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A Study of Evaluation of Rapid Diagnostic Techniques of Malaria in Urban Slums of Vijayawada, Krishna District, Andhra Pradesh, India.

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ABSTRACT

200 clinically suspected malaria patients from Urban Slums of Vijayawada from June to November, 2009 were chosen for the study ranging ,from infants to 80 years old. Maximum number of cases were seen in the age group of 20-30 years. The mean age was 25 and SD of 12.82.The male to female ratio was 2.1: 1, showing a male predominance. 62 cases were positive by peripheral smear.Out of which 50 (81%) were positive for *Plasmodium vivax* and 12 (19%) cases were positive for *Plasmodium falciparum*. One case was positive for both. The cases which were not detected by peripheral smear and SD-Bioline were detected by Paramax-3 kit which showed a sensitivity and specificity of 88% and 100% respectively for pLDH (*Plasmodium vivax*), sensitivity and specificity of 83.3% and 98.9% for HRP-2 (*Plasmodium falciparum*). The SD-Bioline test results indicated that 22% (44 of 200) of the patient samples were positive for malaria parasites,showing a sensitivity and specificity of 74% and 100% for *Plasmodium vivax* ,Sensitivity of 58.33% and specificity of 100% for *Plasmodium falciparum*. The sensitivity of SD-Bioline for *Plasmodium vivax* was as low as 74% while that of Paramax-3 was 88%.The sensitivity of SD-Bioline for *Plasmodium falciparum* was as low as 58.33% while that of Paramax-3 was 88.3%.

Keywords: *Plasmodium*, Specificity, Sensitivity, Peripheral smear.

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INTRODUCTION

Malaria is a major global health problem. It continues to afflict the poor nations and is among the top ten killer diseases of the world. More than 3 million people worldwide live in malaria in endemic areas. Every year 300-500 million people are infected with malaria and 1.5-2 million of these die from disease, 90% of which occur in tropical Sahara. Outside Africa, 70% of the remaining cases occur in just 3 countries, India, Srilanka and Brazil. The global dream of eradicating malaria has remained elusive because of several complex issues that pose a challenge to the health system. In 1992, the global malaria control strategy was introduced with 4 basic elements- early diagnosis, prompt treatment, selective and sustainable vector control and early forecasting of epidemics; yet the desired results could not be achieved. Roll back malaria initiative launched by WHO in 1998 as a global project brought together the biggest players in health system but again with no satisfactory outcome. Clinical diagnosis is imprecise but remains the basis of therapeutic care for the majority of febrile patients in malaria endemic areas, where laboratory support is often out of reach. Scientific quantification or interpretation of the effects of malaria misdiagnosis on the treatment decision, epidemiologic records, or clinical studies has not been adequately investigated. Despite an obvious need for improvement, malaria diagnosis is the most neglected area of research, accounting for an expenditure less than 0.25% ($ 700,000) out of U.S. $323 million which was invested for research and development in 2004 (Chansuda Wongsrichanalai, Trop. Med. Hyg., 2007).

In India malaria has been a serious problem in north eastern states mainly due to topography and climatic conditions which are congenital for perennial malaria transmission. About 70% of infections are reported to be due to *Plasmodium vivax*, 25-30% due to *Plasmodium falciparum* and 4-8% due to mixed infection. *Plasmodium* malariae has a restricted distribution and is responsible for <1% of infections in India.Even with the implementation of modified plan of operation (MPO) which was launched in 1984, the epidemiological situation has not shown any great improvement in our country. During the year 2001, the Annual parasite incidence is 2.04.The identified malaria priority areas are forests, forested foot hills, forest fringe areas and developmental project sites.

One main element of global malaria control strategy for effective management is prompt and accurate diagnosis. In many endemic countries, the current approach to malarial diagnosis, especially, in peripheral health canters, is entirely based on clinical diagnosis, which is of limited accuracy due to the poor specificity of symptoms and signs of malaria. Consequently, presumptive antimalarial treatment is widely administered for any fever with no obvious alternative cause, leading to significant overuse of antimalarial drugs. In addition, over- diagnosis of malaria in the formal health care sector often co-exists with under diagnosis of malaria in the community.With chloroquine and sulfadoxine-pyrimethamine resistance becoming widespread and more effective but expensive antimalarial medicines, including artemisinin-based combination therapies, being used in most of countries, there is a need to improve the diagnosis of acute febrile illness at various levels of health care system; this is now a public health priority, as it would ensure that antimalarial drugs can be targeted to patients who need them.

In general view is that the “gold standard” method for malaria diagnosis is the detection of *Plasmodium* species by microscopic examination of blood films. This method is

relatively simple and has low direct costs, but it is labour-intensive, time-consuming, and requires well- trained personnel who can differentiate between the different *Plasmodium* species. Alternative diagnostic tests for malaria and in particular rapid diagnostic tests (RDTs) have been developed over the past 20 years. These tests are fast easy to perform, and do not require electricity or specific equipment. They include those based on histidine-rich protein 2(HRP2) alone or a modified test format of HRP and parasite-specific aldolase enzyme (pan-malarial antigen), parasite lactate dehydrogenase (pLDH), and qualitative detection of antibodies of all isotypes (IgG, IgM, IgA). The technical performances of these alternative techniques have already been assessed in various populations and epidemiologic settings. Stipulations for these rapid tests include the capability to detect 100 parasites/µl from all *Plasmodium* species and the ability to perform semi quantitative measurements for monitoring drug treatment results. However, the main limitation of the majority of these studies was that microscopy was used as the "gold standard". The majority of malaria cases are found in countries, where cost-effectiveness is an important factor and ease of diagnostic test performance and training of personnel are also major considerations. Most new technology for malaria diagnosis incorporates immunochromatographic capture procedures, with conjugated monoclonal antibodies providing the indicator of infection. Preferred targeted antigens are those which are abundant in all asexual and sexual stages of the parasite.

The main aim of this study was to assess the accuracy of available RTDs for the diagnosis of malaria, as a first step to improve malarial management at different levels of the health care system in Vijayawada, where malaria is endemic. So I made this study to compare 2RDTs (PARAMAX-3 Pan/Pv/Pf and SD BIOLINE Malaria P.f/P.v tests) with the conventional microscopy, using blood samples of patients with clinical suspicion of uncomplicated malaria at urban slums of Vijayawada.

MATERIALS AND METHODS

It is a prospective case study consisting of 200 clinically suspected malaria patients from Urban Slums of Vijayawada . The processing of samples was done in the Department of Microbiology, Siddhartha medical college, Vijayawada. The **inclusion criteria** in this study group consist of fever with chills and rigors followed by sweating. After obtaining brief clinical history about the duration of symptoms, age, sex, occupation, socio-economic status and past medication, the samples were collected. The **exclusion criteria**: Patients who have already taken anti-malarial drugs are not included. Fever with defined cause is excluded.**Collection of Samples:** (Monica Cheesbrough, 2002) 2ml of intravenous blood was collected with a sterile syringe and is transferred into sterile bottle containing EDTA i.e., anticoagulant.**Processing of Blood Samples:** (G.K. Sharma, 1998).Thin and Thick Smears were prepared from the collected blood sample. After smear preparation, smears were stained with Leishman's stain. The blood sample was subjected to serological tests (Rapid Diagnostic Tests):

To detect plasmodial antigen: A third generation Rapid Diagnostic test for malaria (PARAMAX-3Pan/Pv/pf. Manufactured by Zephyr Biomedicals. M.L.No: 558. Lot No: 91061. Mfg.Dt: 10-2008. Exp.Dt: 09-2010) was used. Paramax-3 malaria Pf/Pv/Pan test is an

immunochromatographic test. Procedures were followed strictly as contained in the manufacturer's standard operating manual inserted in the kit.

