Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.7, No.4, 2017

In-vitro activity of artemether, lumefantrine, dihydroartemisinin and piperaquine against *Plasmodium falciparum* clinical isolates transported in a formulated transport medium and EDTA from Chulaimbo sub-county Hospital

Scolastica C. Korir[1], Rose Kakai[1], Hosea M. Akala[2], David Sang[3], Ayub Ofulla[3]
[1]Maseno University, Department of Medical Microbiology, Maseno
[2]Kenya Medical Research Institute, USAMRU- Malaria Drug Research Section, Kisumu
[3]Maseno University, Department of Biomedical Science & Technology, Maseno

ABSTRACT
Introduction: Development of resistance to antimalarials has emerged as one of the greatest challenges facing malaria control today. *In vitro* drug sensitivity relies on the growth of plasmodium in the presence of the antimalarials. Any delay beyond 24 hours in performing the assay results in decreased parasite viability and subsequent IC_{50} values. Fresh isolates brought in for assay from collection sites situated far from the laboratory require a medium to keep the parasites viable in order to produce results which more accurately reflect intrinsic antimalarial drug resistance. **Objective:** To evaluate the use of a formulated transport medium (TM) in maintaining the viability of plasmodium during storage at 4^0C against a panel of antimalarials, compared to the conventional EDTA anticoagulant. **Methods:** This was a cross-sectional study. Blood samples positive for *Plasmodium falciparum* by light microscopy were transported to the laboratory using TM and the conventional EDTA anticoagulant as control. Using 322 *Plasmodium falciparum* isolates from patients attending outpatient clinic at Chulaimbo sub county hospital, SYBR Green 1 *in vitro* assay IC_{50} was done against artemether, lumefantrine, dihydroartemisinin and piperaquine. **Results:** The mean IC_{50} values for drugs were not significantly different in either EDTA or TM samples. Similarly, there was no significant correlation between samples which were transported in EDTA except for the Artemisinin derivative DHA which had a significant correlation with ART (r = 0.123, p=0.03), LUM (r = 0.1382, p=0.01), and PPQ (r = 0.1281, p=0.02) and also in TM between DHA and LUM (r=0.1229, p=0.03). **Conclusion:** Similarity obtained in median IC_{50} between samples in EDTA anticoagulant and TM warrants its continued use. However, DHA significant correlation with most of the drugs used, calls for more research to expound on this finding.

Keywords: Isolates, transport medium, EDTA, susceptibility, inhibitory concentration 50, resistance.

Introduction

In Kenya, malaria continues to cause significant morbidity and mortality, and is often a location where drug resistance emerges in Africa. The development of *P. falciparum* resistance to chloroquine diphosphate (CQ), and later sulfadoxine-pyrimethamine, are well described (Mwai *et al.,* 2009; Khan *et al.,* 1997). This warrants continued *in-vitro* drug IC_{50} monitoring of *P. falciparum* field isolates, which is becoming more practical and safer, as more reliable assays like SYBR Green I become established, combined with simpler field isolate processing such as “immediate *ex-vivo*” (IEV) (Akala *et al.,* 2011).

SYBR green 1 *in vitro* technique is the latest technique which is convenient and can be used in a large parasite density and produces rapid reproducible results (Rengarajan *et al.,* 2002). Its measure of sensitivity depends on the growth of parasites subjected to antimalarials in a medium. Lack of *in vitro* plasmodial growth has been attributed to; the effects of a markedly diminished viability of all parasite populations within the same isolate leading to death of the parasites before reaching the laboratory (Basco, 2004), very low starting parasitaemia due to poor survival rates of the parasites from the field (Bacon *et al.,* 2007), or presence of trace amounts of antimalarial drugs or intake of traditional medicine undetected by urine test (Basco, 2003). Low yield of the *in vitro* assay was also observed in Nigeria, which may have been due to suboptimal storage and transportation conditions of parasitized blood, or the presence of traces of the drug in the sample rather than limitations of the technical approach (Issaka *et al.,* 2013). To enhance the immediate survival and later growth of these parasites, there is need for a proper transition from the natural human host to the artificial medium which will subsequently be used for transportation to the laboratory.

