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SELECTING THE BETTER DIAGNOSTICS FOR MALARIA AT POINT OF CARE AREAS

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ABSTRACT

Background- Malaria is a fatal life threatening parasitic infection with leading cause of morbidity and mortality. Rapid accurate diagnosis is the fundamental for treatment and control of malaria. Immuno chromatographic tests are the only diagnostics that meet the demand. Despite the introduction of rapid devices over a decade ago, no promising studies to evaluate the better diagnostics for malaria. **Aim-** The present study was aimed to evaluate simple, inexpensive, accurate, reliable, easily available better diagnostics for rapid detection of malarial at point of care (POC) areas. **Settings and Design-** The sample collection process and the clinical study were carried out at Department of Research MNR medical college and Hospital Sangareddy. Malarial diagnosis was carried out at Genomix Molecular Diagnostics Pvt. Ltd, Hyderabad. **Methods and Material-** The collected samples were tested with rapid kits and results were confirmed by microscopy and PCR assays. **Results-** The study includes 1403 samples collected from the patients, of which 1227 were clinically suspected cases and 176 from consecutive feverish patients. Over all the microscopy and the malaria antigen ICT exhibit more or less similar sensitivity and specificicty i.e both the assays had 99.70 % sensitivity, 100% specificity, 100 % PPV, 99.88% NPV and 99.91% efficiency respectively. **Conclusion-** Taking all these drawbacks in to consideration rapid test kits are considered to be the best for diagnosis at field level. Hence, we have developed Malaria *pf/pv* antigen rapid kit for field deployable diagnosis which is simple, rapid, accurate, reliable, inexpensive, user friendly, POC, highly sensitive species specific effective diagnostic test compared to other type of diagnostics. Of the two rapid kits developed Malaria *pf/pv* antigen kit is found be more accurate and sensitive then to Malaria *pf/pv* antibody rapid kits.

KEY WORDS: Point of Care (POC), Immuno chromatographic test (ICT), *Plasmodium Lactate Dehydrogenase* (pLDH), Histidine Rich Protein II (HRPII), Polymerase chain reaction (PCR), Positive Predictive Value (PPV), Negative Predictive Value (NPV).

1. INTRODUCTION

Point of care diagnostics (POC) are increasing day by day, due to its cost effectiveness and easy to use in resource limited areas. There is an emergent demand for POC diagnostics in developing countries.[1] The number of deaths occurring in developing countries annually is due to the improper diagnosis despite progress in medical science. Even though development of effective chemotherapeutics has progressed, infectious diseases still continue to affect millions of lives around the world.[2] Malaria is one of the most important tropical infectious disease that causes approximately 350-500 million clinical episodes and more than 1 million deaths occur annually.[3] Malaria is a major cause of morbidity and mortality leading to a number of deaths in 2011.[4] The disease is endemic around the equator keeping approximately 50 % of world's population is at risk.[4] Hence diagnosis is important for the prescribing of effective drugs and treatment.[5] The range of areas inhabited by mosquitoes harbouring malarial parasite is currently expanding due to global climate change.[6] So, proper and accurate diagnosis for malaria is extremely important.[7] The existing tools for the diagnosis of malaria include microscopy, parasite Ag and Ab detection rapid test kits and molecular tools.[8]

More than 100 years, the gold standard method for the diagnosis of malaria is microscopic examination of thick and thin[9] Giemsa, wright or Field stained blood films.[10] However, the microscopy has some limitations

such as time consuming, misdiagnosis of the mixed species infection is common if the microscopist lacks experience and even when the parasitemia is low.[11] The poor sensitivity of smear tests often causes fatal delay in treatment.[11,12] The labor intensive nature of microscopy and the requirement for high levels of operator training, has led to the development of several alternative techniques for laboratory diagnosis of malaria in both endemic and non-endemic areas.[13] Several molecular assays such as PCR assays have also been developed for the detection and identification of malarial parasites based on genus and species specific sequences of the parasites.[12,13] PCR assay is more specific and sensitive than to microscopy[10,14,15], and few as five parasites per microliter of blood can be detected.[16] Regrettably, the PCR assays are beyond the capacity of most malaria endemic areas as they need very expensive, sophisticated laboratory and well trained personals.[10]

