*ORIGINAL ARTICLE*

# Evaluation of ICT Malaria Pf / Pv Immunochromatographic Test for Rapid Diagnosis of *Plasmodium falciparum* and *Plasmodium vivax* Infections at Jimma Malaria Sector, Southwestern Ethiopia.

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## ABSTRACT

**Background:** *Rapid and simple antigen capture tests have been used widely for the diagnosis of only falciparum malaria infection. These methods have a grand insufficiency where* **Plasmodium falciparum** *and* **Plasmodium vivax** *infections are co – endemic. We evaluated an imunochromatographic test, ICT malaria P.f/P.v (AMRAD ICT, Australia) that can distinguish infections due to both species.*

**Methods:** *A cross-sectional study was conducted on 100 patients with a presumptive clinical diagnosis of malaria to evaluate ICT Malaria P.f/P.v test against blinded microscopy Giemsa stained thick film as a "gold standard". The study was done at Jimma Malaria Sector, southwest Ethiopia from 7 to 28 February 2000.*

**Results:** *Out of 100 patients, only 50% of those with presumptive clinical diagnosis of malaria were parasitaemic. Correspondingly, the card test identified 49 positive cases and there was no statistically significant deference between the two methods on the positivity rate for the diagnosis of a malaria infection ($P > 0.9$, $\chi 2 = 0.01$). The sensitivity, specificity, Positive predictive value (PPV), Negative predictive value (NPV) and accuracy of ICT Malaria P.f/P.v test were 87.5%, 91.3%, 46.7%, 98.8% and 91% for the diagnosis of* **P. falciparum***; and 78.6%, 98.3%, 97.1%, 86.4% and 90% for* **P. vivax***, respectively. These parameters were greater than 90% for the diagnosis total malaria infection. The mean time to perform microscopic examination and ICT Malaria P.f/ P.v test were 54 and 10 minutes respectively and the deference was statistically significant ($P < 0.00001$, $\chi 2 = 67.3$).*

**Conclusions:** *In this study the ICT Malaria P.f/P.v was found to be rapid, simple and accurate for the diagnosis of* **P. falciparum** *and* **P. vivax** *infections at primary health care level where there is no laboratory. However, the current market price of the kit will be the major obstacle for routine use.*

**Key words:** *Malaria, Plasmodium falciparum, Plasmodium vivax, rapid antigen capture test, ICT Malaria P.f /P.v*

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## INTRODUCTION

Malaria is one of the major global health problems and most of the morbidity and mortality reports are from Africa (1,2). Malaria has been a great burden for Ethiopian population due to its existence as endemic and epidemic. *Plasmodium falciparum* and *Plasmodium vivax* are widely distributed and these two species roughly account 60% and 40% of all malaria case respectively. *Plasmodium malariae* and *Plasmodium ovale* are rarely reported. However the relative frequency of the species varies from place to place and from season to season (3).

Early detection and promotion of treatment is the cornerstone of Primary Health Care (PHC) service to reduce morbidity and mortality due to malaria (4,5). Presumptive treatment of malaria without laboratory conformation is frequently inconvenient and wastage of resources (6,7).

Microscopic examination of Giemsa stained thin and thick blood film remains the method of choice for the diagnosis of acute and chronic malaria infection. But it has many disadvantages, like the high indirect cost to establish infrastructure and to supply and maintain resources like trained manpower and good quality of microscope. It also need longer time for its examination and it is fair sensitive. As the consequence routine microscopic diagnosis is often poor quality, unreliable, and may not be available at periphery of the health care system (6,8,9).

Thus many research laboratories have concentrated their efforts on the development of alternative methods and they came up with sophisticated techniques like polymerase chain reaction (PCR) with greater than 99% sensitivity and specificity (10). However, these new techniques are generally too expensive and requires too much skilled in put to be used for routine purpose.

A fundamental objective of World Health Organization (WHO) global malaria control strategy is to make alternative diagnostic techniques that are rapid, simple, accurate, affordable and applicable at the village level by community health workers, so that effective therapy can be promptly administered (6).

