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Sensitivity and specificity of parasite lactate dehydrogenase (pLDH)-based rapid diagnostic test (SD Bioline malaria antigen test) in comparison with parasite density

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A cross-sectional hospital-based study (Thanbyuzayat Township Hospital, Mon State) on 103 patients, who were tested with malaria microscopy and parasite lactate dehydrogenase (pLDH)-based rapid diagnostic test strips (RDTs) during 2008. The blood film results indicated that 84 (81.55%) patients were malaria parasite positive and 19 (18.45%) were malaria negative. Forty-two cases diagnosed as *P. falciparum* by RDT were diagnosed by microscopy as 35 cases of *P. falciparum*, 2 cases of *P. malariae*, one case of *P. falciparum* and *P. malariae* mixed infection, and 4 cases of *P. falciparum* and *P. vivax* mixed infection. Forty-one patients diagnosed as non *P. falciparum* (*Plasmodium* species other than *P. falciparum*) by RDT were diagnosed by microscopy as 39 cases of *P. vivax* and 2 cases of mixed infection (*P. falciparum* and *P. vivax*). Twenty cases diagnosed as negative by RDT comprised of nineteen as negative and one as *P. vivax* by microscopy. The sensitivity was 100% and 97%, specificity was 90% and 96%, positive predictive value was 83% and 95% and negative predictive value was 100% and 98% for *P. falciparum* and non *P. falciparum,* respectively. The lowest parasite density detected by test was 109.5 parasites/µl for *P. falciparum* and 160 parasites/µl for *P. vivax*. In this study, the band intensity was significantly related to parasite density and the differences between mean parasite count was significant between faint and intermediate, intermediate and strong. Thus, it can generally estimate the parasite count and can be used in the field for screening of malaria parasite for preparation of quality control sample.

INTRODUCTION

Malaria is the most important parasitic disease of man and remains a major health problem in tropical countries. It is a disease caused by a protozoan of the genus *Plasmodium* namely: *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae* and *Plasmodium ovale*. At present 1326 million people or 83% of population in South East Asia (SEA) are at risk of malaria. The region harbours two main malaria parasites, *Plasmodium falciparum,* which is a well-known killer and *Plasmodium vivax* which causes relapse episodes of malaria [1]. About 95% of the population of moderate to high risk of malaria in SEA region is living in Myanmar, Thailand, India and Indonesia.

Multidrug-resistant *Plasmodium falciparum* is highly prevalent on the Thai-Cambodia and Thai-Myanmar border areas and Myanmar contributes to around 65% of reported malaria deaths [2]. In 2006, the leading causes of morbidity and mortality due to malaria were 7.1 per cent and 9 per cent, respectively [3]. The recommended strategy to control malaria is based on prompt and accurate diagnosis and the use of an artemisinin combination therapy (ACT) [4].

An ideal diagnostic test for malaria must be simple, inexpensive, be able to be performed rapidly, easy to interpret and discriminate between *P. falciparum* and the other malarial species. Microscopic detection of malaria parasites in thick and/ or thin blood smears remains the most appropriate diagnostic method in endemic countries [5]. However, microscopy needs well-qualified personnel and also time-consuming and facilities are not always available in endemic areas. An alternative to the classical thick/ thin blood smear and microscopic examination is the Rapid Diagnostic Test (RDT) which is based on the detection of antigens derived from malaria parasites using immunochromatographic methods. They are available as dipsticks, cards and cassettes and can be easily taken into the field and particularly useful where the electricity supply is non-existent. There are two major classes of RDTs that detect *P. falciparum* only and those that detect *P. falciparum* plus one or more other species of malaria. The RDTs can also detect parasites which are sequestered in the deep vascular compartment whereas peripheral blood smears cannot detect.