So taking all these resources limited settings and point of care areas in to consideration the lateral flow assay (Ab and Ag rapids kits) is the best diagnostic method that directly used at field level or at point of care areas.[10,14,16,17] These lateral flow methods do not offer improved sensitivity over microscopy when parasitemia fall below 100 parasites per µl.[18] The drawbacks of this format include its susceptibility to degradation in suboptimal storage conditions, limited shelf life, an apparent failure to detect some parasites is due to mutations within target antigen[19], and the possibility of false positive results due to persistence of the antigen.[13,14,20,21,22] Though there might be a problem in the stability, sensitivity and specificity of the lateral flow assays, most of the diagnostic approaches are attracted by lateral flow test kits only. The lateral flow kits have a greater potential approach because of its simple, easy and inexpensive point of care handling procedures and infrastructure free settings than the other existing diagnostic methodologies. Due to this reason the lateral flow test kits play a major role in the diagnosis and have become the basis for the most of the commercial diagnostic approaches.[23,24,10] The present study is taken up to develop Malaria *pf/pv* antigen and Malaria *pf/pv* antibody rapid kits and diagnosis of the total 1403 samples using PCR, Malaria *pf/pv* antigen and Malaria *pf/pv* antibody rapid kits and microscopy and the POC diagnosis and field deployable. In addition to study the Malaria *pf/pv* antigen and Malaria *pf/pv* antibody rapid kits sensitivity and specificity than to gold standard microscopy and PCR results.

2. MATERIAL AND METHODS

2.1 Clinical Study Site: The complete clinical study and the collection of the samples was performed at Department of Research, MNR Medical College and the development, validation and diagnostic studies was carried out at Genomix Molecular Diagnostics Pvt.Ltd, Hyderabad, India. The study group include 1227 clinically suspected samples.

2.2 Collection of Samples: The whole blood of the each individual is collected in a vaccutainer that is pre-treated with trace amount of EDTA. The collected samples are examined by microscopy followed by PCR analysis and the remaining sample was plotted on a filter paper and stored at -20^{0}C for further analysis.[10,11]

2.3 Microscopy: Microscopy is the gold standard method used for the specific detection of malaria parasites using a thick and thin blood films stained using Giemsa stain method.[25] The thick smear helps in the identification of malarial parasites to the genus level whereas the thin smear will help in species level identification of the malarial parasites.[1,10,14]

2.4 Lateral flow immune chromatographic Test Kits

Immuno chromatography or lateral flow diagnostic test kits works on the principle of chromatography of antigen and antibodies. The facilities at Genomix Molecular Diagnostics Pvt Ltd, Hyderabad have been used for manufacturing of Malarial *Pf/Pv* Ag and *Pf/Pv* Ab rapid diagnostic test kits and collected blood specimens were diagnosed by using antigen and antibody lateral flow diagnostic test kits. The diagnosis was based on the HRP II (histidine rich protein II) specific antigenic protein for *Plasmodium falciparum* (*pf*) and pLDH (*Plasmodium* lactate dehydrogenase) antigenic protein specific for *Plasmodium vivax* (*pv*) of the malarial species.

2.5 Materials used for Lateral flow kits Preparation

The materials used for development of lateral flow test kits includes the following components, sample pad, nitrocellulose membrane, absorbent pad, supporting matrix and conjugate matrix procured commercially (MDI membranes, Ambala, India). The lateral flow components such as monoclonal and polyclonal antibodies specific for *Plasmodium vivax, Plasmodium falciparum* malarial parasites were developed, purified and characterized for making the malaria antigen lateral flow test kits. Whereas the purified KLH (Keyhole limpet hemocyanin) conjugated peptide antigenic proteins were procured commercially (Genemed Synthesis Inc, USA) that are specific for *Plasmodium falciparum* and *Plasmodium vivax* used to make the malaria antibody lateral flow test kits.

