ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Comparison of SD Bioline Malaria Ag-Pf/pan Test with Microscopic Examination for Detection of *P.Falciparum*, *P.Vivax* and Mixed Infection in South Nias, North Sumatera, Indonesia.

Harurikson Lumban Tobing[1]*, Sumaryati Syukur[2], Endang Purwati[3], Rahmiana Zein[4], Muzahar[1], Endang H Gani[5] and Edy Fachrial[6].

[1]Department of Clinical Pathology, Faculty of Medicine, Sumatera Utara University/RSUP H. Adam Malik Medan, Indonesia.
[2]Laboratory of Biochemistry/Biotechnology, Department of Chemistry, University of Andalas Padang, 25163, Indonesia.
[3]Laboratory of Nutrition, Faculty of Animal Husbandry, University of Andalas Padang, 25163, Indonesia.
[4]Laboratory of Environmental and Toxicology, Department of Chemistry, University of Andalas Padang, 25163, Indonesia.
[5]Department of Parasitology, Faculty of Medicine, Sumatera Utara University, Indonesia.
[6]Laboratory of Molecular Biology, Faculty of Medicine, Prima Indonesia University, Indonesia.

ABSTRACT

Malaria is a parasitic disease with high morbidity and mortality rates in Indonesia and other tropical and sub tropical countries even through these days. Rapid diagnosis and prompt treatment are essential to control the transmission of the disease. Malaria diagnosis by detecting the parasites from thick blood film in endemic areas remains to be a problem by the lack of available means and is highly dependent on the examiner's skill. Alternatively, the SD Bioline Malaria Ag-Pf/pan test is able to detect *Plasmodium* specifically, with rapid result and no special skills required. To determine the SD Bioline Malaria Ag-Pf/pan test quality of results in the diagnosis of *P.falciparum* , *P.vivax* , and mixed malaria infection in an endemic area. 98 blood samples were drawn from suspected malaria patients from the local public health service (Puskesmas) of Pulau Tello, Nias Selatan regency, Sumatera Utara province. The blood samples were analyzed by the SD Bioline Malaria Ag-Pf/pan test and microscopic examination. The study was conducted at Puskesmas Telo of Pulau-Pulau Batu district South Nias in collaboration with Clinical Pathology Department of Medical Faculty, Sumatera Utara University/H. Adam Malik Hospital Medan. The Mc.Nemar' test was used to determine the sensitivity and specificity of both methods.

Keywords: Malaria, SD Bioline Malaria Ag-Pf/pan test, Microscope

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Malaria is transmitted in about 106 counties in tropical even subtropical regions, and about 3,3 billion people or a half of the world's population, are at risk of malaria transmission. The World Health Organization (WHO) estimates that over 219 million cases and about 660,000 deaths caused by malaria (uncertainty range of 490,000 to 836,000) occur worldwide every year and representing an enormous global social and economic burden[1].

The archipelago nation of Indonesia is commonly divided into 2 region based on malaria endemicity, there are Java and Bali, which inhabited by approximately 62% of the total Indonesian population, is classified as hypo-endemic area, whereas the population outer islands, including Sumatera, Kalimantan, Sulawesi, Nusa Tenggara, Maluku and Papua have malaria at much higher levels, ranging from hypo to hyper endemic. All four species of human malaria are found in Indonesia. Formerly, *Plasmodium malariae* and *Plasmodium ovale* were mostly found in eastern part of Indonesia such as East Nusa Tenggara and Papua, but recently *P.malariae* has been detected in western parts of the archipelago [2].

Recent studies have discovered a new species of *Plasmodium* than could infect the humans. This 5th Plasmodium species identified as *Plasmodium knowlesi. Plasmodium falciparum* has a shortest cycle life (asexual) in liver while also attacked all the type of red blood cells thus can lead to severe complications, since it is necessary in rapid and accurate diagnostic that is treatments could be immediately given [1,2].

SD Bioline Malaria Ag-Pf/Pan consists of strip membrane, re-coated with monoclonal antibodies 1 and 1 polyclonal antibodies in the form of two separate lines on the surface of he test kit. The first monoclonal antibody (test line pf) specific to *P. falciparum* HRP2, and the 2nd polyclonal antibodies (test line P.Pan) specific to the species of plasmodium lactate dehydrogenase (*falciparum, vivax, ovale, malariae*). Polyclonal antibody pan specific for lactate dehydrogenase plasmodium species has no cross-reactivity with human lactate dehydrogenase, because this antibody (for lactate dehydrogenase *Plasmodium falciparum and P. vivax*) adsorbed to lactate dehydrogenase in humans. Thus, SD Bioline Malaria Ag-Pf/Pan is designed to diagnose *Plasmodium falciparum* and other Plasmodium species independently[18].