The WHO recommendation is that a RDT should have a sensitivity of ≥95% at a level of ≥100 parasites/µl and should have at least as accurate as results derived from microscopy [5]. RDTs have now been integrated into routine practice in several national malaria control programmes (e.g. Thailand, Cambodia and South Africa) [4]. The objective of National Malaria Control Progamme of Myanmar is to reduce malaria morbidity and mortality mainly through increasing accessibility to quality diagnosis and appropriate treatment [3]. In this connection, studying the relationship between parasite density and band intensity of available RDTs might assist to improve evidence-based clinical management of malaria.

Objectives

- To determine the sensitivity and specificity of pLDH-based RT (SDD Bioline Malaria Antigen Test).
- To determine the parasite density in patients attended the out-patient department (OPD)
- To compare the band intensity of RDT with parasite density.

MATERIALS AND METHODS

Thanbyuzayat Township, Mon State was selected for cross-sectional hospital-based study from January to October, 2008.

Sample size

A minimum sample required was 100 subjects by assuming that prevalence of clinically suspected malaria patients being 0.5 (conservative estimate) at 95% confidence interval and 10% marginal error [6].

Inclusion criteria

All patients with uncomplicated malaria, aged 6 years and above were included. Axillary temperature of ≥37.5°C or history of fever during the previous 48 hours. Informed consent provided by patients or parents.

Exclusion criteria

- Presence of one or more danger signs such as inability to drink or repeated vomiting, recent history of convulsions, lethargy or unconsciousness, inability to sit or stand up, or any sign of severe or complicated malaria
- Presence of pregnancy, lactation
- Presence of underlying diseases (e.g. cardiac, renal, hepatic diseases)

In the study township, 10% Giemsa-stained thick and thin blood films were examined independently by two microscopists (the researcher and an experienced microscopist who was not aware of test results). Parasite densities were measured by counting asexual parasites (rings, trophozoites and schizonts) against 300 WBCs in the thick film. Parasitaemia was calculated as follows [7]:

$$\text{Parasite density/µl} = \frac{\text{number of parasites counted x 6000}}{\text{number of leucocytes counted}}$$

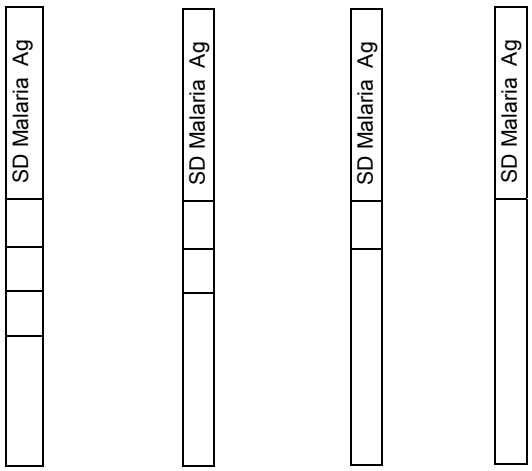
A blood film was recorded as negative after examining 200 thick blood smear fields. All slides showing >20% discrepancies were excluded from this study. All blood samples were also tested with the RDTs according to manufacturer's instructions.

Interpretation of the test:

The test uses two polyclonal antibodies which have been immobilized as two separate lines across a test strip. One antibody (test line 1) is specific to *P. falciparum*. The other antibody (test line 2) is specific to the four Plasmodium species (*falciparum, vivax, malariae, ovale)* (Fig.1). Besides, LDH of *P. falciparum* (control line) is immobilized for the procedural control.

The intensity of band was graded as follows:

0= no band (negative)
1+= faint band, but clearly visible (positive)
2+= medium intensity band, stronger than 1+ but less than control band (positive)
3+= equal or stronger intensity than the control band (positive) [8]



A = *P. falciparum* positive
B = *P. vivax* positive or *P. malariae* positive or *P. ovale* positive
C = Negative
D = Invalid

Fig. 1. SD Bioline Malaria Antigen dipstick (RDT)

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated by Confidence Interval Analysis Software Version 2.0. The correlations between the parasite densities and band intensity were calculated by SPSS version 11.5. Mixed infection cases were excluded by microscopy. A total of 103 blood samples were tested by pLDH test and their results were compared with the results of blood films examined.