2.6 Lateral flow kit preparation Process

2.6.1 Development of Malaria antigen lateral flow kit: The malarial *Pf/Pv* Antigen lateral flow test kits were designed with the polyclonal antibodies specific for *Plasmodium falciparum* and *Plasmodium vivax*. These polyclonal antibodies were coated on the test line positions (T1 and T2) of the nitrocellulose membrane along with biotin at the control position using the Bio Dot Quanti-2000 Biojet antibody coating machine. The gold nano particles were conjugated with disease specific monoclonal antibodies along with streptavidin and were dispensed on to a conjugate matrix and air dried at dehumidified room. The in-house sample diluent buffer was used while testing the kits for lysis the RBC. The

diluent contains 0.2% Tris, 2% casein, 1% triton-X and the pH should be maintained in between 8.0 to 9.0.

2.6.2 Development of Malaria antibody lateral flow kit: The malaria species specific antibody lateral flow test kits were developed with the recombinant antigenic protein (*Pf/Pv*) that were specific for *Plasmodium falciparum* and *Plasmodium vivax* which were coated on the test line (T1 and T2) positions along with biotin and the control line position of the nitrocellulose membrane. *Pf/Pv* specific recombinant proteins were gold conjugated along with streptavidin and were dispensed on the conjugate matrix and were dried in a dehumidified room. The in-house sample diluent buffer was used while testing the kits for lysis the RBC. The diluent contains 0.2% Tris, 2% casein, 1% triton-X and the pH should be maintained in between 8.0 to 9.0.

2.7 Lateral flow Assay Procedure

As per the protocol developed the collected samples were analysed using these lateral flow test kits. A finger prick whole blood or 5µl of collected whole blood specimen on the filter paper is directly added to the sample well using the sample holder dropper and followed by addition of two drops of sample diluent (fig 1). After the addition of sample diluent the sample along with diluent tends to move towards the absorption pad by capillary action on nitrocellulose membrane.

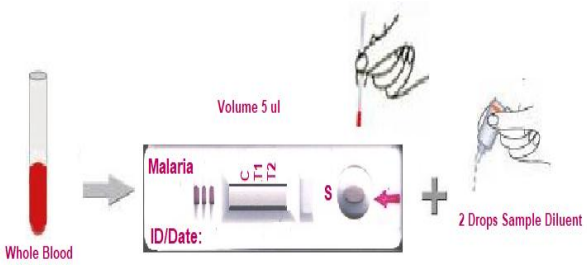

Fig. 1: Diagrammatic representation of test process of Malaria Lateral flow assay.

2.8 Analysis of the Lateral flow Assay: The lateral flow test kit results were analysed by observing the clear purple colored bands that are visible to our naked eye on the results window of the test cassette. If the colored bands appear at control and Test (T1) position it means that the sample is positive for *P. vivax*, whereas the colored lines at the control and Test (T1 and T2) positions indicate that the samples is positive for both the *P. falciparum* and *P. vivax*. If the lines appeared at control and test (T2) position then the sample is positive for *P. falciparum* only. The colored band at control line alone denotes that that sample is negative for malarial parasites as shown in fig 2. If in the absence of control, colored lines formed at T1 and T2 or there is no line at results window then the assay was invalid and it was recommended to repeat the assay with new kit.

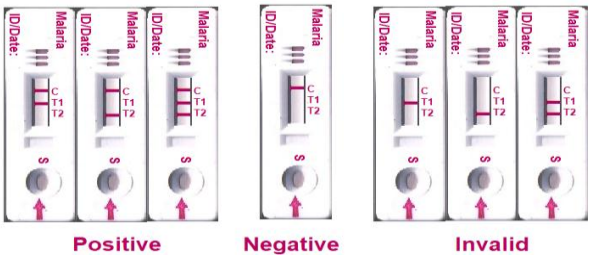

Fig. 2: Interpretation of assay results of Lateral flow test kits.

