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A COMPARISON OF RAPID DIAGNOSTIC CARD TEST WITH GOLD STANDARD MICROSCOPY FOR THE DETECTION OF PLASMODIUM SPECIES IN SOUTH-EAST RAJASTHAN

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ABSTRACT

Background: Malaria is a serious, sometimes fatal, parasitic disease characterized by fever, chills, and anemia and is caused by a parasite that is transmitted from one human to another by the bite of infected Anopheles mosquitoes. At present, malaria is diagnosed microscopically by looking for the parasites in a drop of blood. The Global Malaria Program recommends that suspected clinical malaria could be confirmed using the quality assured Rapid Diagnostic Test (RDT) and Microscopy diagnostic tools. For proper diagnosis of infection as well as disease management and prevention, identification of appropriate test kit is necessary. **Objectives:** The aim of this study was to compare Rapid Card Test with Gold standard Microscopy for the detection of Plasmodium species. The ultimate goal of this study was to recommend most reliable and cost-effective rapid card test for the diagnosis of Plasmodium species in areas where advance diagnostic facilities are not available. **Methodology:** The present study was conducted in the clinical laboratory of NMCH at Govt, Medical College, Kota from period of 1st January, 2018 to 31th December 2018 to comparative evaluate the detection of Plasmodium species by rapid diagnostic tests and Microscopy method. **Result:** In our study, total no. of 20466 samples was tested for suspected Malaria (Plasmodium species) by Microscopy method (gold standard) as a confirmatory methodand rapid test kits. On testing for 20466 samples, 48 samples were reactive by Microscopy but only 46 were reactive in Rapid test. Results by microscopy show that up to 0.3% (34/11460) of the males and 0.15% (14/9006) of the females were infected. With microscopy as the standard, the sensitivity, specificity, positive predictive value and negative predictive value of RDT were; 95.83%, 100%, 100% and 99.99% respectively. Out of 20466 samples, 37 samples and 11 samples were positive for P. vivax and P. falciparum species respectively. **Conclusion:** For proper diagnosis of infection as well as disease management and prevention, identification of appropriate test kit is necessary. The RDT's sensitivity and specificity is comparable with microscopy. These rapid kits are cheaper and easy to perform and their use should be encouraged at rural settings. Microscopy is much more sensitive than rapid tests for screening of infections like Malaria.

KEYWORDS: MALARIA, PLASMODIUM SPECIES, RAPID DIAGNOSTIC TEST, MICROSCOPY.

INTRODUCTION

MALARIA re-emerging as world No.1 infectious Killer. Malaria is the most common infectious tropical disease and an enormous public health problem.[1] There are four species of malaria parasite that can infect human: Plasmodium falciparum, P. vivax, P. ovale, and P. malariae. It is caused by the parasite called plasmodium and is passed to human by mosquito (female anopheles). It is a disease that can be treated in just 2 days, yet it can cause fatal complication if the diagnosis is delayed.[2] The disease now occurs in more than 90 countries worldwide, and it is estimated that there are over 500 million clinical cases and 2.7 million malaria-caused deaths per year.[2] As per WHO estimation, In 2012, there were an approximately 207million cases and an estimated 627000 deaths.[2] Approximately 90% of all malaria deaths occur in sub-Saharan Africa, and 77% occur in children under 5 years.[3] Malaria remains endemic in 104countries, and, while parasite-based diagnosis is increasing, most suspected cases of malaria are still not properly confirmed, resulting in over-use of antimalarial drugs and poor disease monitoring.[4] The use of antigen detecting rapid diagnostic tests (RDTs) is a vital part of malaria case management forming the basis for extending access to malaria diagnosis.[5] Conventional microscopy is regarded as the most commonly used screening technique for general population in Tertiary Care Hospitals of India, but due to

limitations of microscopy like costs, unavailability in many laboratoryand testing sites, involvement of costly instruments, time taking nature and requirement of highly skilled personnel for interpretation, rapid tests that are user friendly are gaining more importance and warrants comparison of performance.[8] Rapid diagnostic kits are a good choice as they are less expensive and do not need high technical manpower or infrastructure were intended for field survey diagnosis, emergency and home testing. The rapid card test is known to have less sensitivity and specificity than microscopy but some have sensitivity and specificity comparable to microscopy.[8] The ultimate goal of this study was to recommend most reliable and cost-effective rapid card test for the diagnosis of Plasmodium species in areas where advance diagnostic facilities are not available. The present study was conducted to compare the efficacy of Microscopy test kits and Rapid test kits for screening in rural area and general population of India.