RESULTS

During one-year study period, 103 clinically suspected malaria patients attending the outpatient clinics of Thanbyuzayat, Mon State were screened for malaria. Around 77% were male patients and 23% were female patients. The age range was 6-58 years. The high incidence was noted in age group between 20 to 29 years. In which, 80 patients (77.67%) were rubber plantation laborers.

Comparison between microscopy and RDTs

When compared with RDT results, 42 cases diagnosed as *P. falciparum* were diagnosed by microscopy as *P. falciparum* 35, *P. malariae* 2, *P. falciparum* and *P. malariae* 1, *P. falciparum* and *P. vivax* 4 cases. Forty-one patients diagnosed as non *P. falciparum* (*Plasmodium* species other than *P. falciparum*) by RDT were diagnosed by microscopy as *P. vivax* 39 and *P. falciparum* and *P. vivax* 2 cases. Twenty cases diagnosed as negative by RDT also have 19 as negative and 1 as *P. vivax* by microscopy. Two cases diagnosed as *P. falciparum* by RDTs were *P. malariae* infection by microscopy. One patient that was diagnosed as *P. vivax* by microscopy (i.e. parasite density 166 parasites/µl) was not detected by pLDH-based RDTs. The lowest intensity that was detected by RDT was 109.5 parasites/µl for *P. falciparum* and 160 parasites/µl for *P. vivax*.

Sensitivity and specificity of RDT

For *P. falciparum*, the sensitivity was 100% (95% CI = 90-100), the specificity was 90% (95% CI=80-95), the PPV was 83% (95% CI=69-92) and the NPV was 100% (95%

CI=94-100). The Kappa value was 0.86 and 95% CI was 75-96.

For non *P. falciparum*, the sensitivity was 97% (95% CI=87-100), the specificity was 96% (95% CI=89-99), the PPV was 95% (95% CI=84-99) and the NPV was 98% (95% CI=91-100). (The Kappa value=0.94 and 95% CI =87-100). All RDT test showed positive control line.

Band intensity and parasite density

For *P. falciparum*, the minimum parasite density that was scored as 1+ (faint) band intensity (in RDT test) was 416.6 parasites/µl and the maximum was 7685.8 parasites/µl, mean value was 4051.19. In 2+ (medium) band intensity, the minimum was 4116 parasites/µl and the maximum was 13182.2 parasites/µl, mean value was 8649.09. In 3+ (strong) band intensity, the minimum parasite density was 16513.3 parasites/µl and the maximum was 49304.1 parasites/µl, the mean value was 32908.7 (Figure 2).