2.9 Statistical Analysis and Data Management

The collected whole blood samples were tested with both the antigen (Ag) and antibody (Ab) lateral flow test kits. Calculation of specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) was carried by using the statistical analysis software SPSS20.0 (Joseph and Fleis, 2003). The statistical analysis for these samples was performed using PRISM (Graph Pad Software, San Diego, CA).

3. RESULTS

Out of 1403 samples collected from the patients, only 1227 are clinically suspected cases and 176 samples were collected from the consecutive feverish patients. Among the suspected cases only 338 samples were conformed positive and 889 samples were negative for *Plasmodium species* by PCR. All the 889 samples showed negative result for *plasmodium species* by microscopy, and malarial *pf/pv* Ag rapid kits but only 867 samples were conformed negative to malarial *pf/pv* Ab rapid kits. Of the 338 PCR positive samples, 337 samples were conformed positive by microscopy and malarial *pf/pv* Ag rapid kits, but only 284 samples were conformed positive using malarial *pf/pv* Ab rapid kits. The mean standard deviation for all 1227 samples was 204 ± 71.96, the mean SD of the total 889 clinically proved malaria negative samples were 148.1 ± 50.26 and the mean SD of 338 clinically suspected malaria positive cases were 56.33 ± 22.85. The Confidential interval (95% CI) value for all these clinically (1227 samples) suspected samples was 128.98 – 280.02, for malaria positive samples the CI value is 32.35 – 80.32 (338 samples) and for negative samples the CI value was 95.41 – 200.92 (889). The Standard Error of Mean (SEM) value for all these suspected samples was 29.38 (1227 samples) and for clinically suspected positive samples the SEM value was 9.33 (338 samples) and for negative samples the SEM value was 20.52 (889 samples) respectively. The *P*-value for suspected positive samples is 0.0007 (338 samples) and the *P*-value for negative cases is 0.1470 (889 samples) as shown in the Table 1.

3.1 Age Distribution: Among all 1227 clinically suspected cases of malaria 197 (16.05%), 285 (23.2%), 255 (20.78%), 226 (18.41%), 186 (115.15%), 78 (6.35%) patients samples were collected from 10 to 20 years, 21 - 30 years, 31-40 years, 41-50 years, 51-60 years and above 61 years as shown in the Table 1.

Table. 1: Age wise distribution among clinically suspected malarial positive and negative cases.

Age (in Years)	Clinically Suspected cases (%) (95% CI)	Malaria Positive (%) (95% CI)	Malaria Negative (%) (95% CI)
≤ 20	197 (16.05%)	44 (13.01%)	153 (17.21%)
21 – 30	285 (23.2%)	88 (26.03%)	197 (22.15%)
31-40	255(20.78%)	72 (21.30%)	183 (20.58%)
41-50	226(18.41%)	60 (17.75%)	166 (18.67%)
51-60	186(15.15%)	52 (15.38%)	134 (15.07%)
> 60	78(6.35%)	22 (6.50%)	56 (6.29%)
Total	1227	338	889
Mean ± SD	204 ± 71.96	56.33 ± 22.85	148.1 ± 50.26
CI (95% CI)	128.98 - 280.02	32.35 - 80.32	95.41 - 200.92
SEM	29.38	9.33	20.52
P-Value		0.0007	0.1470

This table explains the age wise distribution of clinically suspected cases with mean of standard deviation, confidential interval (95% CI), Slandered Error of Mean along with the P- value. The P value is calculated in terms of using the total samples statistically significant with malaria positive and negative cases.

3.2 Gender distribution: In the present work of the total 1227 suspected cases of malaria 640 (52%) are males and 587 (48%) are females (Figure 3). All the male and female patients who are clinically suspected of malaria had the symptoms such as fever, chillness and majority of them had splenomegaly, pallor and convulsions.

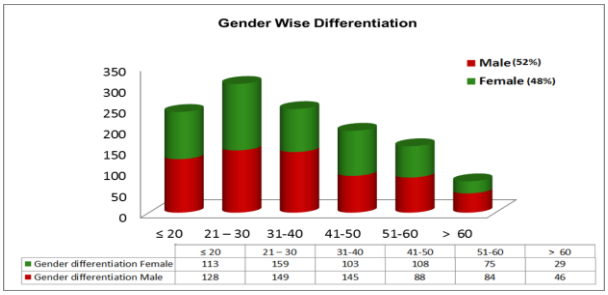

Fig. 3: Gender wise distribution of clinically suspected cases of malaria.