To detect plasmodial antibody: A rapid one step malaria anti-*Plasmodium falciparum* and *Plasmodium vivax* test (SD-Bioline One Step Malaria Anti-P.f/ P.v test. Manufactured by SD BIOLINE STANDARD DIAGNOSTICS PVT.LTD. Lot No: 18043. Mfg.Dt: 21-10-2008. Exp.Dt: 20-04-2010), was used alongside the gold standard (microscopy). The SD Bioline malaria Pf/Pv test is an immunochromatographic test for the qualitative detection of the antibodies of all isotypes (IgG, IgM and IgA) specific to P.*falciparum* and P.*vivax* simultaneously in human serum, plasma or whole blood. The test cassette contains a membrane strip, which is precoated with recombinant malaria P.f capture antigen on test b and 1 region and with recombinant P.v capture antigen on test b and region 2. Procedures were followed strictly as contained in the manufacturer's standard operating manual inserted in the kit. The RDTs for malaria (Paramax-3 and SD-Bioline) are compared with microscopy.

RESULTS

Statistical software: The statistical software namely SPSS11.0 and Systat8.0 were used for the analysis of the data and Microsoft and Excel have been used to generate graphs, tables etc.

A total of 200 blood samples were tested for malaria parasites by both Paramax-3, SD-Bioline methods, and the results were compared to results obtained from reading thin and thick- smear blood films. The blood film results indicated that 31% (62 of 200) of the patients were infected with malaria based on the morphologies of the parasite stages.Table 1: Among them, P. *vivax* was present in 50(25%) samples while P.*falciparum* was present in 12(6%). [The one mixed infection has been tabulated with the P.*falciparum* infection numbers for ease of analysis].Correspondingly, the Paramax-3 test results indicated that 28% (56 of 200) of the patient samples were positive for malaria parasites. Infections with P. *vivax* accounted for 44(22%) samples, while infections with P.*falciparum* accounted for 12(6%)of the total malaria cases. Both methods identified one patient with a mixed infection of P. *falciparum* and P. *vivax*. [The one mixed infection has been tabulated with the P.*falciparum* infection numbers for ease of analysis].

The SD-Bioline test results indicated that 22% (44 of 200) of the patient samples were positive for malaria parasites. Infections with P. *Vivax* accounted for 37(18.5%) samples, while infections with P.*falciparum* accounted for 7(3.5%) of the total malaria cases. Both methods identified one patient with a mixed infection of P. *falciparum* and P. *vivax*. (The one mixed infection has been tabulated with the P.*falciparum* infection numbers for ease of analysis) . In the present study the patient's age group ranged from infants to elderly. According to (Table-2), the maximum number of cases is seen in the age group of 20-30 years .The mean age was 25 years.

Table-3 shows sex-wise distribution of malaria. Out of 102 samples tested in males, 42 were positive for malaria with a percentage of 67.74%. Among 98 samples tested in females 20 were positive for malaria with a percentage of 32.25%. It showed male predominance with male to female ratio of 2.1:1.

Table-4 & 5: Paramax-3 compared to traditional blood films for detection of P. *vivax* (Table-4) and P.*falciparum* (Table-5) infections .Sensitivity= 4400/ 50=88%, Specificity= 15000/ 150= 100%. Positive Predictive Value=4400/ 44= 100%, Negative Predictive Value=150 00/156= 96%.The blood films identified 6 P.*vivax*- positive samples that were not identified by the Paramax-3 test; however, there was 100% agreement between blood film results and Paramax-3 results for the other 44 samples containing P. *vivax*.Paramax-3 had sensitivity of 88% (95% confidence interval, 85.2 to 97.6%) and specificity of 100% (95% CI, 96.2 to 100.0%), when compared to traditional blood films for the detection of P. *vivax* (Table 4). Positive and negative predictive values were 100% (95% CI, 93.9 to 100.0%) and 96% (95% CI, 95.5 to 99.8%), respectively, for P. *vivax*.Sensitivity=1000/12=83.3%, Specificity=18600/188= 98.9%, Positive Predictive Value=1000/12= 83.3%, Negative Predictive Value= 18600/188=98.9%.Although both methods detected 12 cases of P. *falciparum* infection, there were 2 cases detected by Paramax-3 that were not detected by the blood films and 2 cases detected by blood film that were not detected by the Paramax-3 method. Paramax-3 had sensitivity of 85.7% (95% CI, 62.3 to 97.9%) and specificity of 98.9% (95% CI, 95.5 to 99.8%), when compared to traditional blood films for the detection of P.*falciparum* infections (Table 5). Positive and negative predictive values were 85.7% (95%CI, 62.3 to 97.9%) and 98.9% (95% CI, 95.7 to 99.8%), respectively, for P. *falciparum*.

Table 6 & 7: SD-Bioline test compared to traditional blood films for detection of P. *vivax* (Table-6) and P.*falciparum* (Table-7) infections:Sensitivity= 3700/50= 74%, Specificity=15000/150= 100%, Positive Predictive Value= 3700/37=100%, Negative Predictive Value= 15000/180= 83.33%.The blood films identified 13 P.*vivax* positive samples that were not identified by the SD-Bioline test; however, there was 100% agreement between blood film results and SD-Bioline results for the other 37 samples containing P. *vivax*.SD-Bioline had sensitivity of 74% [95% confidence interval [CI], 85.2 to 97.6%] and specificity of 100% (95% CI, 96.2 to 100.0%) when compared to traditional blood films for the detection of P. *vivax* infections (Table 6). Positive and negative predictive values were 100% (95% CI, 93.9 to 100.0%) and 83.33% (95% CI, 95.5 to 99.8%), respectively, for P. *vivax*.Sensitivity= 700/ 12= 58.33%, Specificity=18800/188= 100%, Positive Predictive Value= 700/12= 100%, Negative Predictive Value= 18800/19= 97.4%.The blood films identified 5 P. *falciparum* positive samples that were not identified by the SD-Bioline test; however, there was 100% agreement between blood film results and SD-Bioline results for the other 7 samples contains P. *falciparum*. SD-Bioline had sensitivity of 58.33% (95% CI, 62.3 to 97.9%) and specificity of 100% (95% CI, 95.5 to 99.8%), when compared to traditional blood films for the detection of P.*falciparum* infections (Table 7). Positive and negative predictive values were 100% (95%CI, 62.3 to 97.9%) and 97.4% (95% CI, 95.7 to 99.8%), respectively, for P. *falciparum*.

Table No.8 shows comparison of various methods of malaria diagnosis. The SD-Bioline gave the worst comparative results with a percentage of 22% of total positivity, while Paramax-3 gave a percentage of 28% of positivity when compared with peripheral smear which got a percentage of 31% of positivity.

Table No.9 shows comparison of various methods of malaria diagnosis for P.*vivax*. The SD-Bioline gave the low comparative results with a percentage of 18.5%, while aramax-

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3 gave a percentage of 22% for P.*vivax*, when compared with peripheral smear which got a percentage of 25%.

Table No.10 shows comparison of various methods of malaria diagnosis for P.*falciparum*. The SD-Bioline gave the low comparative results with a percentage of 3.5%, when compared with Paramax-3 and peripheral smear which got a percentage of 6% for P.*falciparum*.