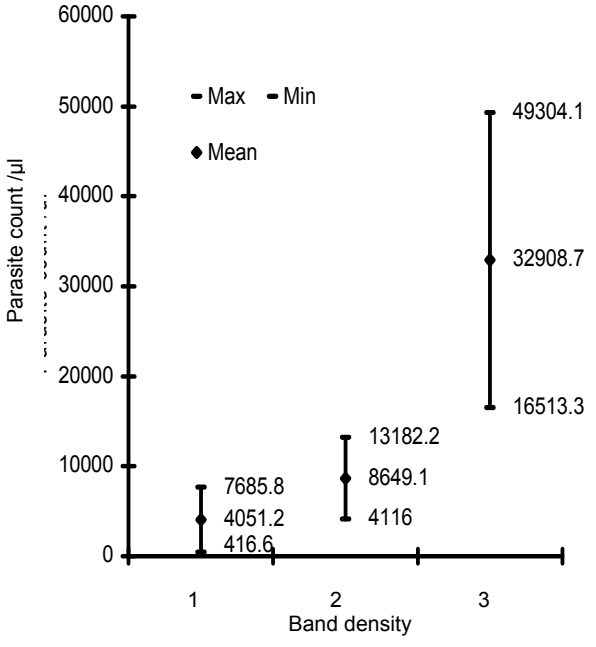


Fig. 2. Mean *P. falciparum* parasite count in different band intensity

For non *P. falciparum*, the minimum parasite density detected in 1+ (faint) band intensity of SD Bioline Malaria Antigen test was 160 parasites/µl and the maximum was 600 parasites/µl, the mean value was 378.90. In 2+ (medium) band intensity, the minimum was 2601.1 parasites/µl and the maximum was 7332.7 parasites/µl, mean value was 4966.86. In 3+ (strong) band intensity, the minimum was 6031.1 parasites/µl and the maximum was 17552.3 parasites/µl, mean value was 11791.75 (Fig. 3).

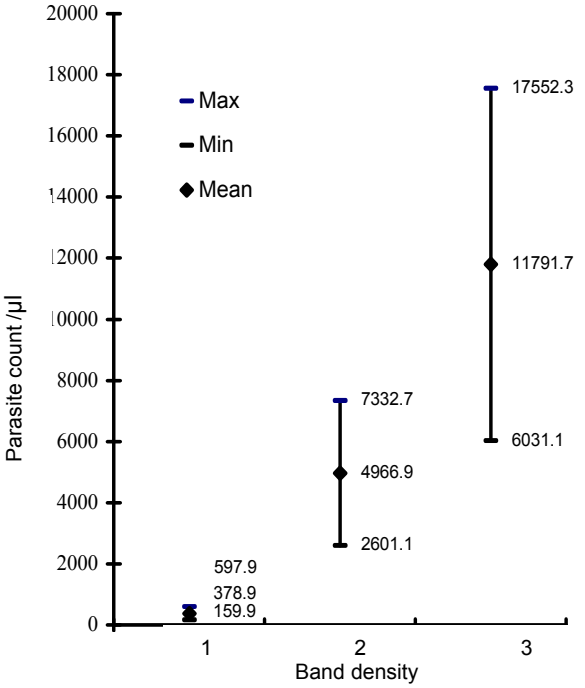


Fig. 3. Mean non *P. falciparum* parasite count in different band intensity

Twelve slides showing more than 20% discrepancies and seven slides with mixed infections were excluded. The lowest parasite density that was also detected by the RDT was 109.5 parasites/µl for *P. falciparum* and 160 parasites/µl for *P. vivax*.

The differences in mean parasitemia between faint and intermediate, and intermediate and strong were significant for *P. falciparum* (P=0.0005 and P=0.0005, respectively) than for non *P. falciparum* (P=0.011 and P=0.001, respectively).

Correlation of band intensity with parasite density

The correlation between band intensity and parasite density of non *P. falciparum* was significant (R = 0.61, P = 0.01) (Fig. 4) and that of *P. falciparum* was also significant (R = 0.66, P = 0.01) (Fig. 5).

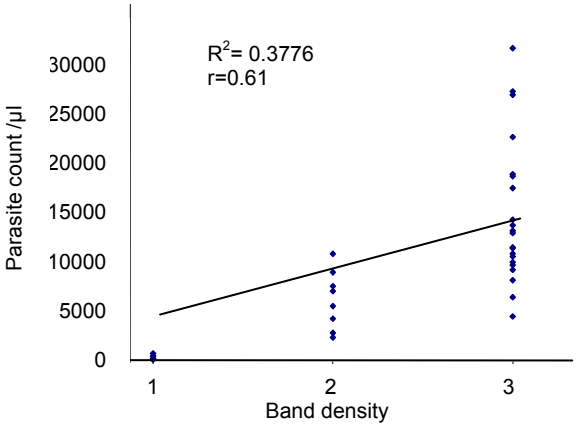


Fig. 4. Correlation between band intensity and parasite count in non-falciparum malaria infection