In response of this, recent advances have now made it possible to detect specific antigens of both *P. falciparum* and *P. vivax* species in blood samples. These antigen capture methods are available in the market with different brand names and many studies were conducted using them. For examples the early methods, ParaSight F (Becton Dickinson, Tropical Disease Diagnostics, USA) (11), ICT Malaria P.f (ICT Diagnostics Brockville, NSW, Australia) (12) and Rapid Test Method (RTM, Quorum Diagnostic Inc. Vancouver, BC, Canada) (13) detect histidine-rich protien-2 (HRP-2) which is a water soluble antigen specifically produced only by *P. falciparum* and it is released from parasitized cells in to the blood circulation (14).

The more advanced and recent techniques which detect infections by *P. falciparum*, and /or *P. vivax* are Opti-Mal (Flow. Inc Portland, Org) which detects parasite lactate dehydrogenase (pLDH) enzyme (15), and ICT Malaria P.f /P.v (AMRAD ICT, Australia) which detects HRP-2 specific for *P. falciparum* and pan-malaria antigen, that is a common antigen for both *P. falciparum* and *P. vivax* (16).

Studies indicated that these antigen capture methods are rapid, very sensitive to detect the antigen even with low parasitaemia, requiring almost no experience and can be carried out in the field situation without the need for slides, microscopes, centralized laboratories and highly trained man power. These enables

diagnosis at the Primary Health Care (PHC) level and by CHW (12.14.17).

In Ethiopia the only method used to confirm malaria infection is microscopic examination of Giemsa stained thick and thin blood films. To alleviate problems associated with this method few studies were conducted to evaluate the application of antigen capture tests in our context (13.18). However these studies were aimed at the methods that detect only *P. falciparum* infection which has little importance in a country where both *P. falciparum* and *P. vivax* infections are co-endemic.

Thus, the main aim of this study was to evaluate ICT Malaria P.f /P.v method (AMRAD ICT. Australia) that can diagnose both *P. falciparum* and *P. vivax* infections.

## MATERIALS AND METHODS

The study was conducted in Jimma town, located 335 km south west of Addis Ababa, at Jimma malaria sector. The ICT malaria P.f/P.v method was evaluated in comparison with Giemsa stained thick blood films as a "gold standard" for the diagnosis of *P. falciparum* and *P. vivax* infections from February 7 to February 28, 2000.

A total of 100 study subjects were selected by simple random sampling method and the selection was made from patients those had presumptive clinical diagnosis of malaria. Information such as age, sex, axial body temperature, and anti malaria drug taken, if any, were collected using structured and pre-tested questionnaire.

Capillary blood sample from finger prick was taken after verbal consent sought from the study participants or their caretaker. For the thick blood film preparation 7µl of blood was taken using a calibrated capillary tube and evenly spread on a pre-coded slide over an area of about 10mm x 10mm. All blood films were air dried and stained for 30 minutes with 3% Giemsa stain (pH 7.2) using the standard procedure (19-21). The time to prepare, stain and examine the thick blood films was recorded for each specimen. Meanwhile, the methanol fixed thin blood films, that were prepared on separate slides were stained in the same manner and used for species identification.

The blood films were examined by experienced microscopists of the malaria sector with out prior knowledge of the ICT Malaria P.f /P.v test results. An Olympus (CH-2, Japan) binocular microscope with built-in illumination and a total magnification of 1000x using an immersion oil objective was employed for the examination of blood films. A thick blood film was reported as negative if no malaria parasite was seen in an average 200 oil immersion fields and for the species identification the thin blood films were examined for each positive thick blood film results. Parasitaemia level was determined based on the method proposed by Green wood and Armstrong, using a thick blood film made from a fixed volume of blood (21). The number of parasites per oil immersion field is multiplied by a factor of 500 and this gives the estimated number of parasites per µl of blood. All slides were examined by two experienced microscopists and the average value was used for calculation. Furthermore, all discordant and 10% of the slides with concordant results were cross-checked by the most experienced microscopist.

