cell count is 3%).
- If lesser red cells are counted, then divide the number parasitized by the total number counted and multiply the result by 100 to obtain a percentage estimate of red blood cells parasitized.
- If occasional parasites are seen when scanning the smear, but none are identified during the process of counting 300 - 500 red blood cells, a percentage value of less than 1% of red blood cells parasitized is assigned.

The 'plus system'

- "The 'plus system' is an old method, which is simple but far less accurate for establishing parasite density in thick blood films" (1).
- "Because of its unreliability, it has been replaced by the method described above and is no longer recommended" (2).

(1), (2): A quote words from WHO publication titled "Basic Malaria Microscopy. Part I Learner's guide – second edition Page No.75. ISBN: 978 92 4 154782 6 (Part).

In this system

+ = 1-10 parasites per 100 oil-immersion thick film fields.

++ = 11-100 parasites per 100 oil-immersion thick film fields.

+++ = 1-10 parasites per single oil-immersion thick film field.

++++ = more than 10 parasites per single oil-immersion thick film field.

I disagree with what has been published regarding the plus system method by WHO, comparison between the three counting methods I see the opposite of what has been published as the plus system method is the most important method which is able to save the life of the infected malaria cases comparing with the other methods because of the following reasons:

1. Both methods Parasites/µL and percentage of parasitized erythrocytes give results by numbers only for example: 200000 parasite/µL or parasitized RBCs 17%.
2. Both methods Parasites/µL and percentage of parasitized erythrocytes results unable to indicate for which kind of plasmodium stages are present nor determine the density for each stage.
3. Presence of some stages in blood film like late stage of trophozoites and both mature and immature schizonts in falciparum infections is considered as a high risk indicator, it might be lead to cerebral malaria (Plus system method able to indicate for that).

Examples confirm the importance of Plus system method

Example one

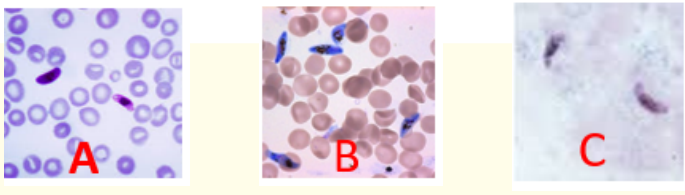

Figure 1: *Slides A, B and C appear the presence of gametocyte form stage only, the nonpathogenic stage. (It is common to see this when follow up the falciparum cases after finishing the treatment dose). Using the percentage method for both slides A and B will show that the density of parasitemia in slid A is more than it in slide B. For the slides A, B and C: Using both "the percentage and parasite/µl method" will appear the amount of parasitemia density but unable to indicate for the presence of the gametocyte stage only. So, using "Plus system method" is the best additional choice. Report result: P.f g +++.*

Example two

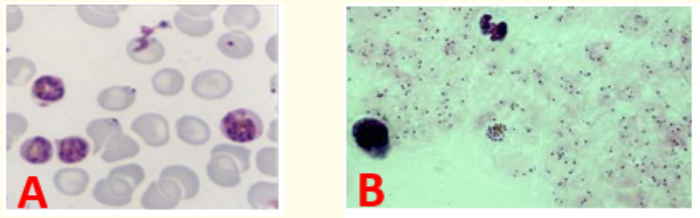

Figure 2: *Both slides A and B are positive slides for a heavy infection of plasmodium falciparum contain early and late trophozoites forms and schizont form stage.*
Slide A (thin film): using "percentage method for estimating parasite load" will represent about the heavy severity of infection but unable to indicate for the presence of the late trophozoites and schizont form stages.
Slide B (thick film): using" Parasite/µl method" for estimating parasite load will represent about the heavy severity of infection but unable to indicate for the presence of the late schizont form stage.
So, status like this, surely, using "Plus system method" is the best additional choice. Report result: P.f r++++ sch +.

Example three

Figure 3: *Both slides A and B are positive slides for a heavy infection of plasmodium falciparum contain early and late trophozoite form stage.*
Slide A (thin film): using "percentage method for estimating parasite load" will represent about the heavy severity of infection but unable to indicate for the presence of the late trophozoite stage.
Slide B (thick film): using" Parasite/µl method" for estimating parasite load will represent about the heavy severity of infection but unable to indicate for the presence of the late trophozoite form.
So, status like this, surely, using "Plus system method" is the best additional choice. Report result: P.f r++++ tr +.

Conclusion

Are early malaria case detection and treatment the key?

This expression is often reverberated, and perhaps there is no reference discuss malaria topic does not mention for this expression, but what is the accuracy is this phrase?

This expression even if it is modified to become the early detection and accurate correct diagnosis in addition to an effective treatment is the key, I will still disagree and criticize this expression and considered it as inaccurate because of the following facts: During 40 years of work in malaria field, I was dealing with a lot of malaria cases which have been detected early and their laboratory diagnosis was true. and have been given effective treatment, although they have entered in complications (due to malaria and not for other reasons), some of which have ended with death.

This leads for thinking about the existence of a real key or keys which will encourage us to know what these keys are?

I think that the determination of parasitic infection intensity should be included in the first request directed to the laboratory by the physician who investigates the result of laboratory testing of malaria to be the result as a standard to compare with the following examination requests to determine the patient's response to treatment in a timely manner in which it can be controlled because the time factor is very important in Malaria cases, especially falciparum, so that what it may be possible now may become impossible after hours.

What is exactly, the meaning of the accurate laboratory test for malaria?

The precise and correct meaning of the laboratory diagnosis of malaria could be summarized as follows:

1. Specifying the type of malaria parasite detected microscopically.
2. Describe all stages of malaria plasmodium parasites are seen in the in the examined blood sample and determine their density. (plus, system).
3. Use appropriate parasite counting methods (percentage of infected red blood cells, number of parasites/μL.

In spite of presence an effective antimalarial drugs and modern accurate lab diagnostic methods, every year more than 400000 people die regarding to malaria, why? and did we could decrease this mortality number and how?

I hope if this manuscript assists to find logic scientific answers for why and how and gives hopes that we could achieve for this global health issue.

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