Based on the Data General Description of Malaria Health Bureau of North Sumatera Province (2010), total population was 13,518,337 inhabitants, number of district/cities were 33 with 533 health services, number of malaria-endemic areas were found in 17 districts/cities with a population of 8,198,463 inhabitants with 327 health service where as malaria-endemic village were 185 villages with a population of 5,202,414 inhabitants [5,6].

According to Health Bureau of Sumatera Utara province, type of malaria in Madina regency endemic with *Plasmodium falciparum*, North Labuhan Batu regency endemic with malaria vivax, while South Nias regency found that both type of *Plasmodium falciparum* and *Plasmodium vivax*.

South Nias regency was a division of Nias regency located in the southern part of Nias island and it is a part of North Sumatera province. Malaria is a major disease in southern Nias regency. The amount of Annual Malaria Incidence (AMI) throughout South Nias regency was fluctuating. In 2005, there was an increase of AMI which exceeded the previous year of 105% and 7,36% of cases malaria clinical examined from blood, obtained Slide Positivity Rate (SPR) number of 11,46% so it can be categorized as High Incidence Area (HIA).

South Nias regency was one of region in Indonesia which have malaria cases in high rate. More over since the earthquake and tsunami happened in March 2005. The disaster caused the outbreak and the emergence of endemic areas. This happens due to the amount of basin ponds formed, and ditches were mixed with the incoming of sea water when the earthquake and tsunami, so that became breeding places of malaria mosquitoes.

Based on data from ten major disease in South Nias regency in 2010 were obtained from Health Bureau of South Nias that the disease that most often found was clinical malaria as many as 42.626 cases.

Bendezu *et al* [3] conducted a research in an endemic area of Peruvian Amazon, Peru against 332 people suspected malaria at age 16-32 years old by Rapid Test Parascreen ^{TM} and then compared with the

microscope. Sensitivity values obtained for *P.falciparum* was 53.5%, specificity was 98,7%, and for non *P.falciparum* sensitivity values obtained was 77,1%. Specificity was 97,6%, while the Parascreen™ test compared to PCR for *P. falciparum*, sensitivity 81,8%, specificity 99,1%, for non *P. falciparum* sensitivity 77,1%, specificity 99,2% .

Afiah *et al* [4], conducted a research in endemic areas of Central Halmahera to 240 persons with suspected malaria with Paracheck Pf test with microscope. The values obtained was sensitivity 88% and specificity 66,6%.

Djalle *et al* [6] conducted a study to 436 people with suspected malaria using 3 methods, Paracheck™-Pf, SD Bioline Malaria Ag-Pf and SD Bioline Malaria Ag-Pf/pan for diagnosis of falciparum malaria. The sensitivity of Paracheck™ was 85,7% (96% Cl, 80,8-89,9%), that of SD Bioline Ag-Pf was 85,4% (95%, 80,5-90,7%), and that of SD Bioline Ag-Pf/pan was 88,2% (95%Cl, 83,2-92,0%). Overall SD Bioline malaria Ag-Pf and SD Bioline malaria Ag-Pf/pan performed slightly better than Paracheck™-Pf [6].

Ima *et al* [5], conducted a study of 604 people with suspected malaria by rapid test immunochromatography compared with microscopic examination and values obtained was sensitivity 100%, specificity 96,7%.

Rapid and accurate diagnosis in detecting Plasmodium parasites in blood of patients is necessary to be done so that they can be treated immediately [7,8,9]. This is a challenge for laboratories throughout the country in order the diagnosis of malaria could be established as soon as possible [10]. As the gold standard, Giemsa staining on blood smears and examination under microscope frequently used because of its relatively low cost, but this examination has several limitations such as requiring skilled laboratory personnel and time consuming result, and it is not uncommon gives false positive and negative result [11,12,13]. WHO also recognizes the need for non-microscopic diagnostic tool to overcome this disadvantages [14]. Several methods for the diagnosis of *Plasmodium falciparum* has developed in detecting the disease process. The immunochromatographic test kit capable of detecting antigen of *Plasmodium falciparum*, and *P.vivax* [15]. The new rapid malaria diagnostic tests are based on immunochromatographic techniques using conjugated monoclonal antibodies as infection indicators. The detected antigens are preferably those present in all forms of the parasite (either sexual or asexual) such as histidine rich protein II (HRPII), lactate dehydrogenase (LDH) and aldolase [16].

