

# Detection of Malaria Parasite Using Different Microscopic Methods and Acomparison Among Them

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**Abstract:** *Malaria is a leading cause of morbidity and mortality worldwide. Prompt diagnosis and treatment are critical factors in reducing morbidity and mortality, as delayed treatment of malaria increases the risk of death. Microscopy has long been the standard for malaria diagnosis. Diagnosis plays a vital role in early detection of malaria which is an essential prerequisite for control of the disease. In the present investigation, different detection techniques were used including microscopic analysis (Giemsa staining, Acridine Orange stain) and Quantitative Buffy Coat Analysis (QBC) and immunochromatographic test (ICT) as a rapid diagnostic test. This prospective study was undertaken for detection of malaria parasite in 565 patients suffering from fever and other constitutional symptoms. All the diagnostic tests like AO staining, Giemsa staining, and QBC have been compared with each other on the basis of their sensitivity and specificity. The QBC shows 100% specificity and sensitivity as compared to AO and Giemsa. Out of the 565 blood smears taken from patients coming from different endemic areas revealed 18 cases positive by Giemsa staining but 19 cases positive by AO and 21 were found to be positive by QBC. It may be difficult to determine whether the cases which are positive by AO but negative by Giemsa are true or false positive such questionable cases should perhaps be evaluated by third method, or perhaps the definition of 'gold standard' need to be modified. I have identified all the four species of Plasmodium, i.e; (P. vivax, P.falciparum, P.malariae and P.ovale) causes a different characteristic fever.*

**Keywords:** Immunochromatographic test (ICT), Quantitative buffy coat (QBC), Giemsa staining, Acridine orange stain (AO)

## 1.Introduction

Malaria is the most important parasitic infection of man, and is associated with a huge burden of morbidity and mortality in many parts of the tropical world. The incidence of malaria worldwide is estimated to be 300–500 million clinical cases each year with a mortality of between one and three million people worldwide annually[1]. Malaria is a mosquito-borne infectious disease of humans caused by eukaryotic protists of the genus *Plasmodium*. It is widespread in tropical and subtropical regions, including much of Sub-Saharan Africa, Asia and the Americas. Malaria is prevalent in these regions because of the significant amounts of rainfall and consistent high temperatures; warm, consistent temperatures and high humidity, along with stagnant waters in which their larvae mature, provide mosquitoes with the environment needed for continuous breeding.

The most economic, preferred, and reliable diagnosis of malaria is microscopic examination of blood films because each of the four major parasite species has distinguishing characteristics [2]. Thin films are similar to usual blood films and allow species identification because the parasite's appearance is best preserved in this preparation. Among all of the above methods, QBC assay is undoubtedly proven to be a highly sensitive, rapid, and reliable test detecting very low number of parasite [3]. In QBC assay, the capillary tube is coated with Acridine Orange (fluorescent dye,) which stains the nucleic acid (DNA as green color and RNA as orange color). A floater is inserted inside the capillary tube, which separates the white blood cells (WBC), red blood cells (RBC) and the parasites according to their densities. The WBCs are arranged in different layers forming visibly a

discrete band above the RBCs layer called buffy coat. During the microscopic examination, parasites appear within the granulocytes and mononuclear cell layer. Some of the authors have commented on grayish black coloration of buffy coat predicting malaria positivity [4,5].

In the recent years, numerous quick and new techniques for malaria diagnosis have been developed, one such being the QBC (quantitative Buffy coat) technique. Giemsa stained blood smears are the most widely used for the microscopical examination of malaria parasite. It shows high sensitivity and specificity, taken as gold standard for malaria diagnosis. It can be used to estimate levels of parasitaemia as well as to identify the *Plasmodium* species. However, it is time consuming (typically taking at least 45 min from blood collection to the result) and requires well trained and experienced personnel [6]. Malaria parasite, unlike their red blood cell host, contain nucleic acid that stains with fluorescent dyes, such as acridine orange. As the parasites within erythrocyte mature, they reduce the buoyant density of infected erythrocyte [7].