Table-1: Results of the Present Study: (n= 200)

Total no. of Samples tested	Number positive for Pv/Pf				Negative for malaria
	Pv alone	Pf alone	Pv+Pf	Total +ves	
200	50(25%)	11(5.5%%)	01(0.5%)	62(31%)	138(69%)

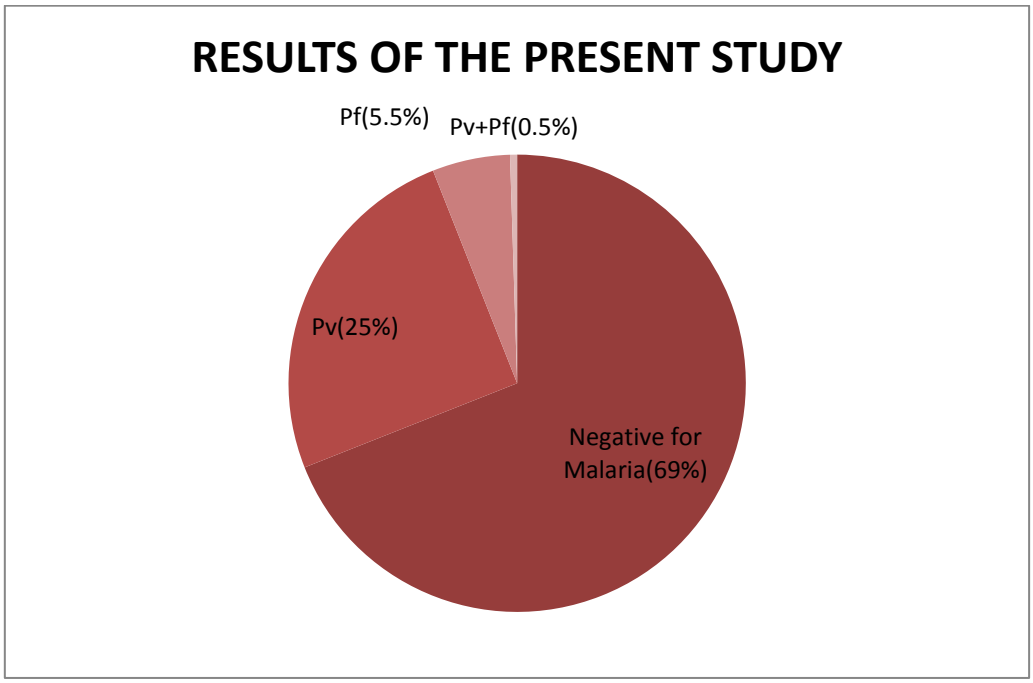

Table-2: Age-wise distribution of malaria in the present study: (n=200)

Age in Years	Total No. Of samples tested (%)	Out of total, No. Of samples positives (%)
00-10	14 (7%)	01 (1.61%)
11-20	20 (10%)	02 (3.22%)
21-30	42 (21%)	23 (37.09%)
31-40	33 (16.5%)	16 (25.80%)
41-50	31 (15.5%)	10 (16.12%)
51-60	30 (15%)	06 (9.67%)
61-70	29 (14.5%)	04 (6.45%)
71-80	01 (0.5%)	00 (0.0%)
Total	200 (100%)	62 (100%)

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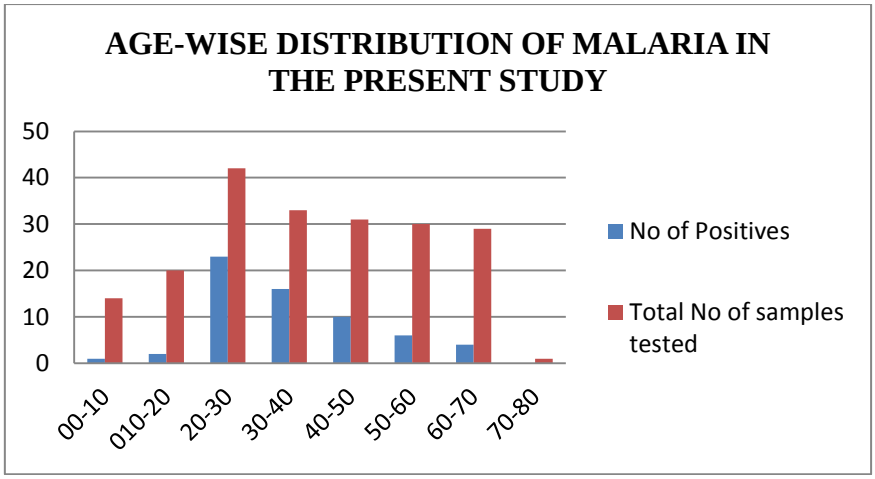

Table-3: Sex-wise distribution of malaria: (n=200)

Sex	Total No. of cases (%)	No. of positives (%)
Males	102 (51%)	42 (67.74%)
Females	98 (49%)	20 (32.25%)
Total	200 (100%)	62 (99.99%)

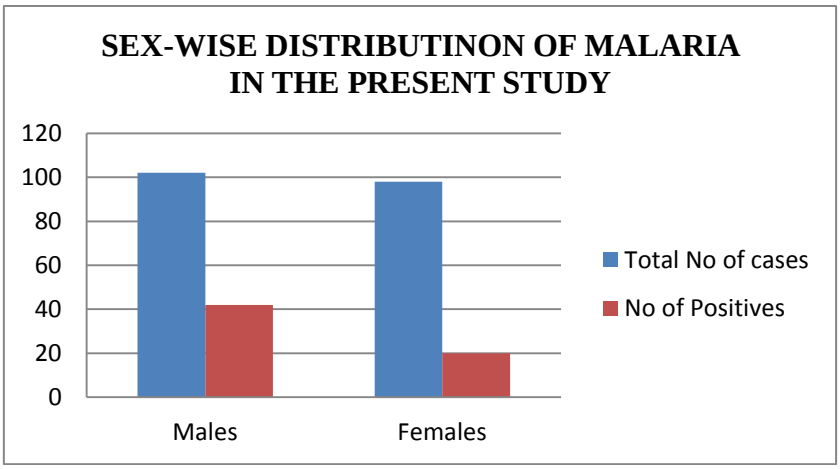

Table-4 & 5: Paramax-3 compared to traditional blood films for detection of P. vivax (Table-4) and P.falciparum (Table-5) infections:

Table-4: For P. vivax: (n=200)

Data	+ve for Pv by Smear Examination	-ve for Pv by Smear Examination	Total
+ve for Pv by Paramax-3	44	00	44
-ve for Pv by Paramax-3	06	150	156
Total	50	150	200

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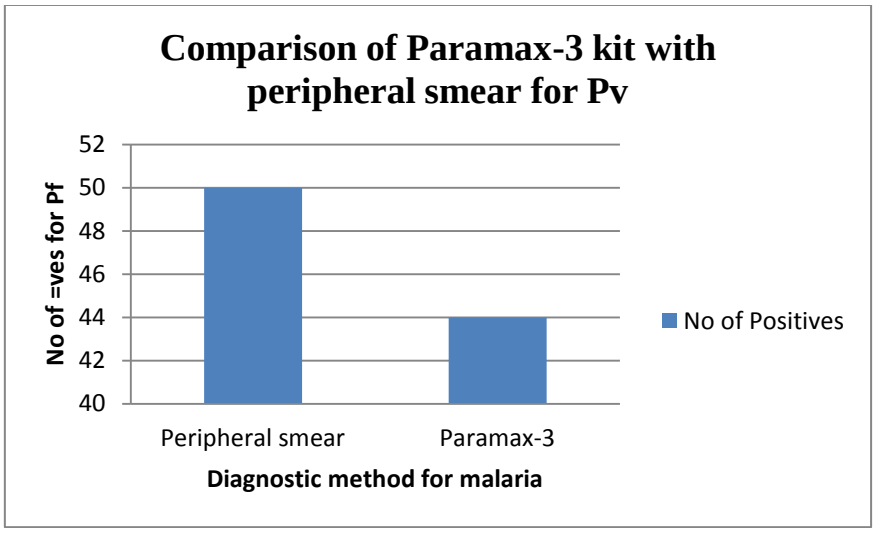
Table-5: For P. falciparum: (n=200)

