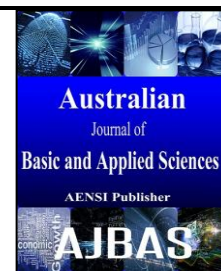




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# Comparison of Two Rapid Diagnostic Tests with Conventional Microscopy for Detection of Malaria parasites

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### ABSTRACT

Malaria is a major cause of death mostly in tropical and sub-tropical countries. The constant increase of population movements, leads to spread of infections previously limited to endemic areas. In this urgent situation, efficient diagnostic method is essential for the management and control of malaria. The aim of the present study was to compare two RDTs with conventional microscopy as diagnostic tools for detection of *falciparum* and non *falciparum* malaria in travelers and migrating people from malaria endemic areas to Egypt. A total of 112 blood samples were studied by light microscopy, microscopy positive malaria samples were tested by two malaria RDTs. Malarial parasites were observed in 28 cases (25%) with conventional light microscopy, of which the OptiMAL test detected 27 positive cases (96%) and only 26 cases (93%) were positive by Bio Tina Pf/Pan Mal test. Microscopy showed 15 *P. falciparum* and 13 *P. vivax* cases. The OptiMAL dipstick revealed 15 positive *P. falciparum* and 12 positive blood samples for non *falciparum* malaria while the Bio Tina Pf/Pan Mal test revealed 14 *P. falciparum* and 12 non *P. falciparum* malaria. The OptiMAL sensitivities in the present study was 100% and 92% for *P. falciparum* and non *falciparum* malaria respectively while the Bio Tina Pf/Pan Mal method sensitivities was 93% and 92% for *P. falciparum* and non *falciparum* malaria respectively. Microscopic examination for malaria parasite by thin and thick blood films is the gold standard technique for malaria diagnosis.

### INTRODUCTION

Malaria is endemic in more than one hundred countries worldwide, still considered the most important parasitic disease (White *et al.*, 2013). The World Health Organization (WHO) in 2014 reported that malaria lead to 197 million cases and 584 thousand deaths during 2013 while in 2014, there were an estimated 214 million cases and an estimated 438 thousand deaths (WHO, 2014; 2015). Most of malaria deaths occur in sub-Saharan Africa, in children (under 5 years) infected with *Plasmodium falciparum* (Cox-Singh *et al.*, 2008; Leiby *et al.*, 2008). *Falciparum* malaria is the commonest cause of severe malaria while *vivax* malaria may lead to severe anaemia in children. Other *Plasmodium* species occasionally reported as a cause of malaria include; *P. ovale*, *P. malariae* and *P. knowlesi* (Cox-Singh *et al.*, 2008). Although primarily limited to tropical and subtropical regions, cases of malaria are increasingly detected in non endemic areas among travelers and immigrants (Thwing *et al.*, 2007; Leiby *et al.*, 2008). In tropical areas, the parasite is transmitted mainly by the bite of some species of female Anopheles mosquito in addition to transfusion transmitted malaria especially with *P. falciparum*. Early and accurate diagnosis is the key to effective reduction of malaria-related morbidity and mortality (WHO, 2003; WHO, 2005; Bell and Peeling, 2006).

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Gustav Giemsa in 1904 reported the Giemsa-stained blood smears as the typical method of malaria detection. Afterward further alternative methods are used by scientists for the detection of malaria antibodies such as the enzyme-linked immunosorbent assays [ELISA] the indirect immunofluorescence antibody assay [IFA] (Sulzer *et al.*, 1969; Spencer *et al.*, 1979; Fleischer, 2004). Later, scientists developed significant methods to detect malaria antigens, and set the basis of commercial malaria RDTs used nowadays by researchers everywhere (Shiff *et al.*, 1994; Moody, 2002). Rapid diagnostic test detects malaria antigen in small amount of blood, it is an immunochromatographic assay that utilize monoclonal antibodies intended for detection of the specific parasite antigen and impregnated on a test strip. The commercially available RDTs either detect *P. falciparum* only, or distinguish *P. falciparum* from the non-*falciparum* species. The optiMAL dipstick test is a rapid immunochromatographic assay which detects *P. falciparum*-specific parasite Lactate Dehydrogenase enzyme (pLDH) and a parasite Lactate Dehydrogenase (pLDH) common to the four Plasmodium species (Piper *et al.*, 1999). Another RDT which is used in malaria surveys is SD Bioline Malaria Ag Pf/Pan test (Kim *et al.*, 2011; Jang *et al.*, 2013; Kosack *et al.*, 2013; Mendoza *et al.*, 2013). Recently synthesized RDTs are manufactured using different target antigens to be suitable for specific detection of malaria in different areas (Parra *et al.*, 1991; Garcia, 1996; Bell, *et al.*, 2006).