3.3 Area distribution: Among the 1227 suspected cases of malaria that are distributed and calculated in terms of socio economic status of the malaria disease 732 (60%) patients are from the rural areas and 495 (40%) patients belong to urban area (Fig 4).

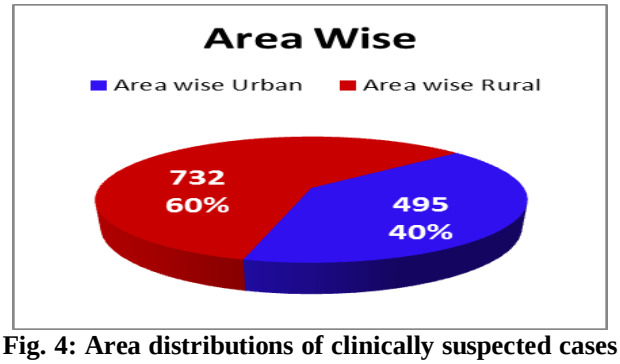

Fig. 4: Area distributions of clinically suspected cases of malaria under socio economic status.

3.4 Occupational distribution

All these clinically suspected cases were distributed based on the type of occupation. 386 patients were daily labour, 327 patients were business people, 106 patients were employees, 273 patients were farmers and the people without work are 135 (Fig 5).

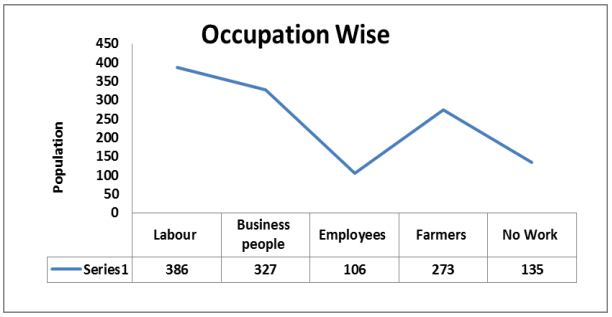

Fig. 5: Occupational wise distributions of clinically suspected cases of malaria.

3.5 Family size distribution: The distribution in terms of family size is, 117 (10%) of patients were < 3 of family size, 365 (30%) patients were with the family size of 3-4 people, 497 (40%) patients with the family size is 4-5 people and the family size is ≥ 6 the number of specimens collected was 248 (20%) (Fig 6).

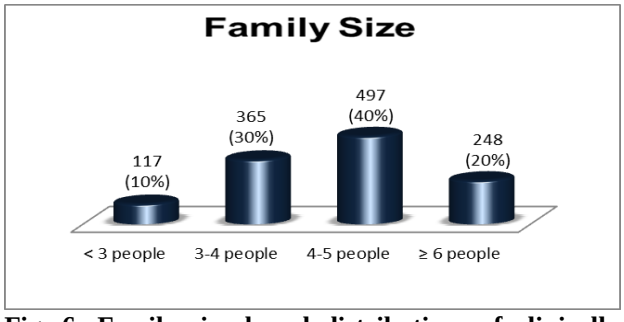

Fig. 6: Family size based distributions of clinically suspected cases of malaria.

3.6 Effect of Literacy on Disease Distribution

The effect of education weather the people are literate or illiterate. 432 (35%) no of patients were illiterate and about 795 (65%) number of patients are literate (Fig 7).

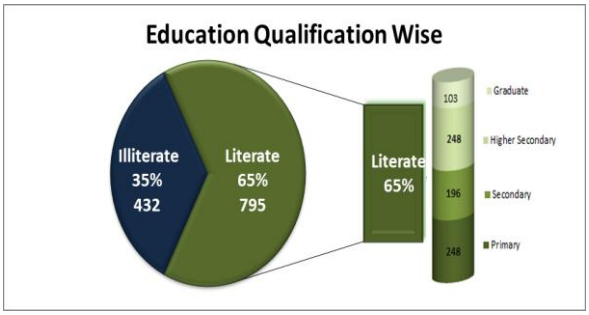

Fig. 7: The effect of education on malaria distribution in clinically suspected cases.