The new technology is necessary to pay attention to the ability of sensitivity and specificity of this tool, it is recommended to use a Rapid Test with a minimum capability of sensitivity 95% and specificity 95%. The World Health Organization has recommended the use of malaria Rapid Diagnostic Tests (RDTs) for prompt and accurate parasitological confirmation of *Plasmodium falciparum* malaria in setting where microscopy services are not available. However, the ability to detect individuals with asymptomatic low density parasitaemia, i.e.,below detection limit o both RDTs and microscopy (~100 parasites/µl blood), in low endemic settings has been increasingly acknowledged as a challenge to achieve malaria elimination [17]. Another important thing is the RDT storage should be in a refrigerator but not in the freezer]. Because that constraints, it is considered the need for the practical/easy and rapid examination to do but it does not reduce the accuracy.

Because of limitations of the microscopic diagnostic device of malaria in malaria endemic areas in Indonesia, it is required the simple RDT diagnostic tool, but have a good quality, so it should be able to diagnose the infection of *P. falciparum*, *P.vivax* and mixed infection. So this study was undertaken by diagnostic test with malaria RDT SD on patients with suspected malaria in *P. falciparum*, *P.vivax* and mixed infection endemic areas.

Based on the background described above, then the problem can be formulated as follows : Does the SD Bioline Malaria Ag-Pf/pan test support tool have a good quality?

The purpose in general is to know the quality of SD Bioline Malaria Ag-Pf/pan in diagnosing a *P. falciparum* infection, *P.vivax* and mixed infection in the endemic malaria and specifically to know the sensitivity and specificity of SD Bioline Malaria Ag-Pf/pan examination in upholding the diagnosis of *P. falciparum* infection,*P. vivax* and mixed infection in endemic areas.

ISSN: 0975-8585

The benefits of this research is to know the quality of SD Bioline Malaria Ag-Pf/pan examination in the diagnose of *Plasmodium falciparum* infection, vivax and mixed infection, so the SD Bioline Malaria Ag-Pf/pan could be widely used as a diagnostic support tool for diagnose the *P. falciparum* infection, *P.vivax* and mixed infection in malaria endemic areas in Indonesia, which does not have microscopic diagnostic devices.

MATERIALS AND METHODS

Study Population

Study area: Nias Selatan located in western part of Sumatera, approximately 92 miles from Sibolga city or central Tapanuli regency. Pulau Tello in one of the island that located in Pulau Pulau Batu district, South Nias regency.The research was conducted with a cross-sectional approach. The study was conducted at the Department of Clinical Pathology, Faculty of Medicine Sumatera Utara University. Adam Malik Hospital Medan, and Pulau Tello Health Service Pulau-Pulau Batu district South Nias regency, from July to September 2014. The population study was malaria patients who visited the health service of Pulau Tello , Pulau-Pulau Batu district South Nias regency. The samples were taken from patients with a clinical diagnosis of malaria, and was taken from all age groups. All participants who took part in this study provided informed-consent and had been briefed about the study procedures and the possibility of an uncomfortable effect that may occur although it was slight.

Sample size : The sample size was obtained using this formula :

$$n = \frac{(Z\alpha \sqrt{2P(1-P)} + Z\beta\sqrt{P1(1-P1) + P2(1-P2)})^2}{(P1 - P2)^2}$$

$$P = (P1 - P2)/2$$

α = significance level
P = 0,5 $Z\alpha$ = 1,96 $Z\beta$ = 0,842 P1 = 0,90 P2= 0,80
= 98

Study criteria

The inclusion factors were patients with fever > 37,5^{o}C, pale, diarrhea, and headache. The exclusion factor were taking anti malarial drugs a week before and if the patients did not want to be examined or not willing to join in this research.

Ethical Clearance and Informed Consent

Ethical clearance was obtained from the Research Committee, Medicine Faculty, Sumatera Utara University with number 213/KOMET/FKUSU/2012. The investigation was approved and informed consent was requested in handwriting from the subject of research that states willing to participate in the study after receiving an explanation of the purpose and objectives of this study.