Data	+ve for Pf by Smear Examination	-ve for Pf by Smear Examination	Total
+ve for Pf by Paramax-3	10	02	12
-ve for Pf by Paramax-3	02	186	188
Total	12	188	200



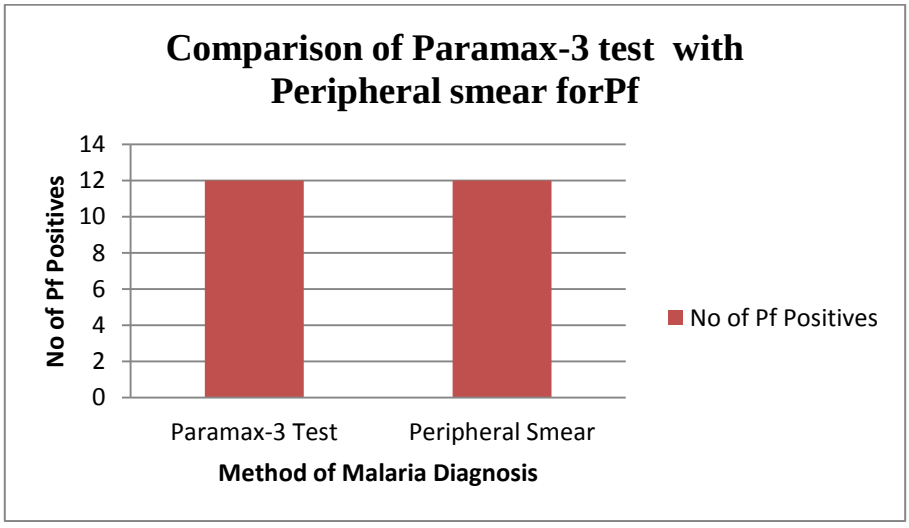


Table 6 & 7: SD-Bioline test compared to traditional blood films for detection of P. vivax (Table-6) and P.falciparum (Table-7) infections:
Table: For P. vivax: (n=200)

Data	+ve for Pv by smear examination	-ve for Pv by smear examination	Total
+ve for Pv by SD-Bioline	37	00	37
-ve for Pv by SD-Bioline	13	150	180
Total	50	150	200

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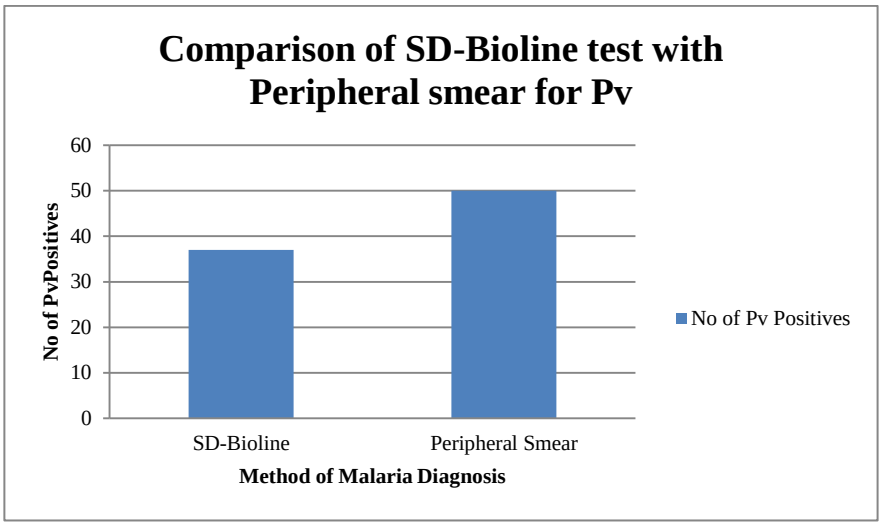
Table 7: For P.falciparum: (n=200)

Data	+ve for Pf by smear examination	-ve for Pf by smear examination	Total
+ve for Pf by SD-Bioline	07	00	07
-ve for Pf by SD-Bioline	05	188	193
Total	12	188	200



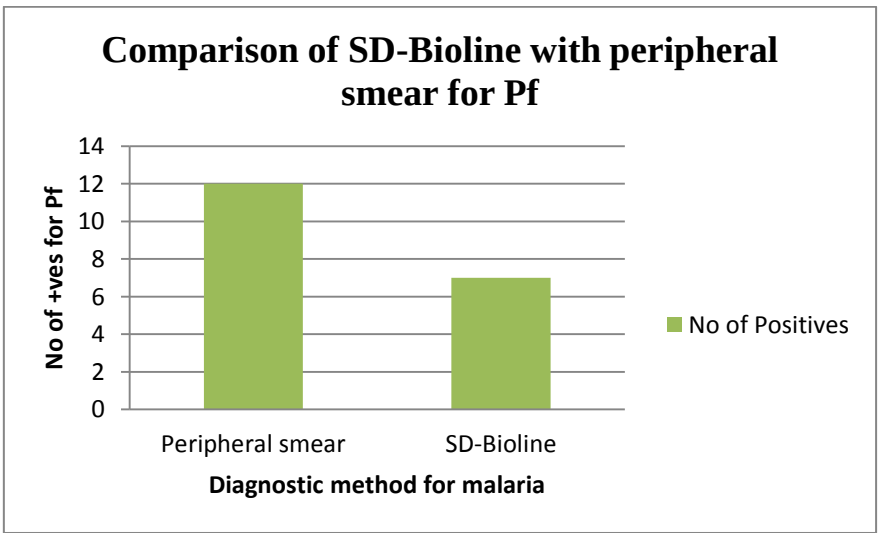


Table-8: Comparison of various methods of malaria diagnosis employed in the present study: (n=200)

Total No. of samples tested	+ve by smear	-ve by smear	+ve by Paramax-3	-ve by Paramax-3	+ve by SD-Bioline	-ve by SD-Bioline
200	62(31%)	168(69%)	56(28%)	144(62%)	44(22%)	156(78%)

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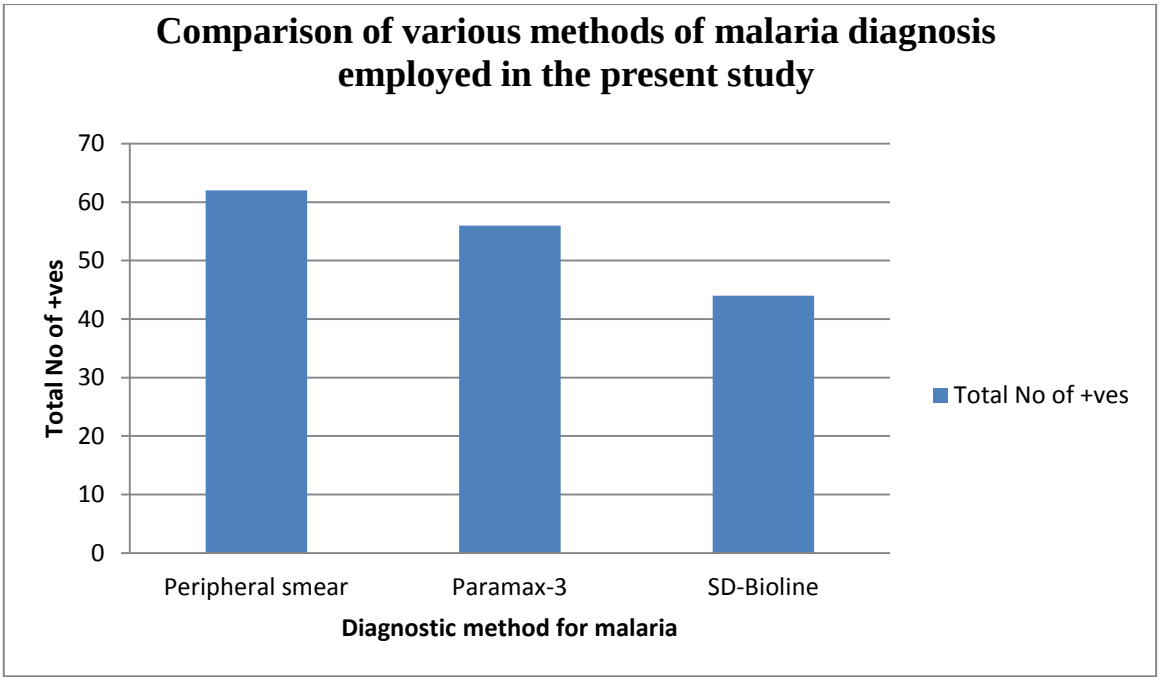

