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**ORIGINAL ARTICLE**

# Validity of Rapid Diagnostic Test Kit Keeping Light Microscopy in Diagnosing Malaria in Febrile patients

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## ABSTRACT

**Background:** Malaria is the common health problematic disease and it causes major number of mortality and morbidity. It must be diagnosed accurately and rapidly. A rapid and correct diagnosis can reduce the sufferings. So it's essential to establish an appropriate and reliable laboratory procedure for malaria diagnosis. Microscopic examination of blood film is taken as the **gold standard** method.
**Aim:** To assess the reliability and validity of rapid diagnostic test kit, so that the rapid and a cost effective diagnostic method may be adopted for the timely diagnosis, and treatment may be offered to patients
**Methodology:** 350 patients having history of fever were taken for this Cross sectional validation study. Tested by ICT rapid diagnostic kit for detection of malarial antigen and results were then compared with gold standard microscopic results
**Results:** for the 350 suspected patients the rate of positivity and negativity were 60% and 40% respectively by microscopic examination and for ICT for detection of malarial antigen were 58% and 42% respectively. Specificity and sensitivity for antigen detection by ICT when it was compared with microscopy of blood smear was 95% and 93.33% respectively. Its overall diagnostic accuracy was good and it was 94%
**Conclusions:** ICT for antigen can diagnose malaria reliably. It is rapid; simple has good sensitivity and specificity. It can detect very low level of parasitic infection and can be performed at point of care and for screening at mass level. No special equipment or high level of setup and skills needed for it.
**Keywords:** Malaria, test kit, microscope

## INTRODUCTION

Malaria is the widespread disease around the world. Approximately 3.3 billion individuals are vulnerable in endemic countries of Asia, Latin America and Africa[1]. Its main signs and symptoms are fever, flu-like sickness and chills. There are 5 well-known species of malaria that can be the source of malarial infection in humans. Amongst all, Plasmodium falciparum is the most fatal[1,2]. P.vivax is extremely rampant in Pakistan having 64% incidence and P. falciparum as the next most prevalent species having 36% incidence. The heavy entry and constant presence of immigrants from Afghanistan, where *P. falciparum* is very common, may also increases the incidence of *P. falciparum* in Pakistan[1]. Studies have revealed that refugees have low level of immunity and therefore more vulnerable to malaria[2,4,5]. These conditions enhance the transmission of malaria in the local population.

Low incidence of malaria in federal area and Punjab is due to better socio economic conditions and better healthcare farcicalities in these areas.

The major problem in controlling the spread and reducing its mortality and morbidity is due to poor socioeconomics and lack of access to healthcare facilities for its diagnosis and management in remote areas[6].

A quick and accurate diagnosis of malaria is the key for the active disease control. There are two diagnostic approaches usually used, one is its clinical diagnosis and widely used approach and the other one is microscopic detection[3]. Malaria sign and symptoms are very non-specific and also overlap with other fever causing conditions. So, diagnosis based on clinical grounds alone is variable and need to be supported and confirmed by laboratory tests[7].

Different techniques are in use for its detection like microscopy, molecular and immunological methods[3]. For the definite diagnosis detection of malaria parasite in peripheral smear is needed.

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Microscopic inspection of blood film is the "gold standard" method[8,9]. It is simple and has low cost but it is overwhelming, need skilled technicians and hematologist and its sensitivity is in doubt for the interpretation of dual infection by the malaria specie as well for low level parasitemia[8]. Furthermore, it is not appropriate for the mass level screening programs[9].

It is a curable disease, with timely and precise diagnosis and treatment we can decrease the illness and its mortalit[7]. For this it is important to have an appropriate and reliable and cost effective laboratory method for its diagnosis.

So we conducted this study for the evaluation of validity of ICT rapid diagnostic method by comparing it with microscopic detection which is taken as gold standard.

## METHODOLOGY

This study is cross sectional validation and was conducted in the hematology department, Benazir Bhutto Hospital for a period of one year from 1st Sept.2017 to 31 Aug.2018. It is a tertiary care reference laboratory. Permission was obtained from Institutional Ethical Committee and consent was taken from all patients fulfilling the inclusion and exclusion criteria. Demographic information like name, age, gender and address were obtained.

Three hundred and fifty patients were taken who fulfills the inclusion criteria like fatigue, abdominal discomfort, fever, Hepatomegaly and splenomegaly. Patients who took antimalarial treatment were excluded from the study. Data then systematically recorded in designed Performa and analyzed by SPSS version 16.0 computer program.

**Sample collection and processing:** Blood samples were collected aseptically. Thick film was made and unfixed dry thick film was put in buffered water until no more hemoglobin can be seen falling from the unfixed film. Thin film was also made and slides were labeled and allowed to be air-dry[28]. Fixation of the thin film was done with ethanol and stained with giemsa stain.

**Examination of the peripheral smear by microscopy[10]:** Both thin and thick films were examined with the microscope. At least 100 filed were examined before labeling the film negative for

malarial parasite. Parasites were counted per 200 white blood cells (WBC) and then it is multiplied by 40 for the parasites per microliter of blood.

**Detection of Antigen:** For antigen detection test were conducted by ICT (immunochromatographic) method with Humasis Malaria P.F/Pan Antigen Test Kit Ref No AMAL-7025.