Due to continually escalating malaria cases in our locality, it becomes imperative to employ a diagnostic test that is sensitive yet specific, rapid and cheap [8]. We therefore evaluated various microscopic methods for malaria diagnosis and attempted to correlate it with the Romanowsky stained thick and thin blood smear microscopy, in terms of sensitivity, specificity, reliability, speed and cost effectiveness.

## 2.Materials and Methods

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The present investigation was conducted on 565 blood samples from patients presenting with pyrexia and/or atypical presentations. All patients attending the Outpatient department and indoor of department of Medicine and department of Pediatrics with a clinical suspicion of malaria, were included in this study. The clinical criteria for diagnosis of malaria are as follows.

- (1) Fever with chill and rigor
- (2) Hepatosplenomegaly.
- (3) Anaemia, and thrombocytopenia.
- (4) Cerebral malaria

The present study was carried out to detect the prevalence of different Plasmodium spp. especially *P. falciparum* and *P. vivax* inhabiting in the peripheral blood of man which can be diagnosed by various laboratory methods. Parasitaemia was also calculated to detect the degree of severity.

### 3. Materials Used

Compound light microscope, Fluorescent microscopes, QBC centrifuge, Refrigerator, simple centrifuge. Glass slides, EDTA vials, Surgical cutting needles, Cotton, Spreader, Staining dishes, Petridishes, Tissue papers, Blotting papers, Slide racks, Slide boxes, Chemical bottles, Gloves, Micropipettes, tips etc. Spirit or antiseptic solution, Giemsa's stain, Acridine orange, Methyl alcohol, Distilled water, Oil immersion.

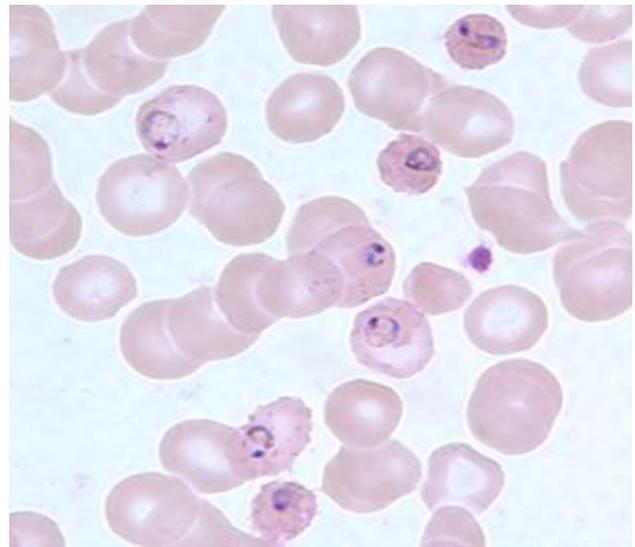
Two milliliters of blood was collected in an EDTA vial and tested for malaria parasite by the conventional methods [Giemsa, AO, QBC and ICT (LDH based optimal)]. A "positive case for malaria" in our study was defined as positive by either of the above described conventional methods (i.e. microscopy and/or ICT). Blood samples tested negative by the conventional methods were taken as controls.

### 4. Results

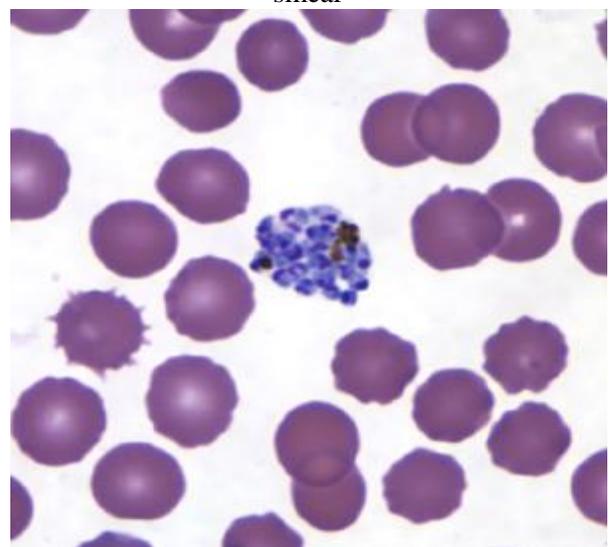
Out of the 565 clinically suspected malaria cases, 21 were found to be positive by conventional methods. A maximum number of malaria positive cases were detected by QBC followed by other methods like ICT, AO and Giemsa [QBC (21/21), AO (19/21), Giemsa (18/21)] (Table 1).