Table-9 Comparison of various methods of malaria in diagnosis of P.vivax in the present study: (n=200)

No. of samples tested	+ve for Pv by smear	-ve for Pv by smear	+ve for Pv by Paramax-3	-ve for Pv by Paramax-3	+ve for Pv by SD-Bioline	+ve for Pv by SD-Bioline
200	50(25%)	150(75%)	44(22%)	156(88%)	37(18.5%)	163(81.5%)

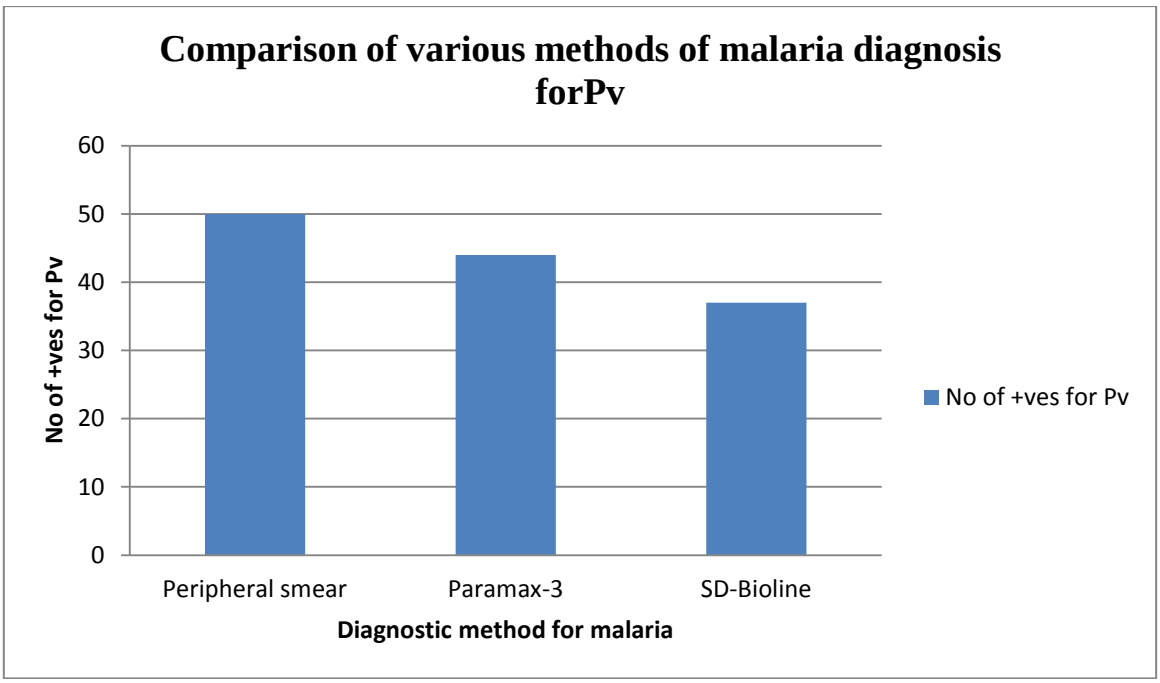

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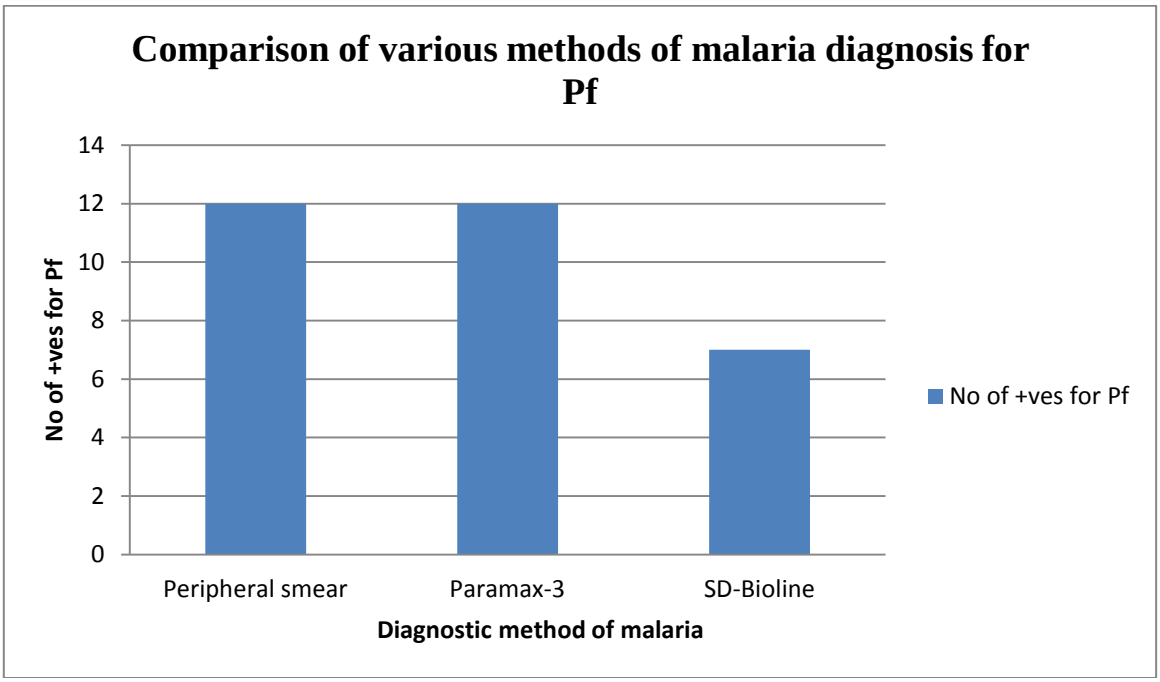
Table-10) Comparison of various methods of malaria diagnosis for P.falciparum in the present study: (n=200)

No. of samples tested	+ve for Pf by smear	-ve for Pf by smear	+ve for Pf by Paramax-3	-ve for Pf by Paramax-3	+ve for Pf by SD-Bioline	-ve for Pf by SD-Bioline
200	12(6%)	188(94%)	12(6%)	188(94%)	7(3.5%)	193(96.5%)