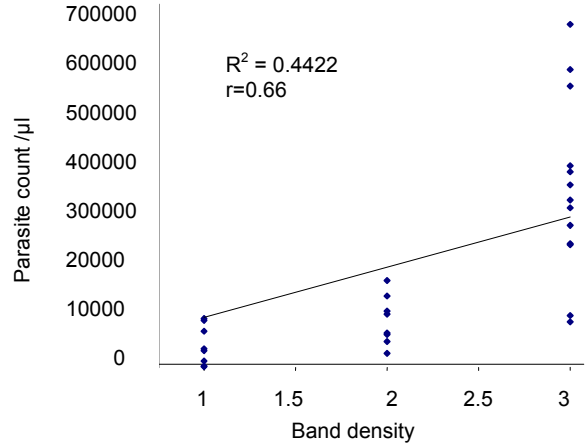


Fig. 5. Correlation between band intensity and parasite count in falciparum malaria infection

DISCUSSION

The resurgence of malaria has renewed interest in many countries and malaria caused by *Plasmodium falciparum* is one of the leading causes of morbidity and mortality in tropical countries. It is one of the priority public health problems in Myanmar. With the introduction of new more costly anti-malarial drugs in Artemisinin combination therapy, confirmation of malaria infection before initiating treatment is essential. Several methods have been developed to supplement and replace the conventional microscopic method. The most promising new malaria diagnostics are the immumochromatographic rapid diagnostic tests.

The current market price of a rapid diagnostic test in developing countries is about U.S$ 1.55-3.5 (depending on the number of targeted species and quantity), compared with microscopy at U.S$0.12-0.4 per malaria smear [9]. But in this study, the price of RDT test was 1,200 kyats per test which was not so expensive compared to other RDT and also easily available. Although the cost of microscopy was not expensive, it was needed to maintain the equipment (e.g. reagent, microscopy, electricity, etc) which was really expensive. The cost of artemisinin combination treatments (ACTs) for negative patients (over-diagnosis) could balance the extra cost of using rapid diagnostic tests. Prompt and accurate diagnosis will not only improve malaria treatment but also possibly reduce morbidity due to other febrile illnesses.

Microscopy is considered as the gold standard for malaria diagnosis and treatment but it has also disadvantages. It requires well-trained microscopists and regular maintenance of functional infrastructures. Moreover, the chance of false positive results increases with poor blood film preparation (i.e. artifacts commonly mistaken for malaria parasite) and normal blood components (e.g. platelet). The chance of false negative results increases with decreasing parasite densities. Errors can occur in differentiating between *P. vivax* and *P. ovale, P. falciparum* and *P. malariae*. However, in this study, *P. ovale* infection was not detected. Underreporting of mixed species infections is also common when the number of parasite density of one type of species involvement is very low.

In this study, the lowest parasite density that was also detected by RDT test was 109.5 parasites/µl for *P. falciparum* and 160 parasites/µl for *P. vivax* that the RDT test is as sensitive as thick film microscopy in the diagnosis of malaria. But the test assay cannot distinguish *P. falciparum* mono-infection from mixed infections (*P. falciparum* and other species) because one of the isozymes of pLDH specific antibody for

P. falciparum had same migrating distance as non *falciparum* bands [10]. Therefore, mixed infections were indistinguishable from *P. falciparum* in this assay. Therefore, false positive results were observed. In two patients, the RDT test had come into view as *P. falciparum* but blood film showed *P. malariae* only, the underlying reason was not known. The RDT test also gave a false negative result for only one patient (i.e. *P. vivax* diagnosed by blood film) who had an estimated parasite densities of 166 parasites/µl which was near to the lowest count of this test strip in this study. This suggests that parasite density can also affect the sensitivity of the RDT test. It is possible that low levels of parasitemia in those patients correspond to low pLDH blood concentration, resulting in failure to capture the antigen by the antibody present in the dipstick. However, the sensitivity of the test was very close to that of the microscopic examination of blood smears i.e. for *P. falciparum* the test showed 100% sensitivity and 90% specificity and for non *P. falciparum*, the sensitivity was 97% and the specificity was 96% and it did not require highly skilled personnel to perform or interpret results. The test has the added advantage that it can detect all four *Plasmodium* species and can be used to follow the efficacy of the drug therapy since it detects an enzyme produced only by live parasites [11].