### **Patients and Methods:**

#### **Patients:**

A total of 112 patients with a clinical suspicion of malaria attending the outpatient clinics of The National Hepatology and Tropical Medicine Research Institute (NHTMRI) were involved in the present study. The study was conducted during the period from April 2014 to October 2016 in Egypt. The majority of patients were workers from tropical countries where malaria infection is endemic, in addition to Egyptian people who travelled to malaria endemic countries for work then return home sick. Informed consent was obtained from each participant, all data were confidential for the research use only. Initial assessment included collection of demographic information by questionnaire, followed by a standard medical history and clinical examination for each case. The clinical presumption of malaria was based on febrile illness (temperature more than 37.5°C) of short duration (at the time of admission or within the previous 48 h) and could be associated with chills, rigors and body aches, with a recent history of living in or visiting a malaria-prone country. Patients were between 22 and 50 years old. Cases having any other obvious reason for fever were excluded from the study, blood sample was collected into a sterile tube containing ethylenediamine tetra acetic-acid anticoagulated (EDTA) and blood smears were prepared from finger prick for each patient.

#### **Microscopy of Giemsa-stained blood films:**

Thin and thick blood films were prepared from finger pricks for all cases, stained with 10% Giemsa stain for 10 minutes and were examined properly by conventional microscopy with subsequent specification of the *Plasmodium* species. In thick blood film, red blood cells were hemolyzed and thus leucocytes and malaria parasites on the smear were detected and specific forms of parasite life cycle (e.g. ring, trophozoite, gametocyte and schizont) were recognized properly, 500 good fields were carefully examined, before considering it as negative, parasite densities were reported and species confirmation were obtained by examining the thin film (Mills *et al.*, 1999; Chotivanich *et al.*, 2006; Cheesbrough, 2009; WHO, 2010).

#### **OptiMAL dipstick test:**

Microscopy positive malaria samples were tested by OptiMAL dipstick method according to manufacture's (Flow inc. Portland, OR, USA) instructions. The immunochromatographic test detects the presence of pLDH antigen in lysed whole blood, pLDH is released from live malarial parasites and discrimination of *plasmodium* species is based on antigen differences between its isoforms. A negative control sample taken from an individual who had not been exposed to malaria (after microscopic examination by expert parasitologist) was included in the study. A positive control samples from cases diagnosed as *P. vivax*, *P. falciparum* and mixed infection of *P. vivax* and *P. falciparum* were also included in the present study. Aside from a control antibody reaction zone at the top of the test strip, the optiMAL dipstick contains two reaction zones or test lines. The first line encountered by the sample comprises of an antibody that is specific for *P. falciparum* pLDH, while the second test line is composed of a pan specific pLDH monoclonal antibody. Tests were counted as valid if a control line was observed. Test results were observed after 10 minutes. Interpretation of the assay test results was done as given below:

- (i) When one control band and two test bands appeared, the test was considered to be positive for *P. falciparum*.
- (ii) When one control band and one test band appeared the test was considered positive for non *P. falciparum* malaria.
- (iii) When only control band appeared at the top of the test strip without test band the test was considered to be negative.

### The Bio Tina GmbH MAL P.f/Pan Test:

CAT No.R 5014 is an in vitro rapid diagnostic test, the Malaria P.f/Pan Rapid test Device (whole blood) is a rapid membrane based immunoassay. Microscopy positive malaria samples were also tested by the Malaria P.f/Pan Rapid test. This test is based on qualitative detection of the *P. falciparum*-specific HRP-2 antigen and or pan-malarial Aldolase antigen. The membrane is pre coated with anti- HRP-2 and anti- Aldolase antibodies. The test utilizes colloid gold conjugate to selectively detect *P. falciparum* specific and pan-malarial antigen in whole blood. The test was performed and the results interpreted according to manufacturer's instructions, tests were counted as valid if a control line was observed. Test results were observed after 15 minutes. A negative control sample taken from an individual who had not been exposed to malaria (after microscopic examination by expert parasitologist) was included. A positive control samples from cases diagnosed as *P.vivax*, *P. falciparum* and mixed infection of *P.vivax* and *P. falciparum* were also included in the present study. In case the control line did not appear, the test was considered as invalid and it was repeated immediately. Interpretation of the assay test results was done as below:

- (i) The test was considered *P. falciparum* positive if control line along with PfHRP-2-specific and pan-malarial antigen lines were visible or if only PfHRP-2-specific lines were seen with the control line.
- (ii) If only control and pan malarial antigen lines were observed, the sample was counted as positive for a malaria parasite other than *P. falciparum*.
- (iii) When only control line appeared, the test was considered to be negative.