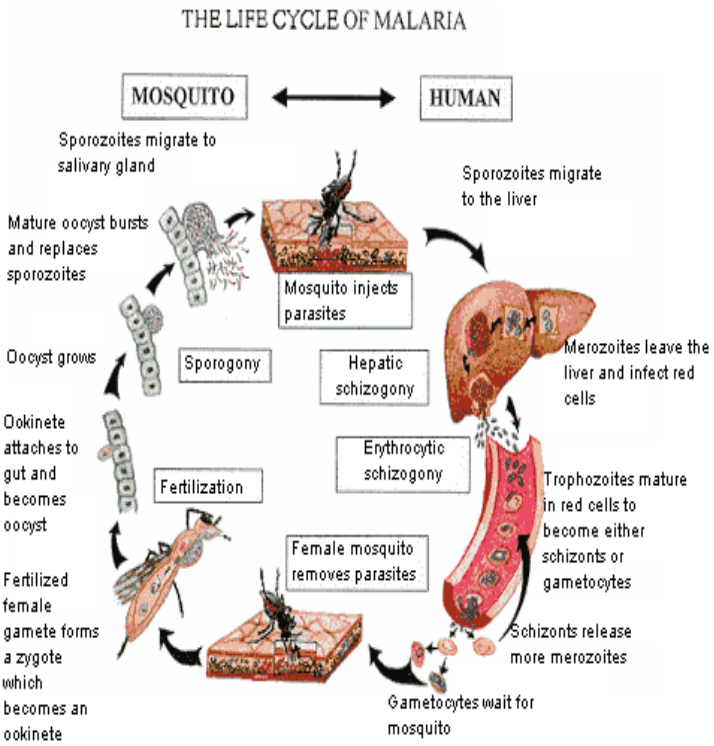


Fig-2, Plasmodium vivax schizonts (Leishman's stain 1000x)

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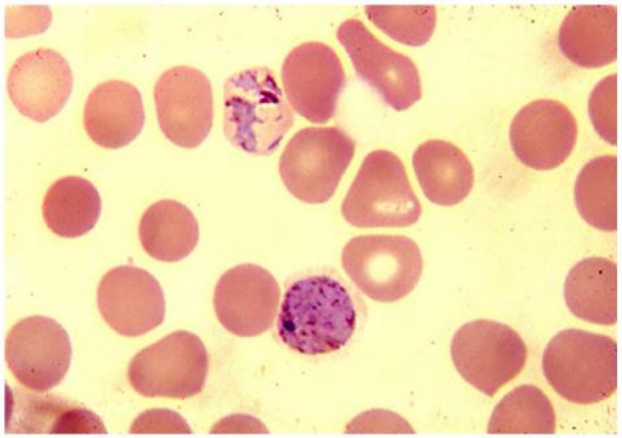


Fig-3, Plasmodium vivax rings and schizont
(Leishman's stain 1000x)
Fig-4, Plasmodium falciparum rings (Leishman's stain,1000x)

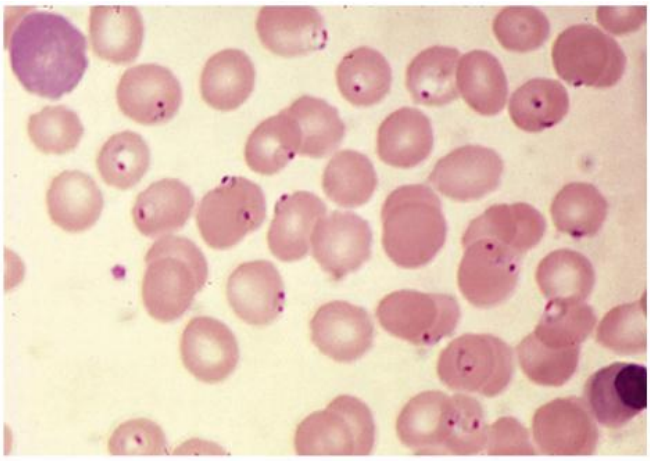
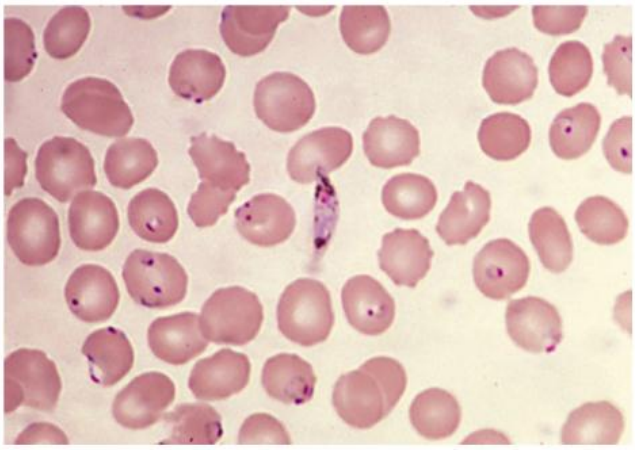


Fig-5, Plasmodium falciparum gametocytes and rings
(Leishman's stain, 1000x)



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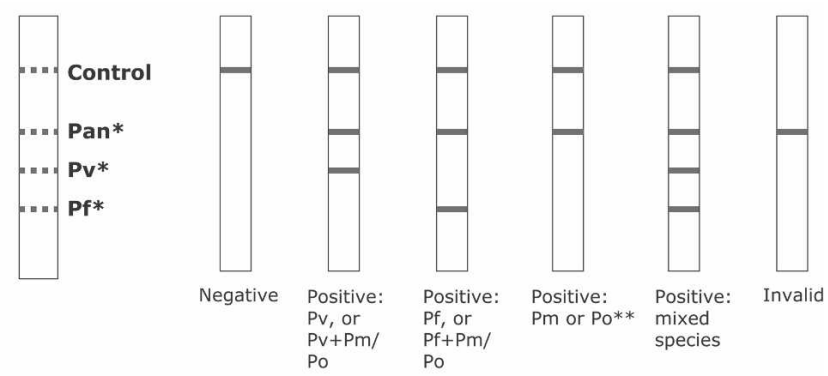
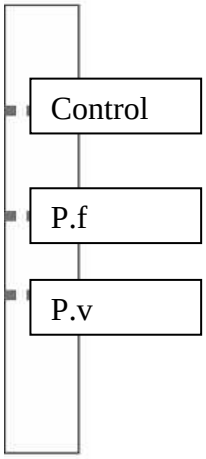
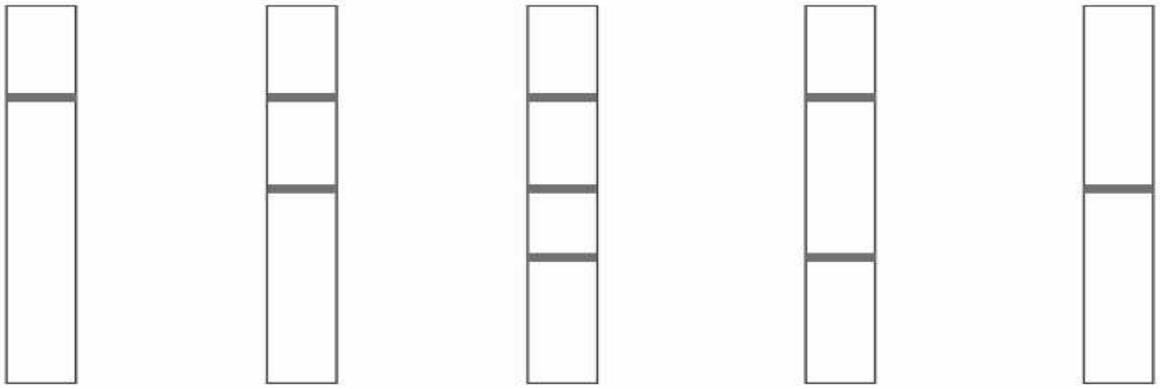


