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**Research Article**

# Evaluation of Immunochromatography test and Quantitative buffy coat against peripheral blood smear examination in diagnosis of malaria

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## Abstract

**Background:** In spite of enormous preventive and control measures, malaria has resurged in many tropical countries including India. The limited access to effective diagnosis and treatment of cases in endemic areas is one of the most important factors hampering the reduction of morbidity and mortality associated with malaria.
**Aims and objective:** The present study was conducted with an aim to evaluate immunochromatography test (ICT) and quantitative buffy coat (QBC) against peripheral blood smear (PBS) examination for diagnosis of malaria.
**Material and methods:** Blood samples collected from 186 clinically suspected cases of malaria was used for preparation of PBS for examination of malarial parasite, QBC and ICT.
**Results:** Out of 186 patients included in study, 122 (65.5%) of were positive for malaria by any of the three tests used. 116 (95.1%) were positive for malarial parasite on the PBS. ICT was positive in 103 (84.4%) cases and QBC was positive in 70 (57.3%) cases.
**Conclusions:** ICT for malaria can be used as rapid, simple and mass screening test in remote and rural areas as skilled professional are not required. ICT may prove as useful tool for malaria control programmes. In established setup where skilled and expert personnel are available PBS remains the test of choice and ICT can be used as an adjuvant to PBS.
**Keywords:** Immunochromatography test, peripheral blood smear, quantitative buffy coat.

## 1. Introduction

The disease malaria has always been the subject of research for medical practitioners. Many ancient medical literatures like Charaka Samhita and Susruta Samhita has mentioned various aspects of malaria and even of its possible link with mosquitoes and insects.

Malaria is a parasitic infection caused by *Plasmodium* spp. Human malarial parasites belong to the subgenera *P.* (*Plasmodium*) and *P.* (*Laverania*). Malarial parasites infecting man are attributed to four species: *P.* (*Laverania*) *falciparum*, *P.* (*Plasmodium*) *vivax*, P. *P.* (*Plasmodium*) *malariae* and *P.* (*Plasmodium*) *ovale.*[1]

Malaria is found throughout the tropical and subtropical regions of the world and causes more than 300 million acute illness and at least one million deaths annually.[2] In spite of enormous preventive and control measures; malaria has resurged in many tropical countries including India. In addition to factors like technical, administrative and operative failures of various control and eradication programmes, the increasing problem of drug resistance in malaria parasite and insecticide resistance in vector has added to malaria resurgence.[3,4]

The limited access to effective diagnosis and treatment of cases in endemic areas is one of the most important

factors hampering the reduction of morbidity and mortality associated with malaria.[5]

Examination of peripheral blood smear (PBS) is considered as the "gold standard" method for the diagnosis of malaria. However, PBS examination is time consuming and labor intensive technique and requires high quality staining, good microscope and skilled microscopist.[6] In contrast to this, the advanced techniques like fluorescent staining, immunochromatography for detection of malarial antigens and detection of specific nucleic acid sequences are rapid and simple methods for diagnosis of malaria.

In immunochromatographic test (ICT) parasitic lactate dehydrogenase (pLDH) and histidine rich protein-2 is detected using monoclonal antibodies.[7] Quantitative buffy coat (QBC) technique is another rapid diagnostic method for malaria. QBC requires fluorescent microscope.[8]

In the present study we evaluated ICT and QBC against PBS examination for diagnosis of malaria.

## 2. Materials and Methods.

The present study was conducted at rural tertiary care hospital of central India. A total of 186 clinically suspected cases of malaria were included in the study. Blood sample collected from these patients was used for preparation of PBS for examination of malarial parasites, QBC and ICT. The clinical details of patients (specifically fever, anaemia, jaundice, splenomegaly and hepatomegaly) were recorded and analyzed.

### 2.1 Peripheral blood smear examination

The thin and thick smears were prepared on a glass slide from a drop of blood. The smear was air dried and thin smear was fixed by methanol. Giemsa stain was used for staining the smear. PBS was examined under light microscope for the presence of malarial parasite. Species identification was done on the basis of morphology in the cases were malarial parasite was seen in PBS.

At least hundred high power fields were carefully and meticulously screened before declaring PBS examination negative for malarial parasite.

### 2.2 QBC

The QBC system (Becton Dickinson, USA) consists of 75 mm long tube internally coated with acridine orange, potassium oxalate, sodium heparin and EDTA. For performing QBC the method suggested by Moody *et al* [9] was followed. The malarial parasite appears green (DNA) and orange (RNA) when observed under fluorescent microscope.

### 2.3 ICT

ICT was performed by using commercially available "Malarial card" Assay (Biomed industry, New Delhi, India). It is a rapid self performing qualitative, two site sandwich immune assay, utilizing whole blood for the detection of *Plasmodium* species. The test uses monoclonal anti *Pf* Plasmodium lactate dehydrogenase (pLDH) antibody (test line F) and monoclonal anti Pan specific pLDH antibody (test line P) immobilized on a nitro cellulose strip. Malaria card detects the presence of pLDH released from the parasitized blood cells, for the detection of all malarial parasites. The test was performed and interpreted as per the instructions of supplier.