SD Bioline malaria Ag-Pf/pan method

Specimen storage: The blood was taken and put it in venipuncture. If blood was not directly used, save in 2-8^{o}C. The blood that has been stored should be conditioned at room temperature before use. The use of samples that have been stored for more than 3 days could cause non-specific reactions.

Examination procedures: Attain whole kit components and specimens to room temperature before use, then remove the test cassette from foil, place it on a flat surface and dry. Clean the tip of finger and take out the blood using the lancet puncture. With disposable loop that has been provided, dip the rounded tip of the loop (capillary tube) into the blood specimen (5µL) and carefully place the rounded tip into the sample well. Then add 4 drops assay diluents into assay diluents well. Read the result within 15 to 30 minutes. Do not read results after 30 minutes because it may give false results.

ISSN: 0975-8585

Anamneses and Physical Examination

Anamneses carried by interviews guided by a list of questions on the status and existing information on the status. Physical examination performed on patients with lying position and check the splenomegaly and anemia. All data and test results are recorded in status of research.

Microscopic Examination

Thick blood and thin blood smear

To make thick blood smear, 3 drops of the blood samples is placed on a clean glass slide, the tip of the second glass objects place on the blood drops, and homogenize by rotating the tip of glass object clockwise, thus formed a circle with a diameter of 1 cm. Allow to dry, then hemolysed, and treated with 10% Giemsa solution and allow 20 minutes. Wash with water and then dried. The result of staining could be seen under the light microscope with 100 x magnification.

For thin blood smear, 1 drop of blood sample is placed on a clean glass slide and then removed with another glass subjects by using the eraser side of object slide. The eraser slide immediately pushed forward slowly without stopping in order to get clear preparations, the allow to do dry, and fixation using methanol, stained with 10% Giemsa and the result could be seen under a light microscope with 100x magnification.

Result interpretation of SD Bioline Malaria Ag-Pf/pan

- Negative : 1 line "C" appear on result window
- P.f Positive : 2 lines (P.f" line and "C" line) appear on result window
- Positive Pan (P.v, P.m, P.o): 2 lines ("Pan" line and "C" line) appear on result window
- Mixed infection P.f and P.v : 3 lines ("P.f", "Pan" and "C" lines) appears on result window
- Invalid : There is no "C"line appear on result window

Statistical Data Analysis

The SD Bioline Malaria Ag-Pf/pan and microscopy examination data that had been collected were tabulated and included into a 2 x 2 table where if malaria parasites was found, included as positive category, and if was not found included as negative category. The true positive result included in a cell, the fake positive result in cell b, fake negative in cell c and true negative in cell d. Then carried out the calculations to find the sensitivity, specificity, PPV, NPV, prevalence and likelihood ratio (**Table 1**).

Table 1: Calculation of sensitivity, specificity, PPV, NPV, prevalence and likelihood ratio

SD Bioline Malaria Ag-Pf/pan test	Result	Microscopy	
		positive	negative
	Positive	a	B
	Negative	c	D

Calculation formula :

- Sensitivity = a : (a+c)
- Specificity = d : (b+d)
- Positive predictive value = a : (a+b)
- Negative predictive value = d : (c+d)
- Prevalence = (a+c) : (a+b+c+d)
- Positive likelihood ratio = sensitivity : (1-spesificity)= a/ (a+c):b/(b+d)
- Negative likelihood ratio = (1-sensitivity): specificity=c/(a+c):d/(b+d)

ISSN: 0975-8585

RESULTS

Sample characteristic

The number of samples in this study were 98 people. The number of women were 50 patients (51%), and the number of men were 48 people (49%). Most symptoms perceived are fever in 73 people (74,5%) and headache in 71 patients (72,4%). Splenomegaly was found in 30 patients (30,6%). The data shown in **table 2**.

Table 2: Sample characteristics

Characteristic	Number (n)	Percentage (%)
Male	48	49,0
Female	50	41,0
Age		
• 0-4	15	15,3
• 5-14	36	36,7
• 15-59	41	41,8
• >60	6	6,1
Symptoms		
• Fever	73	74,5
• Pale	37	37,8
• Stomachache	27	27,6
• Diarrhea	1	1,0
• Nausea	5	5,1
• Loss of appetite	6	6,1
Splenomegaly	30	30,6

Comparison between SD Bioline Malaria Ag-Pf/pan test and P.f microscopy

In this study, after statistical test, the values obtained were sensitivity 97%, specificity 100%, positive predictive value 100%, negative predictive value 99% and prevalence 33%. The data shown in **table 3**.