3.7 Effect of living status on disease distribution

Based on the type of the living conditions of the individual's weather the people were staying in council house or individual houses or huts and in apartments (Fig 8). 193 (16%) were habitats of council houses, 357 (29%) were living in individual houses, 156 (13%) were living in huts and 521 (42%) are living in apartments.

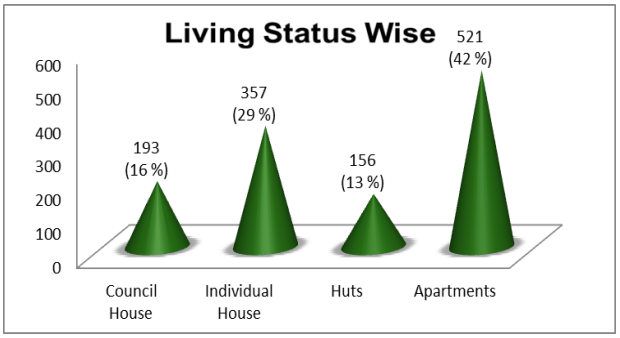

Fig. 8: Effect of living status on clinically suspected cases of malaria.

3.8 Source of water on disease distribution

Based on the source of water consumption for house hold purposes, 440 (36%) patients were source of water is from inside the house premises whereas around 787 (64%) patients source of water is form outside the house premises as described in the Fig 9.

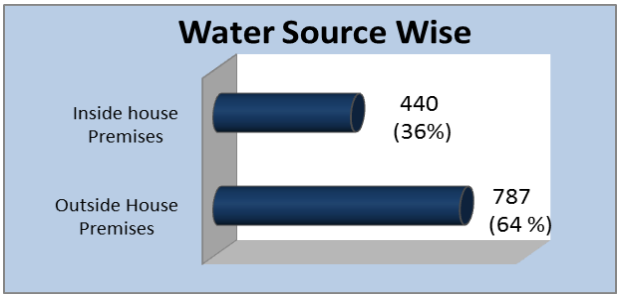

Fig. 9: Water source wise distributions of disease in clinically suspected cases of malaria.

3.9 Effect of prevention & control measures on disease distribution: Based on the prevention of the disease and its control measures from mosquito bite all the 1227 cases were distributed on the type of the preventive methods for controlling the disease (Fig 10).

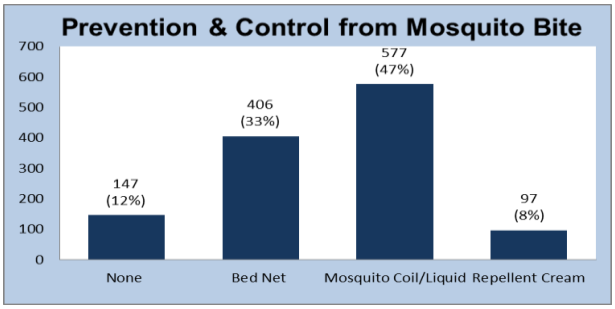

Fig. 10: Prevention and Control from Mosquito Bite.

3.10 Evaluation results of diagnostic assays

All the suspected cases were diagnosed using four different methods such as microscopy, PCR assay, Ag lateral flow assay and Ab lateral flow assay. Among all the 1227 clinically suspected cases of malaria 135 cases were positive for *P. falciparum*, 97 cases were positive for *P. vivax* and 106 number of cases were with mixed infection i.e positive for both *P. falciparum* and *P. vivax* and 889 samples were clearly true negatives for malarial parasites. The evaluation of these diagnostic assays reveals the false positive and false negative results affects the sensitivity and specificity and also the treatment. The microscopy and malaria Ag lateral flow assay showed one false negative result and PCR assay showed 100% negative and 100% positive results. But malaria Ab rapid kits recorded 54 false positive and 22 false negative results (Table 2).