Apart from the medium which the parasites are exposed to, parasites growth rates sometimes depend on the source of serum used. For instance the diet, medication consumed, state of health, and immune status of a donor can inadvertently affect growth rates and modify the 50% inhibitory concentration (IC_{50}) results (ofulla *et. al.,* 1994). The presence of trace amounts of antimalarial drugs or intake of traditional medicine undetected by urine test for commonly used antimalarial drugs may partially explain why some fresh isolates do not grow *in vitro* (Basco, 2003). Furthermore, serum may contain unknown quantities of drug-binding proteins that can skew IC_{50} values. For maximum plasmodial growth, it is important eliminating all these host immune components before test, so as to render maximum growth of the parasites in the antimalarials, thus, making it reliable especially for fresh clinical isolates meant for epidemiological studies (Russell *et al.,* 2003).

This study was carried out in order to shed light on the use of a transport medium with nutrients which can promote better transition and adaptation to enhance prolonged survival and viability of the plasmodium parasites once removed from the human host.

Materials and methods

Study sites: Chulaimbo sub-county hospital located in Maseno division of Kisumu County in western Kenya. The hospital is about 10 kilometers away from the laboratory at Maseno University.

Participant selection procedure: Patients attending outpatient clinic at Chulaimbo subcounty hospital, who were at least 2 years of age and having met the inclusion criteria were invited to participate. Patients with mono infection of *P. falciparum* parasitaemia between 1000 to 200,000 asexual parasites/ul of blood and gave informed consent to participate were included in the study.

Blood sample collection and preparation: Laboratory staffs at Chulaimbo subcounty hospital were trained on the inclusion and exclusion criteria for study participants'. A total of 4ml venous blood was drawn by a trained phlebotomist from patients who gave informed consent to participate in the study. For each participant, 2ml of the blood was collected into a plain vacutainer tube with 2ml of the formulated transport medium while the remaining 2ml was drawn into EDTA coated vacutainer tube. Both tubes were placed at 4^0C until transported to the laboratory at the close of the day to begin immediate *ex vivo* (IEV) drug IC_{50}testing as described below.

Laboratory analysis procedures: SYBR Green 1 based *in vitro* IC_{50} drug sensitivity was used to test each *P. falciparum* isolate transported in TM and EDTA against artemether (ART), lumefantrine (LU), dihydroartemisinin (DHA) and piperaquine (PPQ). The test drugs were obtained from Kenya Medical Research Institute (KEMRI). Briefly, the samples (in TM and EDTA) were washed twice using complete medium by centrifugation as a simple pretest procedure to remove antimalarial immune plasma, white blood cells, and residual drugs which may be present in the test blood, before subjecting it to the drugs. The IC_{50} results obtained were compared with the IC_{50} results obtained from samples collected using the EDTA coated tubes.

To prepare the test drugs, solutions at 1mg/ml were prepared in 5ml 100% dimethyl sulfoxide for ART, LU, DHA and PPQ. Further dilutions were incomplete RPMI 1640 media to the desired starting concentrations followed by two fold serial dilutions to generate 10 concentrations for IC_{50} testing (Mbaisi *et al.,* 2004). The concentrations ranged from 200 to 0.0976ng/ml for ART, LU, and DHA while PPQ was 500 to 0.2441ng/ml. The drug solutions were used immediately after preparation or stored at -80^0C for not more than 1 month before use. A successful assay result was defined as dose concentration relationship across the 10 drug dilutions for one or all drugs per field isolate. Basic and complete RPMI 1640 culture media, the later with glucose and hypoxanthine enrichment, were prepared as described by Johnson *et al.,* (2007). *P. falciparum* isolates processed IEV were placed into assay within 8 hours of phlebotomy, without culture adaptation. The samples were first washed in RPMI 1640 by centrifugation at 2500 revolutions per minute for 3 minutes for three consecutive times. Blood samples with >1% parasitemia were adjusted to 1% parasitemia at 2% hematocrit using uninfected O+ erythrocytes, and those with ≤1% parasitemia were used unadjusted at 2% hematocrit. Transfer of parasite sample and antimalarial drug aliquots in complete RPMI 1640 drug aliquots into 96-well microculture plates and addition of lysis buffer after 72hours incubation was done as previously described (Johnson *et al.,* 2007). The plates were then placed at room temperature in the dark, for 5–15 minutes. Parasite replication inhibition was quantified and the IC_{50} for each drug calculated by an equation generating a sigmoidal concentration response curve (variable slope), with log transformed drug concentrations on the X axis and relative fluorescent units (RFUs) on the Y axis (Graph pad Prism for Windows, version 5.0; Graph pad Software, Inc., San Diego, CA). (Bacon *et al.,* 2007).