AIMS AND OBJECTIVE

The present study was conducted to compare the efficacy of microscopy and Rapid test kits for screening Plasmodium species infection. This study was also designed to assess the performance of Rapid test, with respect to age and parasite density. The present study was designed to check the sensitivity and specificity of rapid card test of which are frequently used in different laboratories and hospitals and to compare with already confirmed cases on microscopy. The ultimate goal of this study was to recommend most reliable and cost-effective RDT for the diagnosis of Plasmodium species in areas where advance diagnostic facilities are not available. This study was conducted to aid in early detection and treatment of Malaria infections and its prevention in community.

MATERIAL AND METHODS

The present study was conducted in the clinical laboratory of NMCH at Govt, Medical College, Kota from period of 1st January, 2018 to 31th December 2018 to comparative evaluate the detection of Plasmodium species by rapid diagnostic test and microscopy method. A total of 20466 blood samples were collected from the outdoor and indoor patients of Govt. Medical College, Kota and its allied hospitals. Total no. of 20466 samples were tested for Plasmodium species by rapid test kits and Microscopy method (gold standard) as a confirmatory method. Samples found reactive by RDT, were again tested by Microscopy and vice versa. The results of the reactive sample by microscopy were compared with the rapid tests. Approximately 2-3 mL of venous blood samples were collected into EDTA anticoagulated test tubes, from a total of 20466 patients who were sent to the laboratory for a malaria test. Blood films (thick and thin) were prepared within a period of 30 minutes. Patients' serum samples were subjected to following tests for detection of Plasmodium species.

(1) Rapid test **(also called RDT or Rapid Diagnostic test, ICT or Immunochromatographic Test)**[6,12]
(2) Microscopy test method:-**Giemsa staining technique**[6,10]

1. RDT method: Advantage Malaria Pan+Pf Card is a visual, rapid qualitative and sensitive solid phase immune chromatographic assay based on antigen detection and is as an aid in differential diagnosis of infection with HRP-2 (Histidine Rich Protein-2) specific P. falciparum and pLDH (Plasmodium Lactate Dehydrogenase) specific Plasmodium Species (P. vivax / P. malariae / P. ovale) in human whole blood specimens. This Kit is manufactures by J. Mitra & Co. Pvt. Ltd. New Delhi, India.[5]

Principle:-Advantage Malaria Pan+Pf Card are an immunoassay based on the "sandwich" principle. The conjugate contains colloidal gold conjugated to P.f specific monoclonal anti-HRP-2 antibody and monoclonal anti-pan specific pLDH antibody.

Interpretation: - Results are noted as per manufactures guidelines and results were interpreted accordingly. Appearance of three purplish pink coloured lines one each in P.f. region (F), Pan region (P) & Control region (C) indicates that the sample is reactive for P. falciparum or mixed infection of P.f and P.v (or P. malarie, P.ovale). Depending on the concentration of pLDH & HRP-2 positive results may be observed within 60 seconds. However, to confirm a negative result the test result should be read only at 20 minutes. Consider a faint test line also as a positive result. The results of the reactive sample by rapid tests were compared with the microscopy and vice versa.

2. Microscopy test method: Thin and thick blood films were prepared. The prepared blood films were processed and stained with 3% Giemsa staining technique. Giemsa staining was used according to standard procedures. Slides were examined until 200 white blood cells (WBCs) were counted if positive. Slides for which parasites were not detected after counting up to 200 WBCs were examined until 400 WBCs were counted before a slide was considered to be negative. Parasite density per microlitre of blood was estimated.[6] Parasite density per microlitre of blood was estimated by multiplying the counts by 8,000 (the approximate number of WBCs per microlitre) and dividing the result by the WBC counts.[6] Duplicate slides were examined independently by two experienced laboratory scientists who were blinded to the RDT results. In the case of a discrepant result, a third laboratory scientist examined both slides. Results from the third laboratory scientist were considered final.

Bio-Safety:- All standard precautions, bio-safety measures & biomedical waste managements in our study according to Biological waste management's Rules 1998 were observed.