*Principle and procedure of ICT malaria P.f/P.v test:*

The card test was carried out by the principal investigator in accordance with the recommended test procedures described

by the manufacturer, which was supplied with the test kit. This ICT Malaria P.f/P.v (AMRAD: ICT, Australia) test detects circulating *P. falciparum (p.f)* and *P. vivax (P.v)* antigens in whole blood. The test uses two antibodies, which have been immobilized as two separate lines across a test strip. One antibody (test area 1) is specific for the histidine-rich protein 2 antigen of *P. falciparum* (Pf HRP2). The other antibody (test area 2) is specific for pan-malarial antigen, which is common for both *P. falciparum* and *P. vivax* species. Whole blood (15µl) is applied to a sample pad impregnated with colloidal gold-labeled antibodies, which are directed against the two malarial antigens. When a positive sample is applied, malarial antigens bind to the gold- coupled antibodies in the pad, and the immune complexes formed migrate along the test strip where they are captured by the immobilized antibodies. When capture occurs, a pink line forms in area 1and/or 2 of the test window (Fig. 1). When a negative sample is applied these lines do not form. A procedural controlling line will appear, in the area of the test window, if the test has been performed correctly. A result is interpreted as positive when any visible line in area 1and/or 2, forms together with a line in area C. The test is positive even when the lines in area 1 or 2 appear lighter or darker than the C line. The test is negative, if only the C line appears and a test is considered invalid if a line does not appear on area C.

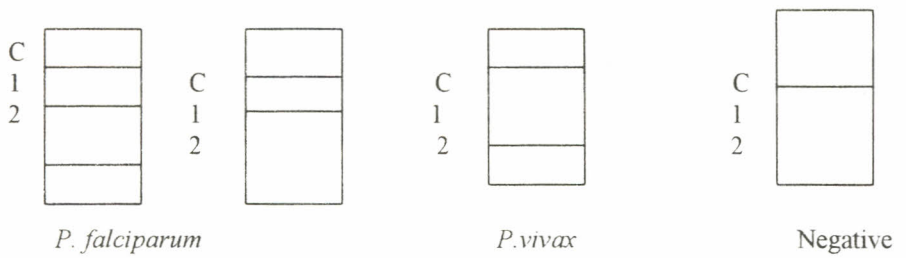


**Fig 1.** Test cards illustrating positive and negative test results for both *P. falciparum* and *P. vivax*

The data were sorted by hand tally and analyzed using computer (EPI-INFO version 6 statistical package). Parameters like sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), accuracy and the time needed to perform the tests were ascertained in comparison with the "gold standard" method. Chi – square test was utilized to check statistical significance deference among the study variables.

## RESULTS

Among the 100 study subjects 50% were females with male to female ratio of 1:1. Seventy six percent of the patients were from Jimma town and the rest came from rural area. Forty four percent of the patients were in 15-24 age group, but highest positivity rate (58%) was seen in the age group of 25-34. Statistically there was no significant association between malaria infection and age, sex, ethnicity, religion, educational status and occupation of the patients ($P > 0.05$, Table 1). Seven of the 100 patients had already started anti malarial drug treatment and there were no statistically significant differences in the mean body temperature of malarious and non-malarious patients ($P > 0.05$).

The ICT Malaria P.f/ P.v method gave

comparable results with Geimsa stained thick blood film for the diagnosis of total malaria infection (Table 2) and the difference in the positivity rate observed by the two methods was not statistically significant ($P > 0.9$, $\chi^2 = 0.01$).

**Table 1**. Socio-demographic distribution of patients suspected to have malaria in Jimma malaria sector, February 2000.