Statistical analysis: Data was analysed using Mann-Whitney test to compare the medians IC_{50} values of antimalarials in EDTA and TM. Pearson correlation coefficient was used to determine the r value which gave the possible correlation of the logarithmic values of IC_{50} between the antimalarials in each EDTA and TM. Data were analysed using Graph Pad Prism 5.00 for Windows (Graph Pad Software, San Diego, CA, and Sigma Plot version 10; Systat Software, Inc., San Jose, CA).

Ethical consideration: The study was approved by Jaramogi Oginga Odinga Teaching and Referral Hospital Ethics and review committee (Ref. 01713). Written informed consent was obtained from participating adults aged at least 18 years or legal guardians for those less than 18 years.

Results

A total of 322 *P. falciparum* field isolates were assayed against four panels of drugs namely ART, LUM, DHA and PPQ. Successful assay defined as a concentration-response across the 10 drug concentrations for 1 or more drugs per each *P. falciparum* field isolate, occurred in more than 50% of all the isolates in EDTA and TM. 70% of the assays had parasitaemia >0.5%. 18% of the plates gave high relative fluorescent units (RFU) for samples in both groups while 82% of the plates gave moderate RFU of between 4000-8000 parasites or even lower. However, all the curves converged to create IC_{50} dose response curves. Those whose curves did not converge were excluded from the study. Table 1 shows that although not statistically significant, the median IC_{50} values obtained from 322 samples transported using EDTA anticoagulant were higher than those in TM for LUM (5.409 vs 4.007ng/ml, p=0.74),

DHA (2.567 vs 1.691ng/ml, *p*=0.68), and PPQ (7.887 vs 6.911ng/ml, *p*= 0.82). On the other hand, the median IC_{50} was slightly higher in TM than EDTA for ART (4.887 vs 4.275ng/ml, *p*=0.99). Notably, in both categories, the least median IC_{50} values were experienced by samples treated with DHA while the highest IC_{50} values were experienced with the samples exposed to PPQ.

Comparison of the mean IC_{50} values gave no significant different (p>0.05) results for the drugs in either EDTA or TM categories except for ART which showed a significant decrease in mean IC_{50} (23.94ng/ml vs 19.25ng/ml, p=0.006) for samples in TM than EDTA. The IC_{50} ranges for all the drugs were in the upper half of the IC_{50} range as shown in Figure 1.

Pearson's correlation coefficient analysis of the antimalarials log IC_{50} values was perfomed separately for samples which were transported in EDTA and TM (Table 2). There was no significant correlation between samples which were transported in EDTA for ART and LUM (r=0.04967, *p*=0.37), ART and PPQ (r=0.0262, *p*=0.64), and finally LUM and PPQ (0.0584, *p*=0.29), However, significantly high correlations were observed in Artemisinin derivatives ART and DHA (r = 0.123, *p*=0.03), LUM and DHA (r = 0.1382, *p*=0.01), and DHA and PPQ (r = 0.1281, *p*=0.02). Among the samples in the TM, significant correlations were observed in ART and LUM (r=0.1260, p=0.02), ART and PPQ (r=0.1382, p<0.001) and finally LUM and DHA (r=0.1229, p=0.03) while the rest showed no significant correlation with r values lower than 0.1.

Discussion

In vitro drug assays in the field are best performed on fresh isolates immediately after collection (Basco, 2004). It is simpler to perform than culture adapted assays (Akala *et al.,* 2011; Bacon *et al.,* 2007) and also reduces concerns on the selection of dominant clones and loss of minor subpopulations (Liu *et al.,* 2008) hence consequent misidentification of a population's susceptibility profile (Mbaisi *et al.,* 2004). However, blood samples containing *P. falciparum* parasites for testing may be stored at collection sites for days or transported long distances to the laboratory before cultivation (Akala *et al.,* 2011), a factor which could cause failure of growth attributed to a prolonged time between the times when samples were collected and when they were tested (Bacon *et al.,* 2007) hence reducing the parasites viability to even zero (Basco, 2004).