Method	No. Of Positive Samples	No. Of Negative Samples	Sensitivity	Specificity
Giemsa	18	3	90%	100%
AO	19	2	90.47%	100%
QBC	21	0	100%	100%

The detection of asexual parasites by light microscopy of Giemsa-stained thick and thin films still remains the gold standard for malaria diagnosis (Fig. 1a,b) However, detection of low parasite densities and mixed infections poses a significant challenge, even to highly experienced microbiologist. The highest achievable levels of sensitivity and specificity are of enormous importance, not just for the early diagnosis of this potentially life-threatening disease, but also for malaria research, such as the validation of new diagnostic devices may provide the required additional sensitivity to augment the gold standard for the diagnosis of falciparum malaria.



**Figure 1a:** aRings and trophozoites in a thin blood smear



**Figure 1b:** Schizont of *P. vivax* in Giemsa stained blood smear

### 5. Discussion

A diagnostic test for malaria not only has good sensitivity and specificity but also needs to be affordable, rapid and easy to use. It should also give a measure of the level of parasitemia, as asymptomatic malarial infections are pervasive in highly endemic areas. Diagnosing malaria by conventional light microscopy – usually by examination of Giemsa – stained blood smears is currently the 'gold standard' in most parts of sub-Saharan Africa [9]. As this method itself reveals limitations of sensitivity, particularly at low parasite densities, the assessment of the diagnostic performance of any new method against Giemsa staining is problematic. For example, here we conclude 565 blood smears taken from patients coming from different endemic areas revealed 18 cases positive by Giemsa but 19 cases positive by AO. It may be difficult to determine whether the cases which are positive by AO but negative by Giemsa are true or false positive such questionable cases should perhaps be evaluated by third method, or perhaps the definition of 'gold standard' need to be modified.

A very large discrepancy was observed between the result based on AO staining and those based on Giemsa staining when the blood samples being checked came from different endemic areas and typically carried >2000 malarial parasites/μl blood; The total of 21 samples confirmed positive by QBC, 19 were positive by AO but only 18 were found positive after Giemsa staining. The two cases where only QBC was positive found to contain gametocytes of Plasmodium species of very low number (Fig.2) Hence, QBC proved to be a more sensitive test in comparison to AO and Giemsa in picking off malaria parasite with a lower count.