Table. 2: Evaluation of the test results of diagnostic assays used for malarial diagnosis.

S.No	Name of the Detection	Test type	Specimen Volume required	Specimen Type Serum/whole blood	Total no. of samples (n=1227)		TP	TN	FP	FN
					Total Positive	Total Negative				
1.	Microscopy	Microscopy	100 µl	Whole Blood	TP- 338	TN- 889	337	889	-	01
2.	PCR based	Molecular Assay	1 µl	DNA	TP- 338	TN- 889	338	889	-	-
3.	Ag based	Lateral flow	5 µl	Wb/Plasma	TP- 338	TN- 889	337	889	-	01
4.	Ab based	Lateral flow	5 µl	Wb/Plasma	TP- 338	TN- 889	284	867	54	22

**Note: TP: True positive (Reactive), TN- True Negative (Non-Reactive), WB-Whole Blood, FP- False positive, FN- False Negative, Wb- Whole blood, Ag based- Malaria Pf/Pv Antigen Based Detection Assay, Ab Based- Malaria Pf/Pv Antibody based Detection Assay.*

3.11 Statistical analysis: The statistical analysis of the evaluated test kit results were tabulated in terms of the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and efficiency. Over all the microscopy and the malaria antigen based lateral flow assay were more or less similar in accuracy. Whereas the PCR assay had 100% sensitivity, specificity, PPV, NPV and efficiency respectively. But the evolutionary data for malaria antibody lateral flow assay was differing with the standards as shown in the Table 3.

Table. 3: Comparison of parameters between diagnostic assays.

S. No.	Assay	Sensitivity %	Specificity %	PPV	NPV	Efficiency
1	Microscopy	99.70%	100%	100%	99.88%	99.91%
2.	PCR assay	100%	100%	100%	100%	100%
2	Ag based	99.70%	100%	100%	99.88%	99.91%
3	Ab based	92.81%	94.13%	84.02%	97.52%	93.80%

3.12 Detection of consecutive feverish patients

Consecutive feverish patients other than malaria were collected MNR Medical College and Hospital, Sangareddy. 176 samples were collected with different age groups, of which 10 samples showed positive with microscopy, PCR assay and malaria *Pf/Pv* Ag test kits and the remaining 166 samples were negative for malarial parasites. But 36 of 176 samples showed positive for malaria with malaria *Pf/Pv* Ab test kits and of the 36 positive samples, 10 samples were conformed positive by Microscopy, PCR and Ag based lateral flow tests and the remaining 26 samples were reported false positive with malaria Ab kits and also the same 26 samples showed negative result with microscopy, PCR assay and Ag based lateral flow tests. The fever of the patients may be due to other diseases. All these clinical information was clearly tabulated as shown in the Table 4.

Table. 4: Results of Consecutive feverish patients other than malaria.

S. No.	Name of the Detection	Total no. of feverish samples (n=176)		TP	TN	FP	FN
		Total Positive	Total Negative				
1.	Microscopy	TP- 10	TN- 166	10	166	-	-
2.	PCR Method	TP- 10	TN- 166	10	166	-	-
3.	Ag based LFT	TP- 10	TN- 166	10	166	-	-
4.	Ab based LFT	TP- 10	TN- 166	10	140	26	-