| Variables | Blood film | | | Statistical test |
|---|---|---|---|---|
| | Positive No (%) | Negative No (%) | Total No (%) | |
| Age | | | | |
| 0-4 | 6 (6) | 6 (6) | 12 (12) | |
| 5-14 | 11 (11) | 12 (12) | 23 (23) | $X^2 = 0.56$ |
| 15-24 | 21 (21) | 23 (23) | 44 (44) | D.f = 3 |
| 25-34 | 7 (7) | 5 (5) | 12 (12) | P = 0.904 |
| 35-44 | 3 (3) | 3 (3) | 6 (6) | |
| 45-54 | 2 (2) | 1 (1) | 3(3) | |
| Total | 50(50) | 50(50) | 100 (100) | |
| Sex | | | | |
| Male | 25 (25) | 25 (25) | 50 (50) | $X^2 = 0$ |
| Female | 25 (25) | 25 (25) | 50 (50) | D.f = 1 |
| Total | 50(50) | 50(50) | 100 (100) | P = 1 |
| Address | | | | |
| Jimma town | 32 (32) | 44 (44) | 76 (76) | $X^2 = 7.98$ |
| Out side Jimma town | 18 (18) | 6 (6) | 24 (24) | D.f = 1 |
| Total | 50(50) | 50(50) | 100 (100) | P = 0.005 |
| Ethnicity | | | | |
| Oromo | 21 (21) | 19 (19) | 40 (40) | |
| Amhara | 8 (8) | 11 (11) | 19 (19) | $X^2 = 4.98$ |
| Dewaro | 9 (9) | 5 (5) | 14 (14) | D.f = 5 |
| Gurage | 2 (2) | 5 (5) | 17 (17) | P = 0.418 |
| Yem | 5 (5) | 2 (2) | 7 (7) | |
| Keffa | 3 (3) | 4 (4) | 7 (7) | |
| Others | 2 (2) | 4 (4) | 6 (6) | |
| Total | 50(50) | 50(50) | 100 (100) | |
| Religion | | | | |
| Christian | 31 (31) | 31 (31) | 62 (62) | $X^2 = 0$ |
| Muslim | 19 (19) | 19 (19) | 38 (38) | D.f = 1 |
| Total | 50(50) | 50(50) | 100 (100) | P = 1= |
| Educational status | | | | |
| Literate | 39 (39) | 43 (43) | 82 (82) | $X^2 = 1.08$ |
| Illiterate | 11 (11) | 7 (7) | 18 (18) | D.f = 1 |
| Total | 50(50) | 50(50) | 100 (100) | P = 0.297 |
| Occupation | | | | |
| Student | 17 (17) | 19 (19) | 36 (36) | |
| House wife | 9 (9) | 11 (11) | 20 (20) | $X^2 = 0.7$ |
| Employed labor | 9 (9) | 7 (7) | 16 (16) | D.f = 3 |
| Others | 15 (15) | 13 (13) | 28 (28) | P = 0.87 |
| Total | 50(50) | 50(50) | 100 (100) | |

D.f = Degree of freedom

**Table 2**. Total malaria parasite detection by microscopic exam77ination and ICT Malaria P.f/P.v methods among malaria suspected patients. Jimma malaria sector. February 2000.

| Microscopic examination | ICT Malaria P.f/ P.v Number of cases | | |
|---|---|---|---|
| | Positive No. | Negative No. | Total No. (%) |
| Positive | 45 | 5 | 50 (50.0) |
| Negative | 4 | 46 | 50 (50.0) |
| Total | 49 | 51 | 100 (100.0) |

Out of the 100 patients examined microscopically, 50 (50%) were found to be infected with malaria parasites; of which, 42 (84%) were infected with *P. vivax* and 8 (16%) by *P. falciparum*. Mixed infection and heamoparasites other than malaria were not detected. Parasitaemia level was in the ranges of 80/µl to 10,050/µl and 80/µl to 48,000 /µl, respectively for *P. falciparum* and *P. vivax* infection. The mean parasitaemia of the patients with malaria was 6913 parasites /µl of blood.

Correspondingly, the *ICT Malaria P.f/P.v* test had 49 (49%) positivity rate, out of this 34 (69.4%) were positive for *P. vivax* and the rest 15 (30.6%) were positive for *P. falciparum*. There were 5 cases detected by microscopy but not by the *ICT Malaria P.f/P.v* tests. All these five false negative cases were *P. vivax*; out of these, 80% (4/5) of them had parasite density between 840/µl to 1500/µl of blood and the remained one had 80 parasites/ µl of blood. On the other hand, ICT Malaria P.f/P.v detected 4 false positive cases that were not detected by microscopy and all of them were *P. falciparum* (Table 3).