### Statistical analysis:

Data were collected and analyzed using the SPSS statistical program. For sensitivity, the test kits results were compared with Giemsa stain-microscopy results. The sensitivity was calculated as the proportion of positive test results obtained among samples containing malaria parasites by microscopy.

### Results:

The results obtained by the OptiMAL test and The Bio Tina GmbH MAL P.f/Pan Test, were compared to those obtained from examination of thin and thick smear blood film. Among 112 blood samples; 28 (25 %) were positive with microscopy, whereas testing for these microscopically positive samples by RDTs revealed, 27 positive with OptiMAL and 26 positive by Bio Tina Pf/Pan Mal test. Out of 28 positive samples by microscopy, 15 were *P. falciparum* and 13 were *P. vivax*. With the OptiMAL, 15 cases were *P. falciparum* and 12 cases were non *P. falciparum* while the Bio Tina Pf/Pan Mal test revealed 14 cases as *P. falciparum* and 12 cases as non *P. falciparum* (Table 1). The present study results showed OptiMAL sensitivities of 100% and 92% for *P. falciparum* and non *P. falciparum* respectively, when compared with conventional light microscopy as the standard diagnostic test. It can detect positive cases as non *P. falciparum* and *P. falciparum* species but can not differentiate non *P. falciparum* species. One case of microscopically positive malaria was not detected by the OptiMAL dipstick method. Concerning the Bio Tina Pf/Pan Mal test, 14 cases were diagnosed as *P. falciparum* while non *P. falciparum* detected cases were 12. Two microscopy positive malaria cases were not detected by the Bio Tina Pf/Pan Mal method. The present study results showed Bio Tina Pf/Pan Mal method sensitivities of 93 % and 92 % for *P. falciparum* and non *P. falciparum* respectively. It can detect positive cases as non *P. falciparum* and *P. falciparum* species but can not differentiate non *P. falciparum* species. Five cases of mixed malarial infection were observed by microscopy, which were also detected by OptiMAL dipstick test and Bio Tina Pf/Pan Mal method. Concerning microscopy positive *P. falciparum* samples, parasitaemia was ranging from 0.2% up to 5%. Three cases showed gametocytes of *P. falciparum* of which 2 cases showed also ring forms while 12 cases showed only ring stage. In comparison to *P. falciparum* samples, the parasitaemia observed in microscopy positive *P.vivax* was ranging from 0.02% to 1% only. As regards microscopy positive *P.vivax* cases, all cases showed ring stage of which two cases showed trophozoite stage also and one case showed gametocyte with the ring stage.

**Table 1:** Plasmodium species detected in clinically suspected malaria patients

| Plasmodium Species                              | Positive malaria cases by Microscopy | Positive malaria cases by OptiMAL test | Positive malaria cases by Bio Tina Pf/Pan Mal test |
|-------------------------------------------------|--------------------------------------|----------------------------------------|----------------------------------------------------|
| <i>P.falciparum</i>                             | 15                                   | 15                                     | 14                                                 |
| <i>P.vivax</i>                                  | 13                                   |                                        |                                                    |
| Mixed( <i>P.falciparum</i> and <i>P.vivax</i> ) | 5                                    | 5                                      | 5                                                  |
| Non <i>P.falciparum</i>                         | 13                                   | 12                                     | 12                                                 |

All positive cases of Malaria infection diagnosed by microscopy and RDTs were recorded and reported to the corresponding clinic for further treatment.