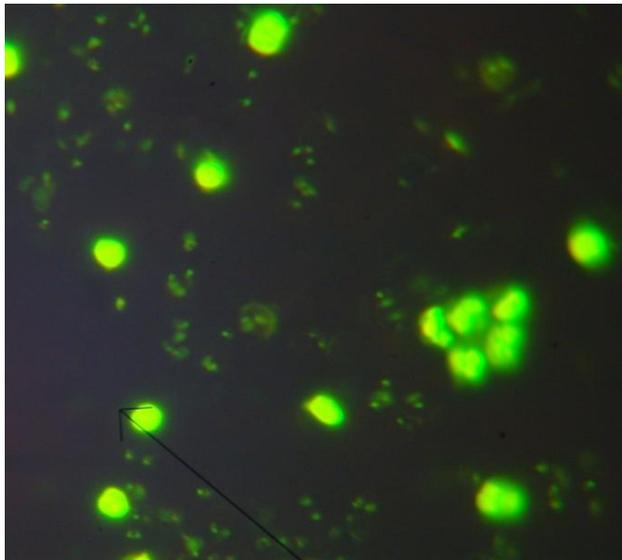


Figure 3: Parasite present in QBC

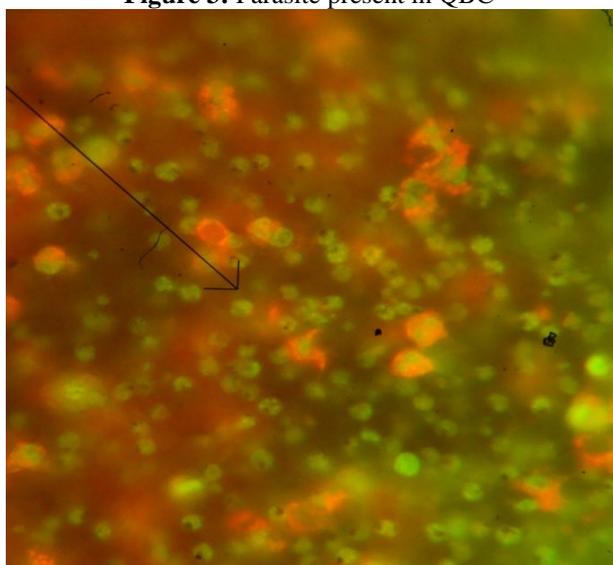


Figure 4: Pigments present in AO staining

This prospective study was undertaken for detection of malaria parasite in 565 patients suffering from fever and other constitutional symptoms. All the diagnostic tests like AO staining, Giemsa staining, and QBC have been compared with each other on the basis of their sensitivity and specificity. The QBC shows 100% specificity and sensitivity as compared to AO (Fig.3) and Giemsa, the sensitivity and specificity of AO and Giemsa were 90.47%, 100% and 90%, 100% respectively [Table 1].

When the diagnostic performance of AO has been evaluated for different levels of parasitaemia, a decrease in sensitivity has always been observed at lower parasite densities. It clearly compromises the usefulness of the AO method in areas where malaria is holoendemic. In such settings, a failure to detect some of the low parasitaemia may have no public-health significance. On the other hand, misdiagnosis is of considerable concern in hypo-endemic areas. The most notable advantages of AO over Giemsa stain is its rapidity. This has some important consequences for malaria diagnosis and control. Firstly, prompt diagnosis facilitates the provision of better and more client-friendly services to the patients, as waiting times are significantly reduced. The AO staining method might be an appropriate diagnostic tool for health centers in developing countries that are visited by many patients with suspected malaria or by many drug-resistant cases, as well as for large-scale screening in epidemiological survey [10]. The third and the most important consequence of the rapidity of AO method for malaria diagnosis and control is that rapid diagnosis could cut the unnecessary use of antimalarial drugs and lead to better treatment of those who do have symptomatic malaria.

Slow diagnosis thus encourages self treatment and the consequences. In general, drug resistance develops most rapidly when sub-therapeutic doses of antimalarial drugs filter out the resistant forms of the parasite [11]. Inadequate doses, inappropriate dose regimes or poor compliance leads to rapid selection of resistant parasites.

Multi drug resistance is already a major threat to effective malaria control in many parts of South-east Asia. The high levels of chloroquine resistance found over large parts of sub-Saharan Africa have prompted several countries to switch to sulfadoxine-pyrimethamine (SP) as the first-line drug, but levels of SP resistance are also rapidly increasing. Cross-resistance between compound classes further amplifies the problem. Over prescribing self-treatment and the treatment of asymptomatic cases should all be reduced. Presumptive malaria diagnosis often results in the treatment with antimalarial drugs of non-malarial cases. Unnecessary adverse effects and high costs accompany the over-administration of antimalarials. Presumptive treatment was less problematic before resistance to chloroquine became widespread, because this drug was cheap, safe and non-toxic. Prescription of quinine and/or SP may cause unnecessary adverse effects among non-infected patients.

## 6. Conclusion

Three different techniques were performed simultaneously for comparative evaluation of their efficacy in diagnosis of Malaria. These tests include

- (i) Giemsa and
- (ii) Acridine Orange (AO) stained smear examinations,
- (iii) Quantitative buffy coat assay (QBC).

Giemsa stained smear examination was assumed as the gold standard for diagnosis of malaria. The sensitivity and specificity of these tests for diagnosis of Malaria were different. The sensitivity and specificity for QBC was found to be 100% in comparison to 90% and 90.47% for Giemsa and AO assay both. However, Acridine Orange stains for

demonstration of malarial parasites after careful evaluation we established its superiority over Giemsa. QBC found to be the best method among all the above tests with high sensitivity and specificity for detection of malaria in comparison to other microscopically methods.

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