Availability of a medium would provide a proper transition from the natural host to an artificial medium and would keep the parasites viable to the laboratory for cultivation. In that regard, this study employed the use of a formulated transport media (TM) with an aim of trying to improve on the viability of parasites during transportation to the laboratory for further cultivation. This is the first study to report a direct comparison between two anticoagulants, evaluating the possible influence of TM on maintaining parasite viability during storage at 4°C against the conventional EDTA anticoagulant. EDTA has the advantages of a lack of inhibitory action on DNA allowing the best preservation of cellular components and morphology of blood cells (Reardon *et al.,* 1993), thus best for hematologic and biochemical investigations (Basco, 2004). Among the assays, the upper half of the IC_{50} was notably higher in our study for all the drugs in both categories, which may reflect greater biological variability among the field isolates with the presence of sub populations of drug resistant and sensitive parasites (Jefari *et al.,* 2004). This factor addressed the fact that some plates produced assays whose curves did not calculate an IC_{50} because the points could not converge to create an ideal IC_{50} dose response curve due to the presence of a subpopulation in low proportions (10%), as also evident elsewhere (CO-EM *et al.,* 2009).

Patient blood samples usually have very different levels of parasitemia, and most drug assays are sensitive to variation in parasitemia. (Liu *et al.,* 2008). Parasitaemia estimation in any *in-vitro* assay is highly dependent on microscopy therefore, variation occurs depending on the expertise of the microscopist in the laboratory. If the starting parasitaemia in an assay is mistakably high then much infected parasites would be found in each well of the plate leading to an increase in the absorption of drugs, (Ritchie *et al.,* 1996) consequently increasing the IC_{50} levels obtained (Geary *et al.,* 1990). This explains the high levels of RFU when curves were plotted against the drug concentration in the present study. The reverse side, a very low starting parasitaemia may lead to a lack of growth and less IC_{50} values (Bacon *et al.,* 2007). Increasing the number of infected erythrocytes lead to higher drug uptake (Basco, 2004) such that there is need for an increased concentration of drugs to inhibit growth when numbers of parasites per volume were inoculated (Duraisinngh *et al.,* 1999; Gluzman *et al.,* 1987). A study in Cambodia also found that parasitaemia level is inversely proportional to the IC_{50} values (Suwanna *et al.,* 2013). These previous studies justified the high IC_{50} values obtained with some plates which consequently raised the mean and median IC_{50} values especially for PPQ in the current study for samples in both EDTA and TM since both used the same amount of parasitaemia.

The DHA was the most active drug with the least median IC_{50} values (2.567ng/ml and 1.691ng/ml in EDTA and TM respectively) and ranges in both EDTA and TM samples which were not significant. This could be attributed by the fact that they are few if any reduced susceptibility cases of the drug in the region and generally in Kenya. This is in line with a study conducted at the Kenyan coast in which DHA emerged the most active drug (Mwai *et al.,* 2009). Highest IC_{50} values of 7.887ng/ml in EDTA and 6.911ng/ml in TM samples obtained by PPQ in our data were unlikely to represent resistance but probably methodological variability, since these values were way below the IC_{50} values from previous studies done in malaria endemic areas (Basco *et al.,*2003; Basco *et al.,* 2007 and Mwai *et al.,* 2009).

In contrast with other studies which have found an IC_{50} LUM activity of 30nm for more than 95% of isolates (Mayxay *et al.,* 2007; Parola *et al.,* 2007; Paradines *et al.,* 2006), our study generated only 24% and 20% from samples in EDTA and TM respectively, a clear indication that the activity of the drug which is used as a partner to ART for treatment of uncomplicated malaria cases remains high in the region. Encouragingly, our ART IC_{50} data which were 4.27nm and 4.88nm in EDTA and TM respectively, were comparable with those found earlier for clinical isolates collected in Cameroon and other African countries where the IC_{50} values ranged between 3.46nm and 5.66nm (Basco and LeBras, 1993; Basco and Ringwald, 2003 and Issaka *et al.,* 2013)