**Procedure:** As per kit manual test was performed after centrifugation of patient sample. 0.05ml serum was placed into sample well and 4 drops of buffer was then added into Assay buffer well immediately. Results were read after 20min. It was interpreted negative if only the control band appeared and positive for *P.falciparum*, if one or two other lines appeared in addition to the control line. Similarly it was interpreted as positive for *P. vivax* and other species of malaria except for *P. falciparum* if one line just following to the control line appeared. The test was interpreted as invalid, if even control (C) line is missing.

## RESULTS

Three hundred and fifty patients were taken for this study results are shown in the tables.

Table 1 shows distribution of age and gender of the studied population. Males were 224 and the remaining 126 were females with ratio of 1.77:1, mean age is 30.21 and standard deviation is 5.0792.

Table 2 showing distribution of detection of malaria by the two comparing methods. 210 were positive and the rest 140 were not detected by microscopic examination of the smear. CT method detects 203 cases as positive and 147as negative.

Table 3 shows comparison of the ICT method for antigen detection and its specificity and sensitivity when it was compared to gold standard microscopy. It was 95% and 93.33% respectively. Diagnostic accuracy was 94%.

Table 1: Suspected cases (n=350)

| Age groups (years) | Study groups | | |
|---|---|---|---|
| | Male | Female | Total |
| 20 to 25 | 22(61.11%) | 14(38.88%) | 36 |
| 26 to 30 | 119(64.32%) | 66(35.67%) | 185 |
| 31 to 35 | 40(64.51%) | 22(35.48%) | 62 |
| 36 to 40 | 43(64.17%) | 24(35.82%) | 67 |
| Total | 224(64%) | 126(36%) | 350 |

Table 2: Detection rate by the two comparing methods.

| Methods | Results | |
|---|---|---|
| | Positive | Negative |
| Microscopy of the smear | 210(60%) | 140(40%) |
| ICT for malarial antigen | 203(58%) | 147(42%) |

Table 3: Specificity and sensitivity of ICT for antigen when compared with gold standard microcopy

| Test results | Disease +ve (MP+) | Disease -Ve (MP-) | Specificity | Sensitivity |
|---|---|---|---|---|
| ICT(+) | 196 (A) | 7(B) | 95.0% | 93.33% |
| ICT(-) | 14(C) | 133(D) | | |
| Total | 210 | 140 | | |

## DISCUSSION

Malaria is the common prevalent health issue especially in under developed countries[11]. Physicians usually diagnose it by the clinical symptoms and its signs but this practice has very poor predictive value leading to its incorrect diagnosis and treatment[12].

It must be diagnosed accurately and rapidly. A rapid and correct diagnosis can reduce the sufferings. So it's essential to establish an appropriate and reliable laboratory procedure for malaria diagnosis. Microscopic examination of blood film is taken as the **gold standard** method. It is cost effective and simple but overwhelming and need highly trained staff and its sensitivity is doubtful for the low level of parasite index. Furthermore, it is not suitable for the screening and control programs where there are huge numbers of blood films have to be examined[13].

Some new methods such as: polymerase chain reaction, Quantitative Buffy Coat, Fluorescence microscopy Immunochromatography, Enzyme Linked Immunosorbent Assay and numerous other molecular techniques are also being in use for its diagnosis. ICT procedure has been recently introduced and is simple and fast[11].

We compared ICT for antigen detection with gold standard microscopy in this study. Specific plasmodium LDH antigen was detected by the ICT method.

Our study shows that the age groups 20-40 years were mostly affected by malaria (Table 1). Similar results were founded by a study that is also from Pakistan[14]. These results can also be compared with another study that is from Saudi Arabia in year 1998 which described that two months to eighty years were affected[15]. Highest suspected cases that are 185 were from 26- 30 years age group. This comprehends with the results from one study of Uganda having majority of the cases <40 years[16]. From our and the various other studies, it may be concluded that 16- 35 years age group commonly get affected by malaria which is due to engagement of this group in outside work where they may get exposed to mosquito[17].

210(60%) were microscopy positive in our study and 203(58%) were by ICT for in the suspected cases (Table 2). These results are comparable with another study from Pakistan which found 45.5% microscopy positive and 43.2% were by Immunochromatography (ICT) for antigen[18]. Similar results were obtained by the study from States of America in 1988 where 48% microscopy positive and 45% by Immunochromatography (ICT)[19].

We found sensitivity of the ICT kit as 93.33% and its specificity as 95.0% when its results are compared with microscopy (Table 3). Similar results were shown by a study from Australia in 2002, having 85% sensitivity and 96% specificity (20). Another study from USA found 94% sensitivity and specificities of 100% when compared with microscopy. As regards to specificity and sensitivity, these findings are comparable to this study.

In our study plasmodium LDH antigen was identified. It is secreted by the living parasite and is plasmodium metabolic enzyme[21]. After effective treatment it vanishes quickly from blood. Hence finding of antigen shows active infection, diagnostic accuracy of ICT for antigen when compared with microscopy was 0.94 which is less than 1 and reliable and suitable method for diagnosis of active infection.

Considering the results of this study, it was established that all age groups can be affected by malaria but bulk of cases were in age group 25-30 years. When comparing with gold standard microscopy method ICT have sensitivity of 93.22% and specificity 94.87% respectively and its diagnostic accuracy is 94%.

## CONCLUSIONS

ICT for antigen can diagnose malaria reliably. It is rapid; simple has good sensitivity and specificity. It can detect very low level of parasitic infection and can be performed at point of care and for screening at mass level. No special equipment or high level of setup and skills needed for it.

**Conflict of interest:** Nil

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