RESULT

In our study, A total of 20466 blood samples were collected from the outdoor and indoor suspected Malaria patients of Govt, Medical College, Kota and its allied hospitals. Total no. of 20466 samples were tested by RDT and Microscopy method (gold standard) as a confirmatory method. On testing for 20466 samples, 48 samples were positive by Microscopy but only 46 were reactive in Rapid test.

Table 1: Results according to sex wise and total no. of positive cases wise by RDT and microscopy.

	Total no. of cases examined (%)	Total no. of cases Positive by RDT (%) (n-20466)	Total no. of cases Positive by Microscopy (%) (n-20466)
Male	11460 (55.99 %)	33(0.29 %)	34(0.3 %)
Female	9006 (44.01 %)	13(0.14 %)	14(0.15 %)
Total no. of cases	20466	46(0.22 %)	48(0.23 %)

Table 2: Distribution of malaria suspected cases by age group wise, plasmodium species wise and comparison of the RDT with PBF.

Age Group (years)	Suspected cases	Peripheral Blood film-Positive (Thick and thin PBF)		Total malaria PBF- Positive (%) (n-48)	RDT Positive (%) (n-46)
		PV +ve (n-48)	PF +ve (n-48)		
1-10	3193	7	2	09 (18.75 %)	09(19.57 %)
10-20	3520	10	2	12(25 %)	12(26.09 %)
20-30	4994	12	4	16(33.34 %)	15(32.61 %)
30-40	3418	3	0	03(6.25 %)	03(6.52 %)
40-50	2947	1	2	03(6.25 %)	03(6.52 %)
50-60	1023	3	1	04(8.33 %)	03(6.52 %)
>60	1371	1	0	01(2.08 %)	01(2.17 %)
Total	20466	37 (77.08 %)	11 (22.92 %)	48	46

P value <0.001

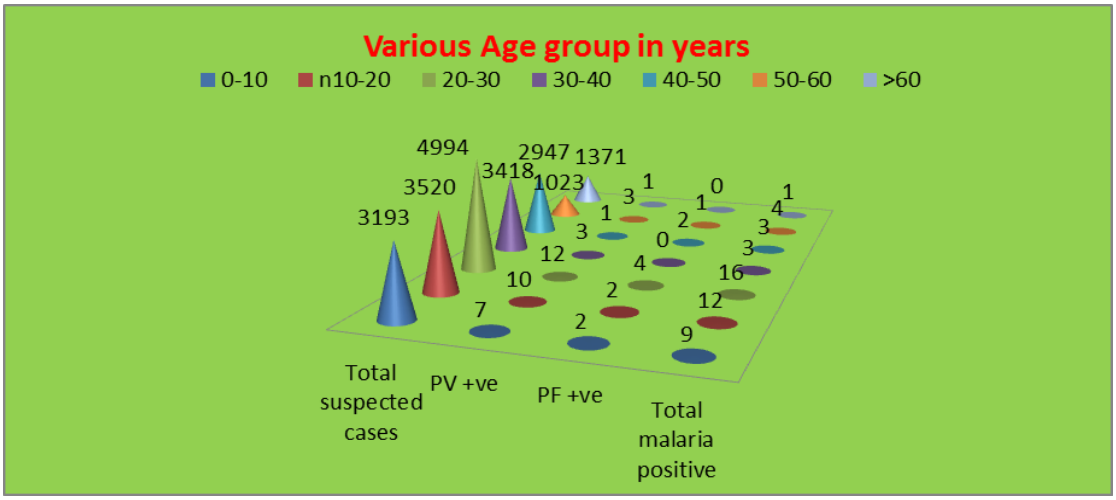

Figure 1: Distribution of malaria suspected cases by age group wise, plasmodium species wise and total no. of Malaria positive cases.

Table 3: Results according to months wise, sex wise and total no. of positive cases wise.

Month	Total suspected cases	Total no. of positive cases(n-48)		Total no. of positive cases (n-48)		
		Male	Female	PV +ve	PF +ve	Total no. of positive cases
January 2018	1357	2	1	3	0	3 (6.25 %)
February 2018	1625	0	0	0	0	0 (0 %)
March 2018	1706	1	0	1	0	1 (2.08 %)
April 2018	1634	3	1	3	1	4 (8.34 %)
May 2018	1580	7	2	7	2	9 (18.75 %)
June 2018	1318	1	1	2	0	2 (4.17 %)