The inoculum size used in *in vitro* testing influences the measured *in vitro* susceptibility to antimalarials, therefore resistance can be overestimated when inoculum effects are not considered. On the other hand, occurrences of cross resistance between antimalarials can also be falsely determined in some cases (Duraisinngh *et al.,* 1999). Positive correlation was observed between DHA and all the tested drugs (ART, LUM and PPQ) for samples in EDTA, unlike the same samples in TM where DHA had statistically significant correlation of r=0.1229 (p=0.02) with LUM only while others were not significant. The variation observed in cross resistance of DHA in the samples in EDTA and TM would have been attributed by the inoculum effect. However, further research need to be conducted to rule out the possible cross resistance between these drugs in the region. A study at the coast in Kenya found a positive correlation between DHA and LUM, as well as PPQ and LUM (Mwai *et al.,* 1999), in line with the results of the current study.

Conclusion

Results from this study suggests that the formulated transport medium (TM) holds promise for future use having shown similar IC_{50} results with no significant difference with the samples which were transported in EDTA anticoagulant tubes. Variations in the positive correlations experienced by DHA indicate possible cross resistance between the drugs which need further research to elucidate on the finding.

References

1. Akala, H.M., Eyase, F.L., Cheruiyot, A.R., Omondi, A.A., Ogutu, B.R., Waters, N.C., Johnson, J.D., Polhemus, M.E., Schnabel, D.C., Walsh, D.S., (2011): Anti-malarial drug sensitivity profile of western Kenya *Plasmodium falciparum* field isolates determined by a SYBR Green I *in vitro* assay, and molecular analysis. *American Journal of Tropical medicine and hygiene* **85***:* 34 – 41.
2. Bacon, D.J., Latour C., Lucas C., Colina O., Ringwald P, Picot S., (2007). Comparison of a SYBR green I-based assay with a histidine-rich protein II enzyme-linked immunosorbent assay for *in-vitro* antimalarial drug efficacy testing and application to clinical isolates. *Antimicrob Agents Chemother***51***:*1172–1178.
3. Basco, L. K. (2003). Molecular epidemiology of malaria in cameroon.xv. Experimental studies on serum substitutes and supplements and alternative culture media for *in vitro* drug sensitivity assays using fresh isolates of *plasmodium falciparum. American Journal of Tropical Medicine and Hygiene,* 69(2), 168–173.
4. Basco, L. K. (2004). Molecular epidemiology of malaria in Cameroon. xx. Experimental studies on various factors of *in vitro* drug sensitivity assays using fresh isolates of *Plasmodium falciparum. American Journal of Tropical Medicine and Hygiene,* 70(5), 474–480
5. Basco, LK, and Le Bras J. (1993). *In vitro* activity of artemisinin derivatives against African isolates and clones of *Plasmodium falciparum.Am. J. Trop.Med. Hyg.***49**:301–307
6. Co EM, Dennull R.A., Reinbold, D.D., Waters, N.C., Johnson, J.D., (2009). Assessment of malaria *invitro* drug combination screening and mixed-strain infections using the malaria Sybr green I-based fluorescence assay. *Antimicrob Agents Chemother* **53***:* 2557–2563.
7. Duraisingh, M.T., Jones, P.,Sambou, I., Seidlein, L.,Pinder, M., Warhurst, D.C., (1999). Inoculum effect leads to overestimation of *invitro* resistance for artemisinin derivatives and standard antimalarials: a Gambian field study. *Parasitology.***119***:*435–440
8. Geary, T.G., Divo, A.D., Jensen, J.B., Zangwill, M. and Ginsburg, H. (1990). Kinetic modelling of the response of *Plasmodium falciparum* to chloroquine and its experimental testing *in vitro.BiochemicalPharmacology* **40**, 685-691.
9. Gluzman, I.Y., Schlesinger, P.H. and Krogstad, D.J. (1987). Inoculum effect with chloroquine and *Plasmodium falciparum*. *Antimicrobial Agents and Chemotherapy* **31**; 32-36.
10. Jafari S, Le Bras J, Bouchaud O, Durand R,2004. *Plasmodium falciparum* clonal population dynamics during malaria treatment. *J Infect Dis 189:*195–203.
11. Johnson JD, Dennull RA, GerenaL, Lopez-Sanchez M, Roncal NE, Waters NC, (2007). Assessment and continued validation of the malaria SYBR green I-based fluorescence assay for use in malaria drug screening. *Antimicrob Agents Chemother* **51***:*1926–1933.
12. Khan B, Omar S, Kanyara JN, Warren-Perry M, Nyalwidhe J, Peterson DS, Wellems T, Kaniaru S, Gitonga J, Mulaa FJ, Koech DK, 1997. Antifolate drug resistance and point mutations in *Plasmodium falciparum* in Kenya. *Trans R Soc Trop Med Hyg*. **91***:*456–460.
13. Liu, S., J. Mu, H. Jiang, and X.-Z. Su. 2008. Effects of *Plasmodium falciparum* mixed infections on *in vitro* antimalarial drug tests and genotyping. Am. J. Trop. Med. Hyg. 79:178–184.