### Discussion:

WHO estimates that 3.2 billion people are at risk for malaria. Malaria is still endemic in 97 countries, and cases of malaria are more and more reported in non endemic areas. Just about 35% of suspected malaria cases in Africa were not recognized with a diagnostic test during 2014, consequential lead to over-use of anti malarial drugs and poor disease monitor (WHO, 2015). WHO recommends that malaria management in all patients should be based on parasite diagnosis (WHO, 2010). In the present study the performance of the OptiMAL test for the diagnosis of *P. falciparum* infection was superior to the Bio Tina Pf/Pan Mal test performance while the performance of both RDTs was the same concerning the detection of *P. vivax* infection in symptomatically diagnosed patients. The OptiMAL test showed 100% and 92% sensitivities for *P. falciparum* and non *falciparum* malaria respectively while the Bio Tina Pf/Pan Mal method sensitivities were 93% and 92 % for *P. falciparum* and non *falciparum* malaria respectively. Malaria diagnosis trials using different RDTs were carried on by researchers from different countries with more or less similar results to the results obtained in the present study. Palmer *et al.* (1998) had reported OptiMAL sensitivity of 94% for *P. vivax* and 88% for *P. falciparum*. Moody *et al.* (2000) carried on a study in sub-Saharan Africa, Asia and South America, reported OptiMAL sensitivity of 95.3% for *P. falciparum* and 96% for *P. vivax*. John *et al.* (1998) concluded that OptiMAL sensitivity was about 94% for *P. falciparum* and 98.2% for *P. vivax* in their study in Southern India. In Gambia, Hunt-Cook *et al.* (1999), concluded 91.3% OptiMAL sensitivity for *P. falciparum*. Another study that was performed at Combined Military Hospital Quetta by Ahmad *et al.* (2003), concluded 100% OptiMAL sensitivity for both malaria species. Another study was performed in Belgium( non-endemic country for malaria ), reported sensitivity of 92.9% for the SD Bioline Malaria Ag Pf/Pan (Maltha *et al.*, 2011) while in Korea, the researchers reported a sensitivity of RDT for *P. vivax* that ranged from 86.8% to 92.7% (Kim *et al.*, 2011 ; Jang *et al.*, 2013). The results of the present study are more or less similar in sensitivity to most of the above mentioned studies performed worldwide for malaria diagnosis. One case of microscopically diagnosed *P. vivax* infection was not detected by both RDTs, this case was only detected by the blood film (parasitemia 0.02%). The results obtained by both the Bio Tina Pf/Pan Mal and OptiMAL tests is significantly affected by the level of malaria parasite in peripheral blood which is concluded by other researchers in previous studies ( Iqbal *et al.*, 1999; Fryauff *et al.*, 2000; Ricci *et al.*, 2000; Singh *et al.*, 2000; Proux *et al.*, 2001). Furthermore, one case of *P. falciparum* infection diagnosed by microscopy and OptiMAL test in the present study was not detected by the Bio Tina Pf/Pan Mal test, the same finding had been observed in earlier studies which concluded that false-negative dipstick test results may be detected even in samples with higher parasitemias, but the underlying reason is not known (Anonymous, 1996; Karbwang *et al.*, 1996 ; Humar *et al.*, 1997 ; Palmer *et al.*, 1998 ;Van den *et al.*, 1998; Cooke *et al.*, 1999 ). The diagnostic efficacy of malaria is dependent on many factors as the different malaria species with different stages, signs and symptoms for each patient, the presence of viable or non-viable parasite, host immunity, deep sequestration of the parasite in tissues, and treatment of suspected cases without definite malaria diagnosis (Shiff *et al.*, 1993; Beadle *et al.*, 1994; Singh *et al.*, 1997; Tangpukdee *et al.*, 2009).

The preliminary records concluded that PfHRP-2 antigen, detected by the Bio Tina Pf/Pan Mal test, could be still detected in blood for up to 10 days after the parasites are cleared from the peripheral blood ( Shiff *et al.*, 1993; Eisen and Saul, 2000 ), while pLDH activity that is detected by the OptiMAL test, showed rapid significant decrease after the parasites clearance( Piper *et al.*, 1999), beside that the OptiMAL can detect an enzyme produced only by living parasites(Cheesbrough, 2009). The sensitivity of the RDTs was studied and reported by earlier studies to be influenced by the level of malaria parasite in peripheral blood( Iqbal *et al.*, 1999; Fryauff *et al.*, 2000; Ricci *et al.*, 2000; Singh *et al.*, 2000; Proux *et al.*, 2001), which could be dangerous, as to ignore malaria diagnosis in patient that could lead to serious complications as proper treatment not instituted in time. The assessment of a negative result in this condition will be judged mainly by the clinical features of the patient.

### Conclusion:

The results of the present study found that *Plasmodium* antigens detection by the non microscopic RDTs could be considered as one of the essential diagnostic malaria methods under certain situations in particular when work load is too high or in absence of expert parasitologists. These RDT require no equipment, seem simple and rapid to carry out (the results are available within 15-20 minutes), unfortunately these tests are much more expensive. However, the cost aspect is considered less significant owing to the reduction in mortality, and hospital admissions. The OptiMAL has another benefit as it is used to follow the effective therapy in view of the fact that it detects an enzyme produced only by alive parasites. In the present study, we can conclude that the OptiMAL test performance was better than that of the Bio Tina Pf/Pan Mal test for the detection of *Plasmodium* infection in clinically suspected cases. Microscopy is still considered the gold standard for malaria diagnosis, which is critical for therapeutic decisions. Examination of stained blood film is the most important technique by which we can observe different *Plasmodium* species with their growth stages and detect the level of malaria parasite in peripheral blood. Thick blood film is of great help in diagnosis of cases of low parasitemia, it is the

gold standard technique for malaria diagnosis. Even though malaria diagnosis by microscopy could be delayed as dealing with one blood sample could take minimum sixty minutes, it is inexpensive and precise if performed by a patient, expert parasitologist.

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