*Pan-malaria, *P. falciparum* and *P. vivax* lines: target antigens = pLDH

** Or positive non-Pf, non-Pv





2. Plasmodium vivax schizont and rings

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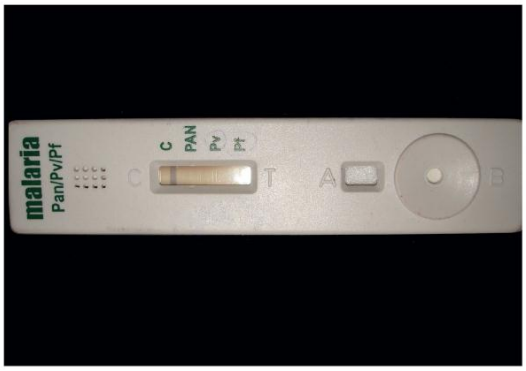
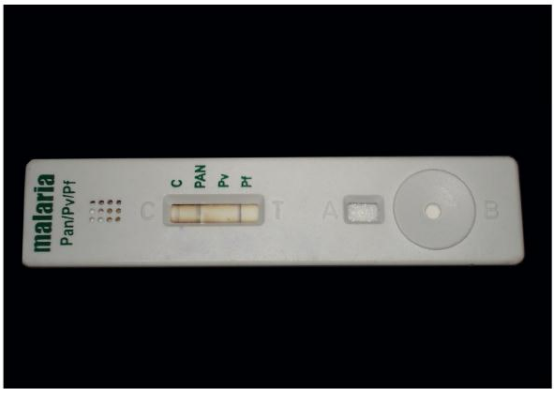
Paramax-3 Kit

SD-Bioline Kit



Negative (Paramax-3 kit)



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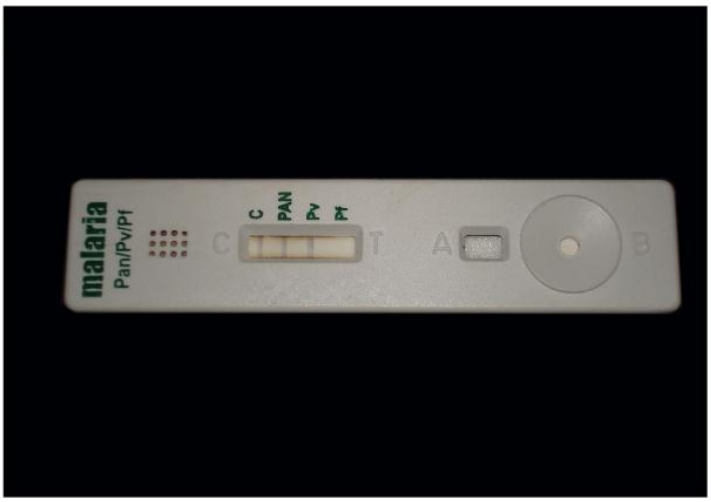
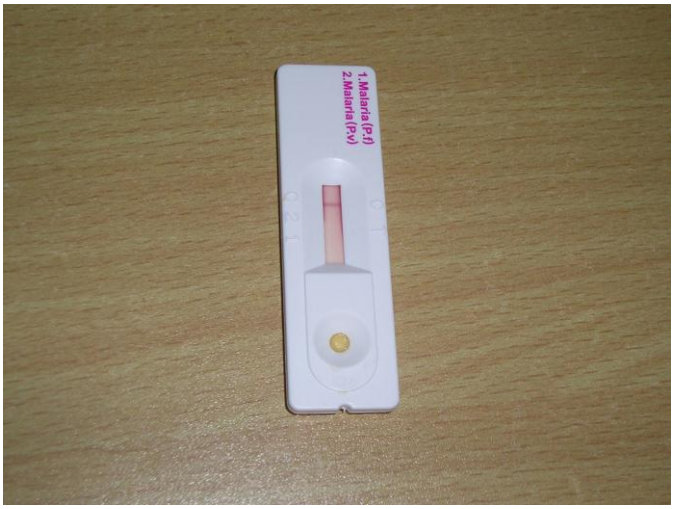
Plasmodium falciparum positive (Paramax-3 kit)



Plasmodium vivax positive (Paramax-3 kit)

Mixed infection (Paramax-3 kit)



Negative (SD-Bioline kit)

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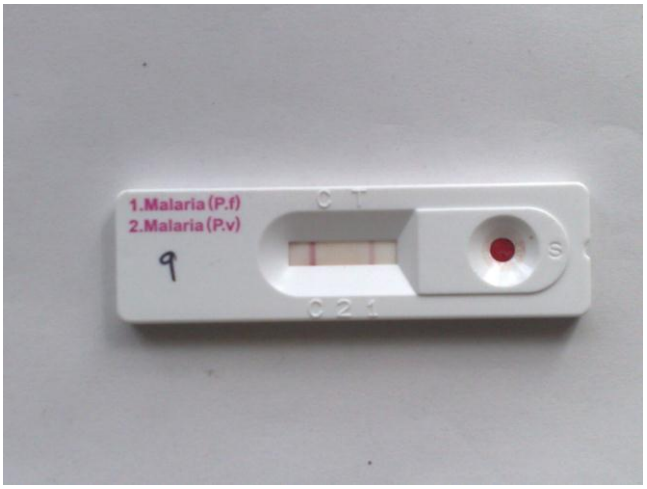

Plasmodium falciparum positive (SD-Bioline kit)

Mixed infection (SD-Bioline kit)

DISCUSSION

Malaria is a life threatening infection with a global impact extending from the most developed countries to regions of the world with only the most basic of health care infrastructure and the regions where malaria is highly endemic are increasing the need for rapid, prompt and accurate diagnosis. Endemic malaria, population movements and foreign travel – all contribute to malaria diagnostic problems in the Laboratory. Changing patterns of accepted morphological appearances of parasites, possibly due to drug pressure, strain variation, or approach to blood collection, have created diagnostic problems that cannot easily be resolved merely by reference to an Atlas. Even today microscopy still remains the most widely used method and the gold standard in malaria diagnosis in India.

In remote urban slums, control of malaria is a great challenge. Strengthening national capabilities to provide early diagnosis and treatment both within and outside the health services is of highest priority in WHO's action plan for malaria control 1995 – 2000 (WHO 1996).Currently, management of malaria by the Indian National Anti-Malarial programme (NAMP) is based on presumptive treatment of fever cases. Because symptoms lack specificity, moat diagnoses are inaccurate, resulting in both overtreatment with

antimalarial agents and undertreatment of those with other illnesses (Taylor & Mtambu, 1986). This places the population at undue risk of side-effects with no resulting benefit from the treatment and wastes drug supplies.

In view of this I have taken the recently developed Immunochromatographic tests i.e Paramax-3 (P.f/P.v/Pan malaria test) and the SD-Bioline(P.f/P.v malaria test) that was launched into the market and used in urban slums of Vijayawada. The RDTs results are compared with traditional blood film examination. I was able to carry out the test without any difficulty and achieved excellent results.Blood film examination still remains the gold standard in the diagnosis of malaria (Crooke et. al., 1999). Blood obtained by a finger or ear lobe is ideal because the density of parasites is greater in blood from this capillary rich area. The thick and thin smears can be utilized for diagnosis alone (or) in combination by employing malaria stains available (Giemsa, Leishman's Wright's, Field's stain, JSB stain). Peripheral smear is the standard, cost effective diagnostic technique for detection and differential of *Plasmodium* species.

It has several limitations like time consuming, labour intensive and requires the service of skilled technician. Further diagnosis of malaria can be missed if the parasite count is less than 60/µL of blood. This could be the possible reason for the failure to detect malaria in two samples which were smear negative and Paramax-3 positive in the present Study (Table-5). More over as P.*falciparum* may sequester in the deep capillaries the infection may easily be missed because there are insufficient number of parasites for detection in blood films (Moody, 2002).