## 3. Results

**Figure 1 Age and sex distribution of patients.**

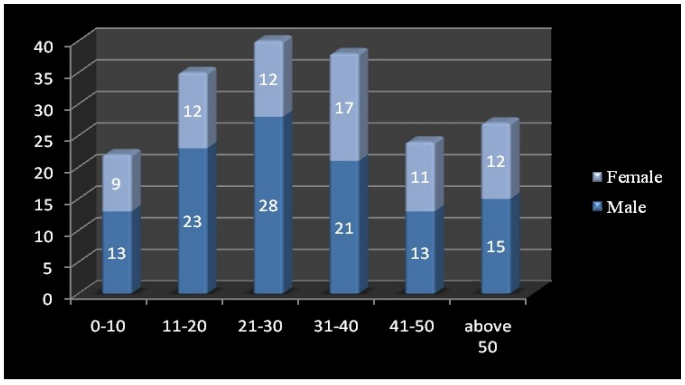

**Table 1. Comparison of positive results of PBS, ICT and QBC.**

| Tests | No. of positive results |
|---|---|
| PBS+ICT+QBC | 58 |
| PBS+ICT | 39 |
| PBS+QBC | 12 |
| ICT+QBC | 00 |
| Only PBS | 07 |
| Only ICT | 06 |
| Only QBC | 00 |

In the present study, out of 186 patients 113 (60.7%) were males and 73 (39.3%) were females. In males the maximum number of patients belonged to 21-30 years age group, whereas in females the maximum number of patients belonged to 31-40 years age group (Figure 1).

Fever with chills was the most common clinical presentation followed by hepatomegaly, splenomegaly and jaundice.

As shown in Table 1, out of 186 patients included in study, 122 (65.5%) of were positive for malaria by any of the three tests used. Out of 122 cases positive for malaria, 116 (95.1%) were positive for malarial parasite on the PBS, 98 (84.5%) for *P. falciparum* and 18 (15.5%) for *P. vivax*. ICT was positive in 103 (84.4%) cases, 95 (92.2%) were positive for *P. falciparum* and 08 (7.8%) for *P. vivax*. QBC was positive in 70 (57.3%) cases, 66 (94.2%) for *P. falciparum* and 04 (5.8%) for *P. vivax*. 07 cases were positive only on PBS which included 6 cases of *P. falciparum* and 1 case of *P. vivax*. 06 cases were positive only by ICT and all of them were *P. falciparum*.

## 4. Discussion

The prompt and effective diagnosis of malaria is not only important in treatment and but also have epidemiological significance in developing countries like India.

In malaria examination of PBS is considered as a “Gold Standard” test universally. This method was introduced by Sir Ronald Ross in 1903.[8] PBS is stained with Romanowsky stains. In our study maximum number of cases were positive by PBS examination. In PBS examination, the thick smear has high sensitivity whereas thin smear is highly specific for detection of malarial parasite.[8] The thick smear detects the low level of parasitaemia and reappearance of circulating parasites during infections, recrudescence and relapse.

Although a labor intensive and time consuming PBS has several advantages. PBS examination is cost effective method for diagnosis of malaria. The evaluation of intensity of parasitaemia is also possible by PBS examination and hence the efficacy of treatment can be monitored.[10] In addition to this, it also allows the detection of other haemo-parasites like *Leishmania donovani* (LD bodies), *Babesia*, *Microfilaria* and *Trypanosoma*.[10]

In our study the commercially available kit “Malaria Card” assay was used. Malaria Card is non microscopic rapid diagnostic test for malaria. In the present study ICT was positive in 84.4% of cases. The sensitivity and specificity of test is shown to be more than 90% in the presence of more than 100 parasites per µl.[8,10,11] Sensitivity of the test decreases with decrease in level of parasitaemia. The values of pLDH fall to undetectable levels about maximum 3-5 days after successful therapy, therefore this test can be used to monitor prognosis and serves as an indication of recrudescence and possible drug resistance.[8,10] It is user friendly and obviates the need of microscope and skilled microscopist. The test can be carried out bedside on onsite in the field. No false positivity is seen in this test since there is no cross reactivity with LDH from other haemoparasites and pathogenic bacteria and fungi. Due to cross reactivity in pan specific band accurate species identification is not possible in mixed infection.[8]

The centrifugal buffy coat or QBC was introduced by Spielman *et al* in 1988.[12] In QBC the parasites are seen through the capillary tube using a special long focal length objective with a fluorescent microscope. In our study only 57.3% of cases were positive by QBC. QBC is costly technique and requires fluorescent microscope with skilled and experienced microscopist. In QBC *P. falciparum* gametocyte with buoyant density similar to that of leucocytes are found within the buffy coat, where it is difficult to distinguish parasite from leucocyte.[9]

The only advantage of QBC method over immunological method for diagnosing malaria is its ability to detect other haemoparasites like Babesia and microfilaria.

## 5. Conclusion

From our study it can be concluded that ICT for malaria can be used as rapid, simple and mass screening test in remote and rural areas as skilled professional are not required. ICT may prove as useful tool for malaria control programmes. In established setup where skilled and expert personnel are available PBS remains the test of choice and ICT can be used as an adjuvant to PBS.

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