4. DISCUSSION

The re-emergence of parasitic malaria has renewed interest not only in the preventive measures of the disease, but also in developing a better POC, rapid diagnostic test kit for the diagnosis.[11] At present a number of detecting and diagnosing methods has replaced the conventional microscopy. The impact of malaria is increasing day to day especially in the tropical endemic areas and so there is a huge demand for rapid diagnostics. Hence, WHO announced the need for developing a rapid, simple, easy to use, highly sensitive, and accurate, in expensive POC diagnostic test to determine the existence of parasitic disease in human healthcare.[27] According to the scientific research and experts reviews reveals that rapid kit can only meet the present need for diagnosis in the market. The use of rapid kit is very simple, easy to perform, reliable and helps in improving the diagnosis rate in rural endemic areas.[20,28] The rapid test kits diagnosis in non-endemic regions is becoming more feasible, which may reduce time-to-treatment for cases of imported malaria.[29] Currently 100 types of malaria rapid diagnostic test kits are available from different manufacturers all over the world (http://www.wpro.who.int/sites/rdt). Most of these kits are antibody based are specific to *P. falciparum*, hence can't distinguish the differences between the parasitic species of the malaria individually. The sensitivity and specificity of antigen based rapid test for diagnosis of malaria has been reported to be an excellent kit compared to the other type of tests.[11,30,31] The present study explains the draw backs of improper diagnostic kits causing misdiagnosis for malaria.[20,32] Mainly the disease diagnosis should focus on which diagnostic method will give proper and accurate diagnosis results. Because of the presence of the species specific antigenic protein in blood of the effected people the use of antigen based kits will execute proper diagnosis. The statistical analysis of the evaluated test kit results were tabulated in terms of sensitivity, specificity, PPV (Positive Predictive Value), NPV (Negative Predictive Value) and efficiency. Over all the microscopy and the malaria antigen based lateral flow assay exhibit similar sensitivity i.e both the assays had 99.70 % sensitivity, 100% specificity, 100 % PPV, 99.88% NPV and 99.91% efficiency respectively. Whereas the PCR assay had 100% sensitivity, 100% specificity, 100% PPV, 100% NPV and 100% efficiency.

But the evolutionary data for malaria antibody lateral flow assay has 92.81% sensitivity, 94.13% specificity, 84.02% PPV, 97.52% NPV and 93.80% efficiency as shown in the Table 3. The results obtained in the present study were in agreement with the study carried out by Bell et al., 2005, Murray et al., 2003 in terms of variation in the sensitivity and specificity with antibody kits.[33] In our study the antibody kits were compared with gold standard microscopy, PCR assay and with antigen rapid kits. The antibody test kits did not show promising results and variation in the sensitivity and specificity and false positive and false negative results were reported. The major problem with antibody based kits was the cross reactivity. The study of cross reactivity with malaria antibody rapid kits was explained with rheumatoid factor in blood that generates a false positive test line at early stages of the diagnosis.[34,35] The cross reactivity that occurs is may be due to heterophile antibodies.[14,36,37] Chansuda et al., 2008, Laferl et al., 1997, and Grobusch et al., 1999, reported cross reactive results with antibody kits that the false positive results occur in a limited number of antibodies rapid test kits of malaria.[34,35,38] But the malarial *pf/pf* Ag rapid diagnostic test kit showed promising results to that of microscopy and PCR assay which were similar to the results reported by Prudhvi et al., 2016, Beadle et al., 1994; Bharti et al., 2008.[11,17,22] The present study proved that antigen rapid test kits for malaria had promising results with high levels of specificity and sensitivity that were compared with microscopy and PCR assays which are considered more sensitive and gold standard methods for malarial diagnosis. Therefore the present study results proved that the malaria *pf/pv* antigen rapid detection test kit is an effective and sensitive tool and is the better diagnostic tool for detecting the malarial parasites especially in resource limited point of care areas.

5. CONCLUSION

The present work reveals that rapid diagnostic kits are a very simple, inexpensive, user-friendly, point of care and effective diagnostic assays that can be executed at the bedside for detection of malaria. The sensitivity, specificity, PPV, NPV, and efficiency of Ag based malaria rapid kits were similar to that of conventional microscopy and do not require highly skilled personnel to perform or interpret the results. Early diagnosis and treatment are imperative in preventing the complications. Microscopy is the gold standard for malaria parasite detection but laborious and requires experts to interpret the results. Rapid immunochromatographic antigen based test that detects specific antigens produced by malaria parasite in the blood can be performed at bedside in 10-15 minutes.

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Ethical Clearance: The study was approved by the Organizational Human Ethical Committee.

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Conflict of Interest: The authors declare that there is no conflict of interest related to this study.

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