Table 3: Comparison SD Bioline Malaria Ag-Pf/pan test with P.f Microscopy

	Result	P.f microscopy		Number
		Positive	Negative	
SD Bioline Malaria Ag-Pf/pan test	Positive	31	0	31
	negative	1	66	67
Amount		32	66	98

Comparison between SD Bioline Malaria Ag-Pf/pan test and P.v microscopy

In this study, after statistical test, the values obtained were sensitivity 100%, specificity 97%, positive predictive value 91%, negative predictive value 100% and prevalence 21%. The data shown in **table 4.**

Table 4: Comparison SD Bioline Malaria Ag-Pf/pan test with P.v Microscopy

	Result	P.v microscopy		Number
		Positive	Negative	
SD Bioline Malaria Ag-Pf/pan test	Positive	21	2	23
	Negative	0	75	75
Amount		21	77	98

Comparison between SD Bioline Malaria Ag-Pf/pan test and Mixed Infection Microscopy

In this study, after statistical test, the values obtained were sensitivity 97%, specificity 100%, positive predictive value 100%, negative predictive value 98% and prevalence 37%. The data shown in **table 5.**

Table 5: Comparison SD Bioline Malaria Ag-Pf/pan test with mixed infection Microscopy

	Result	Mixed infection		Number
		Positive	Negative	
SD Bioline Malaria Ag-Pf/pan test	Positive	35	0	35
	negative	1	62	63
Amount		36	62	98

DISCUSSION

In this study shows that the number of malaria patients more women than men which 51% and 49%. The age distribution was relatively uneven, the highest was the age of group 15-59 years old was 41,8% and 5-14years old was 36,7%.

The highest malaria cases occurred in the age of 5-14 years old. The difference in malaria morbidity in men or women or various age groups due to several factors such as employment, education level, residential, migration and immunity [8].

The diagnosis of malaria is set based on anamnesis, clinical symptoms and laboratory result. The gold standard are the appearance of malaria parasite on microscopic examination (thick and thin blood smear). This examination has many disadvantages, which requires the availability of light microscope and skilled examiner. Based on the evaluation of Stabilization Program External Quality Health Laboratory, from 19 laboratories in West Nusa Tenggara that assessed using positive preparations malaria, only 79% of laboratory technician who can read properly preparations.

In this study has been examined with Standard Diagnostic Malaria RDT and microscopy of the 98 samples that meet the criteria of clinical malaria. On microscopic examination of 21 samples obtained *Plasmodium vivax*, *Plasmodium falciparum* 32 samples, while the samples without Plasmodium infection were 28 samples and mix non-falciparum infection were 17 samples.

In this study, SD Bioline Malaria Ag-Pf/pan test compared to microscopic examination, an the sensitivity obtained was 100%, specificity 97%, positive predictive value 91% and negative predictive value 100%.

SD Bioline Malaria Ag-Pf/pan test is one of malaria rapid diagnostic test that has the ability to know the *Plasmodium falciparum*, *Plasmodium vivax* and mixed infections in blood circulation. Different from the immunochromatography test that is just only able to know *Plasmodium falciparum* and *Plasmodium vivax*. Parasight F (Becton Dickinson Advanced Diagnostic, Franklin lakes, NJ) and the IC *Plasmodium falciparum* (Amrad-ICT, Sydney Australia) is an example of the immunochromatography test that in only able to know *Plasmodium falciparum*. OneMed Optimal (Flow Inc, Portland, Oregon), ICT *Plasmodium falciparum*/ *Plasmodium vivax* (ICT pf/pv), Amrad-ICT could only detect *Plasmodium falciparum* and pan malaria.

Based on this study, the results showed that the SD Bioline Malaria Ag-Pf/pan test from Clinical Pathology Laboratory, Medicine Faculty, Sumatera Utara University/ Adam Malik Hospital Medan, North Sumatera, had a sensitivity of 100%, specificity 97%, positive predictive value 91%, negative predictive value 100%. A similar study conducted by Ima *et al* [5], the immunochromatography test from hepatitis laboratory NTB had a sensitivity of 100%, a specificity of 96,99%, positive predictive value 83,2%, negative predictive value 100%.

The study of Tjitra *et al* [11], using ICT Pf and Pv obtained a sensitivity of 95%, specificity 89,6%, positive predictive value 96,2% and negative predictive value of 88,1%.