July 2018	1658	3	4	7	0	7 (14.58 %)
August 2018	1860	9	3	12	0	12 (25 %)
September 2018	2723	1	0	0	1	1 (2.08 %)
October 2018	2386	3	0	1	2	3 (6.25 %)
November 2108	1625	3	2	1	4	5 (10.42 %)
December 2018	994	1	0	0	1	1 (2.08 %)
Total no. cases	20466	34 (70.83%)	14 (29.17%)	37(77.08%)	11(22.92 %)	48

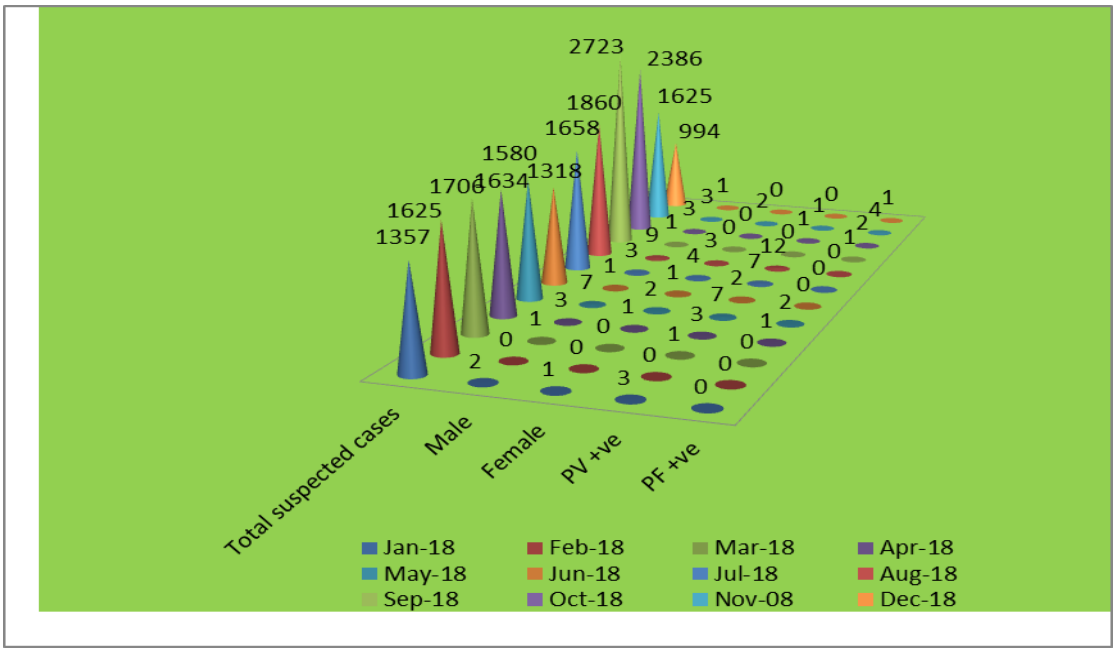

Figure 2: Results according to months wise, sex wise and total no. of positive Plasmodium species wise.

Table 4: RDT results according to ages of the patients and Degree of malaria parasitaemia.

	Different Age groups (in years)							Total no. Positive (%) (n-48)
	1-10	10-20	20-30	30-40	40-50	50-60	>60	
Total Positive Cases Examined	09	12	16	03	03	04	01	48
Mild Parasitaemia (100 to 500)*	05	08	08	02	00	01	00	24(50 %)
Mild Parasitaemia (>500 to <1000)	01	03	03	01	01	01	00	10(20.83 %)
Moderate Parasitaemia(≥ 1000<10000)	01	00	02	00	00	01	01	05(10.42 %)
Severe Parasitaemia (≥ 10000)	02	01	03	00	02	01	00	09(18.75 %)
Total Positive by Microscopy (%)	09	12	16	03	03	04	01	48
Total Positive by RDT (%)	09	12	15	03	03	03	01	46

***Units for parasitaemia is parasites/µL of blood**

Table 5: Comparative Evaluation of Rapid Test Kits with Microscopy.

TEST (n-20466)	Positive by Microscopy	Percentage Reactivity	Reactive by RAPID TEST	Percentage Reactivity	False Negative by RAPID TEST
MALARIA TEST (Plasmodium species)	48	0.23%	46	0.22%	0.01%

Table 6: Comparison of Rapid Card Test with Microscopy.

Rapid Card Test	MALARIA Test (Plasmodium species)	
	Microscopy Positive	Microscopy Negative
Rapid Reactive	46	0
Rapid Non reactive	02	20418
Total cases	48	20418

P value <0.001

Table 7: Comparative evaluation of Rapid Test Kit with Microscopy as a gold standard confirmatory method.