14. Mariama Issaka, Ibrahim Arzika, Julia Guillebaud, AbaniMaazou, Sabine Specht, Halima Zamanka, Thierry Fandeur and Adamou Salissou.(2013). *Ex Vivo* Responses of *Plasmodium falciparum* Clinical Isolates to Conventional and New Antimalarial Drugs in Niger.*Antimicrobial Agents and Chemotherapy*.**57**; 7 3415–3419.
15. Mayxay, M.,M. Barends, A. Brockman, A. Jaidee, S. Nair, D. Sudimack, T. Pongvongsa, S. Phompida, R. Phetsouvanh, T. Anderson, N.J. White, and P.N. Newton. (2007). Invitro antimalarial drug susceptibility and *pfcrt* mutation among fresh *Plasmodium falciparum* isolates from the Lao PDR (Laos). *Am. J. Trop. Med. Hyg*. **76**:245–250.
16. Mbaisi A, Liyala P, Eyase F, Achilla R, Akala H, Wangui J, Mwangi J, Osuna F,A lam U,S moak BL, Davis JM, Kyle DE, Coldren RL, Mason C, Waters NC, (2004). Drugs susceptibility and genetic evaluation of *Plasmodium falciparum* isolates obtained in four distinct geographical regions of Kenya. *AntimicrobAgentsChemother***48***:*3598–3601.
17. Mbaisi, A., Liyala, P., Eyase, F., Achilla, R., Akala, H., Wangui, J., Waters, N. C. (2004). Drug susceptibility and genetic evaluation of *Plasmodium falciparum* isolates obtained in four distinct geographical regions of Kenya. *Antimicrobial Agents Chemotherapy, 48*, 3598-3601.
18. Mwai L, Kiara SM, Abdirahman A, Pole L, Rippert A, Diriye A, Bull P, Marsh K, Borrmann S, Nzila A, 2009. *In vitro* activities of piperaquine, lumefantrine, and dihydroartemisinin in Kenyan *Plasmodium falciparum* isolates and polymorphisms in pfcrt and pfmdr 1. *Antimicrob Agents Chemother* **53***:*5069–5073.
19. Ofulla, A. O., Orago, A. S., Githure, J. I., Burans, J. P., Aleman, G. M., Johnson, A. J., & Martin, S. K. (1994). Determination of fifty percent inhibitory concentrations (IC_{50}) of antimalarial drugs against *Plasmodium falciparum* parasites in a serum-free medium. *51*, 214-218.
20. Parola, P. ,B. Pradines, F. Simon, M.P. Carlotti, P. Minodier, M.P. Ranjeva, S. Badiaga, L. Bertaux, J. Delmont, M. Morillon, R. Silai, P. Brouqui, and D. Parzy. (2007). Antimalarial drug susceptibility and point mutations associated with drug resistance in 248 *Plasmodium falciparum* isolates imported from Comoros to Marseille, France in 2004–2006. *Am. J. Trop. Med. Hyg.* **77**:431–437.
21. Pradines, B., P. Hovette, T. Fusai, H.L. Atanda, E. Baret, P. Cheval, J. Mosnier, A. Callec, J. Cren, R. Amalvict, J. P. Gardair, and C. Rogier. (2006). Prevalence of invitro resistance to eleven standard or new antimalarial drugs among *Plasmodium falciparum* isolates from Pointe-Noire, Republic of the Congo. *J. Clin. Microbiol*. **44**:2404–2408.
22. Reardon, D.M., Warner, B., Trowbridge, E.A. (1993). EDTA, the traditional anticoagulant of haematology: with increased automation is it time for review? Med Lab Sci, 48:72–5
23. Rengarajan, K., Cristol, S., Mehta, M., and Nickerson, J. (2002). Quantifying DNA concentrations using fluorometry: a comparison of fluorophores. *Molecular Vision,* 8, 416-421.
24. Russell, B. M., Udomsangpetch, R., Rieckmann, K. H., Kotecka, B. M., Coleman, R. E., & Sattabongkot, J. (2003). Simple *in vitro* assay for determining the sensitivity of *Plasmodium vivax* isolates from fresh human blood to antimalarials in areas where P. vivax is endemic. *Antimicrobial Agents Chemotherapy,* 47, 170-173
25. Suwanna, C., Stuart, D. T. Chanthap, L., Kritsanai, Y., Wiriya,R.,S., Sundrakes, P. S., Jacob D. Johnson, Douglas S. Walsh, David L. Saunders and Charlotte A Lanteri. (2013). Direct comparison of the histidine-rich protein-2 enzyme-linked immunosorbent assay (HRP-2ELISA) and malaria SYBR green I fluorescence (MSF) drug sensitivity tests in Plasmodium falciparum reference clones and fresh ex vivo field isolates from Cambodia. *Malaria Journal* **12**:239