After doing the microscopic test and SD Bioline Malaria Ag-Pf/pan test against 98 samples, the negative result obtained on microscopic examination and SD Bioline Malaria Ag-Pf/pan test. The decreasing of sensitivity of SD Bioline Malaria Ag-Pf/pan test affected by the type of parasites and the level of parasitaemia.

CONCLUSION

Finally it may be concluded that the SD Bioline test has a high sensitivity and specificity so that it can be used as an alternative diagnostic for *Plasmodium falciparum*, *Plasmodium vivax* and mixed infection.

ACKNOWLEDGEMENT

I thanks to my Malaria group research in Medan, and also Prof.Edison Munaf, M.Eng as a Dean of Mathematic and Natural Science Andalas University, for encouragement of this manuscript. My best gratitude also addressed to the Dean of Medicine Faculty Sumatera Utara University, Prof Gontar A.Siregar, Head of Department of Clinical Pathology Medicine Faculty Sumatera Utara University, Prof.Adi Koesoma Aman, Deputy Head of Department of Clinical Pathology Medicine Faculty Sumatera Utara University, Prof.Herman Hariman, Head of Clinical Pathology Study Program Medicine Faculty Sumatera Utara University, Prof.Ratna Akbari Ganie, Secretary of Clinical Pathology Study Program Medicine Faculty Sumatera Utara University, dr Ricke Loesnihari, MKed, SpPK(K), Director of Tropical and Infectious Disease Clinic, Dr.dr.Umar Zein,SpPD DTM&H KPTI for supporting this research.

REFERENCES

[1] Herrera S, Vallejo AF, Quintero JP, Herrera MA, Cancino M, Ferro S. Malaria J 2014; 13 : 2-10
[2] Syaruddin D, Hidayati APN, Asih PBS, Hawley WA, Sukowati S, Lobo NF. Malaria J 2010; 9 : 2-8
[3] Bendezu J, Rosas A, Grande T, Rodriguez H, Cuentas AL, Escobedo J, Gamboa D. Malaria J 2010; 9 :1-6.
[4] Afiah N, Windarwati, Hardjoeno. The Indonesian J Med Sci 2009; 5(1) : 275-280.
[5] Ima AL, Purwanto AP, Arfi S, Tetrawindu H, Octora M, Mulyanto, Surayah K, Amanukarti. Indonesian J Clin Pathol Med Laborat 2006; 12(3) : 118-112.
[6] Djalle D, Gody JC, Moyen JM, Tekpa G, Ipero J, Madji N, Breurec S, Manirakiza A 2014. BMC Inf Dis 14 :2-7
[7] Shujatullah F, Malik A, Khan HM. J Vector Borne 2006; 43(4) : 86-90
[8] Khan SA, Anwar M, Hussain S, Qureshi H, Ahmad, Afzal S. J Pakistan Med Assoc 2004; 1-4.
[9] Jelinek T, Grobusch MP, Schwenke S, Steidi S, Sonneburg FV, Nothdurft HD, Loscher T. J Clin Microbiol 1999; 37:721-723
[10] Moody A. Clin Microbiol Rev 2002; 15 : 66-78.
[11] Tjitra E, Suprianto S, Dyer M, Currie BJ, Anstey NM. J Clin Microbiol 1999; 37: 2412-2417.
[12] Palmer CJ, Lindo JF, Klaskala, Kaminssky R, Quesada JA, Baum MK, Ager AL. J Clin Microbiol 1998; 36 : 203-206.
[13] Richter J, Harms G, Muller-Stover I, Gobels K, Haussinger D. Parasitol Res 2004; 92(6) : 518-519.
[14] Richardson DC, Ciach M, Zhing KJY, Crandall I, Kain KC. J Clin Microbiol 2002. 40(12) : 4528-4530
[15] Harani MS, Beg MA, Khaleeq L, Adil SN, Kakepoto GN, Khursid M. J Pak Med Assoc 2006; 56(4) : 167-171
[16] Portero JL, Yuste MR, Descalzo, Raso J, Lwanga M, Obono J, Nseng G, Benito A, Cano J. Malaria Res Treat 2010; 10 : 1-6.
[17] Morris K, Schmidt BA, Shakely D, Martensson A, Jornhagel L, Ali AS, Msellem MI, Petzold M, Ferreira PE, Bjorkman A. Malaria J 2013; 12:106.