Test	Sensitivity	Specificity	PPV	NPV	Diagnostic effectiveness (Diagnostic accuracy)	Youden's index	F1SCORE
MALARIA by Rapid card test	95.83%	100%	100%	99.99%	99.99%	0.958	0.979

*__Sensitivity-(also called the true positive rate, the recall, power, hit rate or probability of detection)-__refers to a test's ability to designate an individual with disease as positive.

__Specificity-(also called the true negative rate or selectivity)-__refers to a test's ability to designate an individual who does not have a disease as negative.

__Positive predictive value-(PPV also called the precision)-__is the ability of an assay to identify actual infected individuals among all persons.

__Negative predictive value-(NPV)-__is the ability of an assay to identify correctly the real non-infected individuals among persons.

__Diagnostic accuracy-(DA also called the Diagnostic effectiveness)-__The accuracy of a test is its ability to differentiate the patient and healthy cases correctly. DA is affected by disease prevalence.

__Youden's index-(also called Yuoden J satstices)-__It is a single statistic that captures the performance of a dichotomous diagnostic test. DA is affected by spectrum of disease, not by disease prevalence.

__F1Score-(also called the aka, F-Score/F-Measure)-__It is the harmonic mean (average) of the PPV and Sensitivity. It can be used as a single measure of performance of the test for the positive class.

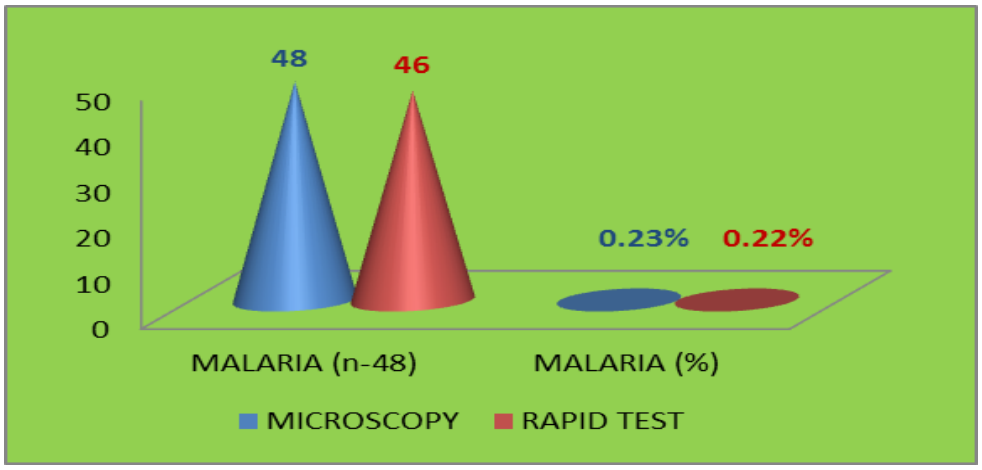

Figure 3: Comparative Evaluation of Rapid Test Kits with Microscopy.

Table 1 shows the distribution of study participants according to sex. A total of 20466 patients were examined, out of which 11460(55.99%) were males while 9006 (44.01%) were females. Results by microscopy show that up to 0.3% (34/11460) of the males and 0.15% (14/9006) of the females were infected. But by RDT, 0.29% (33/11460) and 0.14% (13/9006) of the females were infected. Table 2 and Figure 1 shows distribution of malaria suspected cases by age groups wise, Plasmodium species wise and comparison of the RDT with PBF. The PBF indicated that 77.08% (37/48) of the patients were positive for P. vivax and 22.92% (11/48) were infected with P. falciparum. Out of 48 positive patients by microscopy 34(70.83%) were males while 14(29.17%) were females. Age group mostly affected were 20-30 years, 10-20 years and 1-10 year 33.34%, 25% and 18.75% respectively. Table 3 and Figure 2 shows results according to months wise, sex wise and total no. of positive cases wise. Most of suspected cases of malaria were come in October (2723) September (2386) August (1860) and March (1706) months whereas total no. of positive cases were come in August (12), May (9) and November (5) in decreasing order. Table 4 shows the degree of malaria parasitaemia and RDT results, against ages of the patients. Out of the 48 patients who tested positive by microscopy mostly affected 20-30 years,10-20 years, 1-10 year, 50-60 years, 30-40 years, 40-50 years and > 60 years with 33.34%, 25%, 18.75%, 8.33%, 6.25%, 6.25% and 2.08% respectively in decreasing order. In terms of parasite density, out of the positive cases, 50% were mild parasitaemia (100 to 500 parasites/ µL of blood), 20.83% Mild Parasitaemia (>500 to <1000 parasites/µL of blood), 10.42% moderate parasitaemia (≥ 1000<10000 parasites/uL of blood) of blood) and 18.75% severe parasitaemia (≥10000 parasites/uL of blood). Considering microscopy as standard, there were 02 false positive results. Table 5, Table 6 and Figure 3 shows comparative evaluation of rapid test kits with microscopy for Plasmodium species. Total no. of 20466 samples