Table 1: Drug susceptibility testing of *Plasmodium falciparum* isolates in EDTA and TM against a panel of antimalarials

	Median IC_{50} (ng/ml) for *P. falciparum* isolates transported in:		
Drugs	**EDTA**	**TM**	**p-value**
Artemether (ART)	4.275	4.887	0.9873
Lumefatrine (LUM)	5.409	4.007	0.7448
Diydroartemisinin (DHA)	2.567	1.691	0.6839
Piperaquine (PPQ)	7.887	6.911	0.8153

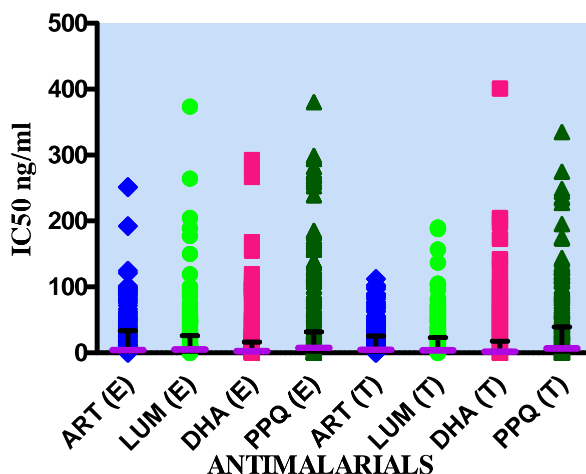
Comparison of the medians using Mann - whitney test
Key: TM= Transport medium, p = significance level of the test

Table 2: Correlation of *in vitro* responses of antimalarial drugs against field isolates of *P. falciparum* in EDTA and TM

	In vitro correlation of drugs against *P. falciparum* isolates transported in:			
	EDTA		TM	
Drug pairs	Correlation coefficient (r)	*p*-value	Correlation coefficient (r)	*p*-value
ART - LUM	0.04967	0.3699	0.1260	**0.0225**
ART - DHA	0.123	**0.0259**	0.09014	0.1032
ART - PPQ	0.0262	0.6364	0.1834	**0.0008**
LUM - DHA	0.1382	**0.0122**	0.1229	**0.0261**
LUM - PPQ	0.0584	0.2916	0.0758	0.1709
DHA - PPQ	0.1281	**0.0203**	0.0372	0.5024

Correlation of the drug pairs using pearsons correlation coefficient
Key: ART = Artemether, LUM = Lumefantrine, DHA = Dihydroartemisinin, PPQ =Piperaquine, p = significance level of the test, r = Pearson's correlation coefficient of log IC_{50} values.TM= Transport medium

Figure 1: *In vitro* activities of ART, LUM, DHA and PPQ against *P. falciparum* field isolates transported in EDTA and TM



Key: E = EDTA, T = Transport medium. Purple bars = Medians IC_{50}, Black bars = median inter - quartile ranges. ART = Artemether, LUM = Lumefantrine, DHA = Dihydroartemisinin, PPQ Piperaquine