In the present study out of 200 samples, the blood film results indicated that 31% (62 of 200) of the patients were infected with malaria based on the morphologies of the parasitic stages. Among them, P. *vivax* was present in 50(25%) samples while P.*falciparum* was present in 12(6%). [The one mixed infection has been tabulated with the P.*falciparum* infection numbers for ease of analysis].

The prevalence rate of P. *falciparum*, in this study found to be 6%, while the overall malaria prevalence was 31%. This prevalence rate was found to be lower than the earlier studies. Since the subjects were selected randomly from clinically suspected patients, it is possible that this could have influenced the prevalence rate of malaria infection in this study. The malaria prevalence rate in this study is however in consonance with previous studies.

The percentage of positivity by peripheral smear compared with other studies.

Study Series	Percentage (%)
Shiff et al., (1993)	50.80%
Kodsingh et al., (1997)	46.90%
Tarimo et al.,(1999)	52.00%
Pawan et al., (2000)	26%
P.U.Agomo et al., (2003)	35.83%
Das et al., (2003)	18%
Jeremaih et al., (2005)	40.98%
Zaccheaus AJ et al., (2007)	27.5%
Present Study	**31.00%**

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Malaria effects all ages. In the present study, it is commonly seen in the age group 20-30 years with mean age of 25 and SD of 12.82.

Mean and Standard Deviation

Study Series	Mean	S.D (Years)
Rickman et al.,(1989)	30.6	15.9
Kodsingh et al.,(1997)	26	15.8
Mills et al., (1999)	39	-
Present Study	**25**	**12.82**

Males are more frequently exposed to the risk of acquiring malaria than females. Present study showed male predominance with ratio 2.1:1

Study Series	Ratio
Ugen et al., (1995)	1.3:1
Kodsinghe et al., (1997)	2:1
Mishra et al., (1999)	3:1
Singh et al.,(2001)	1.9:1
Present Study	**2.1:1**

Paramax-3 Kit

In the current scenario, Paramax-3 test is more accurate diagnostic technique as it is based on presence or absence of antigen of the parasite, HRP-2 & pLDH. In areas where microscopy is not readily accessible and it can take 4-6 weeks before slide results are available (Singh et al, 1996) which causes delay in the diagnosis and treatment of cases contribute to the continuing transmission. Whereas this RDT method requires a small amount of (5-6oμl) blood for the test to be performed, the results are obtained within 3-5 minutes and interpretation is easy depending on the presence or absence of a line on the test strip.

Further its need is increased in case of cerebral malaria, intravascular haemolysis where immediate and reliable diagnosis is very important. It can also be used for post treatment evaluation of P.*vivax* cases as pLDH antigen becomes negative immediately after effective treatment because antigen can be detected only in live parasites. The disadvantages include cost factor (the cost of the ICT malaria P.f/P.v (US$ 1.2 per test) is too high (Tjitra et al, 1999), and the test remains positive even after one week of Antimalarial treatment. This persistence can be due to persistent viable asexual stage of parasite below the detection limit of microscopy and delayed clearance of circulating antigen.

Sensitivity and Specificity of pLDH compared with other studies

pLDH	Sensitivity	Specificity
Robert Piper et al., (1994)	100%	100%
Carol J Palmer et al.,(1998)	94%	100%
Makler MT et al., (1998)	95%	100%
Anthony Moody et al., (2002)	100%	100%
Huong N M et al., (2002)	100%	100%
Moody,Anthonyet al., (2002)	96%	100%
Present Study	**88%**	**100%**

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The sensitivity and specificity of HRP-2 antigen

Study Series	Sensitivity	Specificity
Premji et al., (1994)	89%	84%
Dietz et al., (1995)	89%	97%
Carballic and Acheet al., (1996)	86%	99%
Humar et al., (1997)	88%	77%
Singh et al (1997)	93%	92%
Anthony Moody et al.,(2002)	100%	100%
Huong NM et al.,(2002)	95%	97.2%
Present Study	**83.3%**	**98.9%**

SD- Bioline Kit

The SD Bioline malaria Pf/Pv test is an immunochromatographic test for the qualitative detection of the antibodies of all isotypes (IgG, IgM and IgA) specific to P.*falciparum* and P.*vivax* simultaneously in human serum, plasma or whole blood.

SD-BIOLINE was not able to detect some positive cases which Paramax-3 and microscopy could detect. However one interesting aspect of SD-Bioline is that the manufacturers used two surface proteins, namely, merozoite surface protein (MSP-1) and circumsporozoite protein (CSP) which has previously been documented to induce potent antibodies. They have been put forward as strong candidates for malaria vaccine development.

Several studies have been done on some of the rapid strips like Optimal (Jelinek et al., 1999; Cooke et al., 1999), SD-Bioline (Cavanagh et al., 1998), ICT (Singh et al., 2000) and others. Irrespective of the type of rapid malaria screening strip used, it was found that the only measurement for evaluating the diagnostic value of the strip to determine the sensitivity and specificity using the gold standard as a basis of comparison (Tarazon et al., 2004; WHO, 1995). The sensitivity of most of the reagent srips were found to reduce with decrease in parasite density, thus suggesting that they are most useful in areas where malarial endemicity prevails (Tarazon et al., 2004).

In this study, the SD-Bioline P.f/P.v RDT sensitivity was found to be low (58.33%) while the specificity was high (100%) for the diagnosis of P.*falciparum*.

This finding is similar to the observation of Agomo P.V et al.,(2003) in whose report, the SD-Bioline's sensitivty was reported to be 54.84%. The PPV and NPV of 58.0% and 68.0% respectevely, are however at variance with the PPV (100%), NPV (97.4%) which is obtained in this study. The specificity value of 42.9% in his report is also at varience with the specificity of 100% obtained in this study.

My study shows more or less similar results to that of Zaccheaus Awortu Teremiah et al., (2007) in Port Hercourt, who has reported a sensitivity of 47%, specificitity of 100%.a positive predictive value of 100% and a negative predictive value of 83.2% for P.*falciparum*

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CONCLUSION

The major advantages of peripheral smear study are , it is least expensive, species differentiation is clear and quantisation of parasitaemia is possible. Through the technique of staining is simple, it is time consuming, labour intensive and requires the service of a skilled technician.

Paramax-3 test meets many of the criteria for an ideal diagnostic test, it is simple, rapid, sensitive, specific easy to perform and does not require any equipment.

This test can be used as an epidemiological tool because, in areas where both P.*vivax* and P.*falciparum* are prevalent, it can be used to identify the *Plasmodium* species infecting the patients in epidemics quickly and it allows public health workers to deliver the appropriate chemotherapy rather than just give chloroquine to each and everyone with malarial symptoms. This is particularly important where the preferred therapies for the two infections are different.

Further implementation of this test will result in time saving and reduce the use of expensive drugs unnecessarily by the clinically suspected malaria patients. This test can be used in urgent or epidemic situations for spot diagnosis and treatment of both P.*vivax* and P.*falciparum* infection by nonmedical staff. Hence this test method could help to attain the goals of the Roll Back malaria initiative.

The SD-Bioline RDT has an advantage of differenting P.*falciparum* from P.*vivax* malaria. Despite the few limitations(low sensitivity), the test can be used as an epidemiological tool in areas where P.*vivax* and P.*falciparum* are prevalent. It can also be used to identify the *plasmodium* species infecting the patients in the slums rapidly by nonmedical staff. The cost of the SD-Bioline P.f/P.v (Rs.33/- per test) at the time of study was quite affordable and would favour its widespread use in malaria endemic areas of developing countries where most of the patients need a fever screen.

Finally I want to conclude that the peripheral smear is gold standard, Paramax-3(antigen detection) kit is more sensitive and specific, and SD-Bioline (antibody detection) kit is specific and cost effective.

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