**Table 3**. Malaria parasite detection by microscopic examination and ICT Malaria P.f/P.v methods among malaria suspected patients of Jimma malaria sector. February, 2000.

| Microscopic examination | ICT Malaria P.v / P.v | | | |
|---|---|---|---|---|
| | *P. falciparum* | *P. vivax* | Negative | Total |
| *P. falciparum* | 7 | 1 | 0 | 8 |
| *P. vivax* | 4 | 33 | 5 | 42 |
| Negative | 4 | 0 | 46 | 50 |
| Total | 15 | 34 | 51 | 100 |

Except the PPV for the diagnosis of *P. falciparum*, the ICT Malaria P.f/P.v test gave satisfactory result for the diagnosis of total malaria, *P. falciparum* and *P. vivax* infection (Figure 1). The sensitivity, PPV and accuracy were 50%, 20% & 16% respectively when the parasite density was less than 100 /µl of blood and these parameters raised to 100% when it was above 10,000 /µl for the diagnosis of total malaria infection (Figure 2). The highest sensitivity, PPV, and accuracy was obtained when the parasite density in the range of 101- 10,000/µl of blood for both species (Table 4).

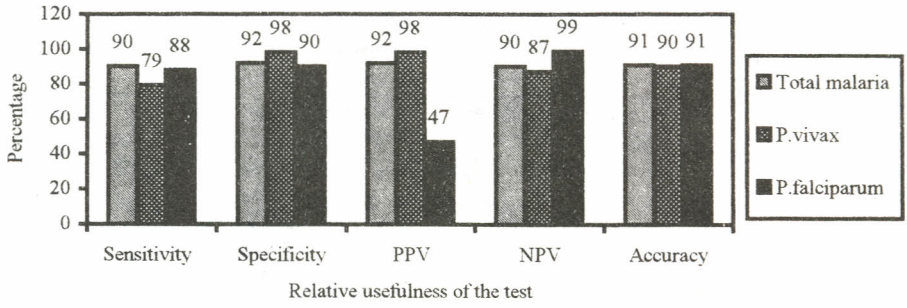


**Figure 2.** the relative usefulness of the ICT Malaria P.f/p.v test for the detection of total malaria. *P. vivax* and *P. falciparum* infection among malaria suspected patients of Jimma malaria sector, February 2000.

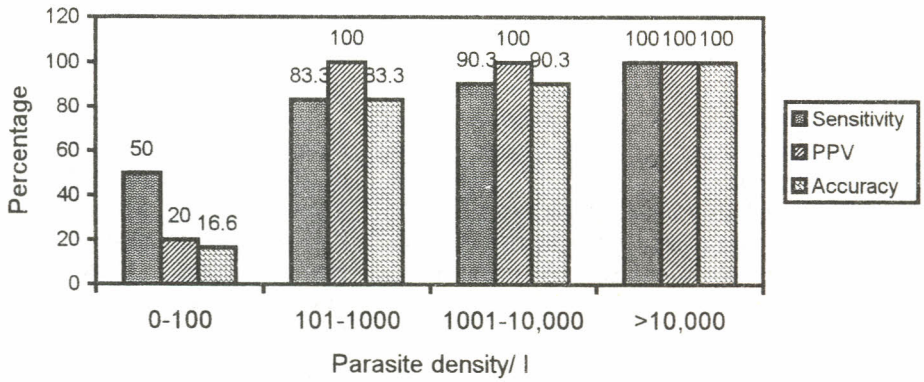


**Figure 3**. Total malaria sensitivity. PPV and accuracy of ICT Malaria P.f/p.v test by parasitaemia range among malaria suspected patients of Jimma Malaria Sector Feb. 2000.

**Table 4.** Sensitivity, PPV and accuracy of ICT Malaria P.f/P.v for *P.vivax* and *P. falciparum* versus parasite density per μ l of blood among malaria suspected patients of Jimma malaria sector, February 2000.

| No of parasites /μl of blood | Species | Sensitivity | PPV | Accuracy |
|---|---|---|---|---|
| 0-100 | *P. vivax* | NA* | NA | NA |
| | *P. falciparum* | 100% | 20% | 20% |
| 101-1,000 | P. vivax | 83.3% | 100% | 83.3% |
| | *P. falciparum* | NA | NA | NA |
| 1,001-10,000 | *P. vivax* | 80.8% | 100% | 80.8% |
| | *P. falciparum* | 100% | 71% | 71.4% |
| > 10, 000 | *P.vivax* | 77.8% | 87.5% | 70% |
| | *P. falciparum* | 50% | 33.3% | 25% |

*NA: Not applicable.