were tested by RDT and ELISA method (gold standard) as a confirmatory method. On testing for 20466 samples, 48 samples were reactive by Microscopy but only 46 were reactive in Rapid test. Table 7 shows that using Microscopy as a gold standard confirmatory method, sensitivity of RDT for Plasmodium species was 95.83%, specificity was 100%, PPV(positive predictive value) was 100%, NPV(negative predictive value) was 99.99%, Diagnostic accuracy was 99.99%, Youden's index was 0.958 and F1 Score was 0.979.

DISCUSSION

In the present study Microscopy was compared with the RDT for the screening of Plasmodium species. For Plasmodium species screening, rapid tests are equally sensitive to Microscopy and yet they are cheaper and quicker. Within the rapid tests, the sensitivity and specificity was same but there were variations in the cost.[19] Microscopy and other advanced methods is laboratory based, time consuming and require trained personnel. Rapid test enables early detection at sites where laboratory facilities or trained manpower are not available or there is issue of accessibility.[20,21] The rapid tests reduce the potential for loss of follow up of a case when results are not given straight away. The high laboratory cost is another factor that reduces the willingness to screen the general population. Ideally rapid devices should have a high degree of sensitivity and a reasonable specificity so as to minimize false positive and false negative results.[22] This study agrees with previous studies in India and other countries, which have stated that rapid test kits are not sensitive enough to be used solely for the detection of Plasmodium species.[2] Some studies suggest that the diagnostic performance of Rapid Test is comparable to Microscopy. However in our study we found Microscopy to be much more sensitive than Rapid Test. The our results were compare with studies of Baker J, et al 2005[9], Endeshaw T, et al 2008(Ethopia)[11], Pathak S, et al 2012(India)[18] Khadanga S et al 2014(India)[13], Ayogu E, et al 2016(Nigeria)[7] and Komlosi M et al 2017(Burundi).[14] Comparative assessment of microscopy and rapid diagnostic test were compare with studies of Maltha J, et al 2010[16], Oyeyemi OT et al 2015[17] and Mahende C et al 2016.[16] Comparison of studies conducted by other researchers showed slight variations in results. Results were varies with geographical distribution and social characteristic of population groups. Our study is a step ahead in this direction with the purpose of providing authentic scientific data based on the affected population. We conclude that Malaria directly affects epidemiology, morbidity, mortality, socioeconomic and preventive aspects, So particularly in developing countries like India, the present study and other similar studies by early detection of Plasmodium species prevalence for in assessment of disease burden in community and in controlling the complications of Malaria.

SUMMARY AND CONCLUSION

The ultimate goal of this study was to recommend most reliable and cost-effective rapid kits for the diagnosis of Plasmodium species in areas where advance diagnostic facilities are not available. We reported that rapid test is less efficient than Microscopy compared with conventional Microscopy which needs long time; Rapid card test results are available within minutes. This will be very helpful in initiating immediate treatment and minimizing the serious complications and mortality. Rapid card test are quite susceptible to unfavourable storage conditions, so this is essential to do periodic quality control checks to avoid false positive or false negative results. These rapid card tests should be recommended only in resource limited poor settings, remote areas and peripheral health facilities for screening purpose. Malaria are highly dangerous infection for community; false negative results leave a threat of silent transmission and spreading of diseases among people and also create an urge for more sensitive assays such as Microscopy. A major concern in utilizing rapid screening tests is that these tests should have a high degree of sensitivity and a reasonable level of specificity to minimize false positive and false negative results. Therefore, it is recommended that, rapid test kit should be used in conjunction with other method particularly Microscopy method or advance technique methods.

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