Another objective of this study was to compare the band intensity of rapid diagnostic tests with parasite density. Twelve slides checked by two microscopists showing more than 20% discrepancies and 7 mixed infections were excluded from this study to get any correlation between band intensity and parasite density. The minimum and maximum parasite densities were 160 parasites/µl and 7685.8 parasites/µl (i.e. score as 1+), 2601.1 parasites/µl and 13182.2 parasites/µl (i.e. score as 2+), 6031.1 parasites/µl and 49304.1 parasites/µl (i.e. score as 3+), respectively. The differences in mean parasitemia between faint and intermediate, and intermediate and strong were significant for *P. falciparum* (P=0.0005 and P=0.0005, respectively) and also for *P. vivax* (P=0.011 and P=0.001, respectively). The correlation between band intensity and parasite density of *P. falciparum* was R^2=0.4422, r=0.66, P=0.012 and non *P. falciparum* was R^2=0.3776, r=0.61, P=0.012 (Bivariate, two tailed, Pearson correlation, by SPSS 11.5).

Therefore, this test can be used in the field for screening of malaria parasite and also for preparation of quality control sample to ensure adequate performance of the RDT. If this observation is validated in larger multi-center clinical studies, the pLDH dipstick may prove to be a reliable alternative for the diagnosis of *P. falciparum* or non *P. falciparum* malaria. In contrast to dipsticks based on the histidine rich proteins, the pLDH assay measures the enzyme levels of intact viable parasites and so can be used to monitor response to specific treatment [11]. It may be a valuable adjunct to clinical assessment of the patient and blood film microscopy. However, inability to distinguish mixed infection from *P. falciparum* with this dipstick remains a problem in clinical utility. Finally, its final resolution must await a non falciparum specific antibody that does not cross-react with *P. falciparum* and the RDTs should be easily feasible in public especially in remote areas where malaria is common.

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REFERENCES

1. WHO. The occasion of World Malaria Day. WHO Southeast Asia Region. Retrieved September 1, 2008 *http://www. searo. who.int/ LinkFiles/Malaria-WMDO8-RD-Message.pdf.*

2. WHO. Malaria: Disease burden in South-east Asia region. WHO Regional Office for South-east Asia, World Health Organization, Geneva, Switzerland. Retrieved September 18, 2007 from *http://www.searo.who.int/EN.html.*
3. Ministry of Health. Health in Myanmar 2008.
4. WHO. *Meeting Report on Informal Consultation on Fields and Guidelines on Malaria Rapid Diagnostic Tests, 20-23 January 2003*. World Health Organization, Geneva, Switzerland.
5. WHO. New Perspectives: Malaria Diagnosis. *Report of a joint WHO/USAID informal consultation, 25-27 October 1999,* World Health Organization, Geneva, Switzerland, 2000.
6. Lwanga SK & Lemeshow S. Sample size determination in health studies: a practical manual. *World Health Organization*, Geneva, Switzerland, 1991.
7. WHO. Template protocol for the assessment of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria. World Health Organization, Geneva, Switzerland, 2005.
8. WHO. Methods manual for laboratory quality control testing of malaria rapid diagnostic tests. World Health Organization, Geneva, Switzerland, 2008.
9. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A & Wernsdorfer WH. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). *American Journal of Tropical Medicine and Hygiene* 2007; 77 (6): 119-127.
10. Myint Oo. Isoenzyme variation in schizonts of *Plasmodium vivax* from Burma. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1986; 80: 1-4.
11. Moody A. Rapid diagnostic tests for malaria parasites. *Clinical Microbiology Reviews* 2002; 15 (1): 66-78.