The average time to prepare, dry, stain and examine the blood films for microscopical examination was 54 minutes. The minimum, maximum and the mean time to perform the ICT Malaria *P.f/P.v* test were 5, 15, and 10 minutes respectively and the time deference to perform the two methods is statistically significant (P< 0.00001, $\chi 2$ =67.3).

## DISCUSSION

The positivity rate of malaria was high among patients who came from outside Jimma town. This might be because of shortage of accessible health service unit at rural areas and the patients might came when the disease becomes severe and also it might be due to uneven distributions of the sample. In agreement with previous investigation (22), malaria infection was independent of age, sex, ethnicity, religion, educational status and occupation of the patients. There is no statistically significant difference between the mean body temperature of malarious and non-malarious patients. Thus diagnosis of patients mainly based on clinical symptoms is unreliable, because viral and bacterial infection can present with similar clinical signs (7).

Blood films taken from 100 malarial symptomatic patients were tested and 50% of them were identified as positive for malaria parasite while the *ICT malaria P.f/P.v* test detected 49% as malarial positive cases. There was statically insignificant deference between the two tests on the proportion of the positive results thus: we can use the two methods interchangeably for the diagnosis of a malaria infection. (P >0.9, $\chi^2$ =0.01).

Out of the 14% (14/100) discordant results, 28.6% (4/14) were positive for *P. falciparum* by the ICT Malaria P.f/P.v test but negative by microscopic blood film examinations. Other studies (12,13,23) also reported "false positive" results and there are several possible explanations. It may be that the superior sensitivity of ICT Malaria *P.f/P.v* test provides a more precise diagnosis of patients infected with *P. falciparum* by detecting parasites that could be missed by traditional blood film investigation. This may be because of the sequestering of *P. faliciparum* infected red blood cells in microcirculation (24), which allows HRP-2 antigen to be detected by the antigen capture method, no parasite to be

seen by microscopic examination. Even if this was not seen in our case, also the persistence of HRP –2 antigen that is specific for *P. falciparum* in blood circulation may continue for several days after anti-malaria treatment of infected cases (12,14). It is also possible that the presence of rheumatoid factor gives rise to genuine false positive result by the ICT Malaria *P.f/P.v* test (25).

The 35% (5/14) false negative *P. vivax* results might be due to many reasons. The first is when the concentration of parasites is below the ICT Malaria *P.f/P.v* detection level. The other plausible explanations might be the deletion of the gene that is responsible for the pan-malaria antigen expression and variation in the expression of pan-malaria antigen. The presence of blocking antibody, the formation of immune complexes or other possible factors which affect immunological reaction like pre or post zone reaction phenomena also might give false negative result.

In this study 28.6% (4 of 14) cases reported as *P. vivax* by microscopy were *P. falciparum* by the ICT Malaria P.f/P.v test. This phenomena occurred at high level of parasitaemia (> 4050 /µl) and the sensitivity, specificity and other parameters for each species affected significantly in spite of the presence of high parasite load. These findings were justified by the fact that the ICT Malaria P.f/P.v test can not detect mixed infection and if there is mixed infection it indicates the investigation as *P. falciparum* infection. This is because of the cross reactivity at the common antigen band. Even though there were no mixed infections observed by microscopy in this study, previous studies indicated that many *P.vivax* results by microscopy were found to be mixed infection when tested by PCR (10). Hence, the four P.vivax cases in the study might be mixed infections with *P. falciparum*.

An alternative explanation for the false positive *P. falciparum* result by ICT Malaria P.f/P.v while the microscopic examination was *P. vivax* could be that the HRP-2 antigen (specific for *P. falciparum*) capture assay shows some cross reactivity with infection caused by *P. vivax* (13,18,23). Thus, the true *P.vivax* infection would give false positive *P. falciparum* result by the ICT Malaria P.f/P.v method.

The single discordant result which was detected as *P. falciparum* by the microscopic examination but as *P.vivax* by the *ICT Malaria P.f/P.v* test method may be explained by a study conducted in Mali which indicated that 2-3% wild type of *P.falciparum* lack the specific gene for the synthesis of this HRP-2 antigen (26). The common band appeared in the absence of HRP-2 band that is specific antigen for *P. falciparum*. Other possible explanations forwarded are antigen variation in HRP-2, genetic heterogeneity for the expression of HRP-2, the presence of blocking antibody or the formation of immune complex (22). It is known that immunological reactions give false negative result when the antigen or the antibody is in excess. Thus, this may also be a possible explanation for the false negative appearance of the HRP-2 antigen band on the test card.

This study indicated that the sensitivity, specificity, PPV, NPV and accuracy of ICT Malaria P.f/P.v test for the diagnosis of total malaria infection were greater than 90%. This will also support the idea of the card test as an alternative method for the diagnosis of a malaria infection. The sensitivity of the ICT Malaria P.f/P.v for diagnosing of total malaria infection were 60% and 100%, respectively when the parasite density were below and above 100 /µl and 10,000/µl of blood. Furthermore, the PPV and accuracy were increased linearly with the increment of parasite density and this confirmed that the card test could give optimum total

malaria diagnosing capacity when the parasite density is high.

Optimum sensitivity, PPV and accuracy of ICT Malaria P.f/P.v test results for each species were seen when the parasite density was between 101-10.000/µl of blood. This might be due to small number of cases at low and high level of parasitaemia, occurrence of high false positive result for *P. falciparum*, and discordant species reading by the two methods at high level of parasitaemia density. The threshold of parasite detection level obtained by the ICT Malaria P.f/P.v test in this study was 80 *P. falciparum* organisms per µl of blood, and for the P. vivax it was 110 parasites / µl of blood which is close to the average microscopist detection limit that is 100 parasites / µl of blood (6).

A study conducted in Radamata Primary Health Centre, Indonesia, using the ICT Malaria P.f./P.v test showed 95.5%, 89.8%, 88.1% and 96.2% sensitivity, specificity, PPV and NPV, respectively for the diagnosis of *P. falciparum*. The specificity, negative predictive value, sensitivity, and positive predictive value were 94.8%, 98.2%, 75% and 50%, respectively for diagnosis of *P. vivax* (16).

This study (16) was conducted on symptomatic adults and children and the method showed an agreement on its sensitivity, specificity and NPV for the diagnosis of *P. falciparum* and *P. vivax* infections with our study. However, the PPV for diagnosis of *P.vivax* in their study was considerably lower (50%) than our finding (97.7%). On the contrary for diagnosing of *P. falciparum*, the PPV in our case was much lower (46.7%) than their finding (88.1%), and less than the desirable level. This might be due to the presence of high false positive *P. vivax* cases in their study whereas there were high false positive cases for *P. falciparum* in ours.

Though, association between intensity of line and level of parasitaemia was not statistically analysed due to low number of samples in this study, other studies done else where showed that intensity of the colour line is roughly related to the level of parasitaemia (16). This may help partially to estimate the level of parasitaemia that is beneficial for the follow up of patients on monitoring of drug response.

The average time to carry out preparation and examination of microscopic in this study was 54 minutes which is similar to previous studies (13), and it makes microscopic examination tedious and lowering the efficiency in situation where a large number of blood films have to be examined.

The mean time to perform the ICT Malaria P.f/P.v test was 10 minutes, similar with other antigen capture test methods, which detect only *P. falciparum* infection (13,14). This rapidity has significant importance especially during emergency situation and in identification of the causative agent during epidemics. On top of these, ICT malaria P.f/P.v test can be carried out simply by following the instructions (11,12) and needs only a demonstration.

In conclusion, in a country where malaria is endemic, the parasite density of symptomatic patients is high enough for the ICT Malaria P.f/P.v test to get optimum sensitivity and specificity. Also it identifies both *P. falciparum* and *P. vivax* infection which accounts more than 99%causes of malaria in Ethiopia. In contrast to light microscopy, the ICT Malaria P.f/P.v test found to be faster, easier, comfortable to transport to the field and has a long shelf life at ambient temperature. Further more, it can be easily labeled and stored for clinical or epidemiological study purposes.

and the test can be carried out by community health workers.

However before this ICT Malaria P.f/P.v test replace the thick and thin blood film especially in poor resource setting countries and in places with co-endemic *P. vivax* and *P. falciparum* infections occurs like Ethiopia, it has to be cost effective and have no/or minimal cross-reaction between Plasmodium species. It should also be able to detect all Plasmodium species using their respective specific antigens to diagnose mixed infections.

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