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Performance evaluation of Care Start™ Malaria Pf/Pv Combo Test at Arbaminch General Hospital and Health center, Gamo Gofa zone, South Ethiopia.

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This is to certify that the thesis prepared by **Temesgen Eticha**, entitled:

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List of abbreviations

CSA	-	Central Statistics Agency
DBS	-	Dried Blood Sample
GMP	-	Good Manufacturing Practices
HRP-2	-	Histidine Rich Protein-2
LDH	-	Lactate Dehydrogenase
MCH	-	Maternal and Child Health
NPV	-	Negative Predictive Value
PPV	-	Positive Predictive Value
NGO	-	Non-Governmental Organization
PCR	-	Polymerase Chain Reaction
pLDH	-	plasmodium lactate dehydrogenase
RDT	-	Rapid Diagnostic Test
mRDT	-	malaria Rapid Diagnostic Test
SNNPR	-	South Nations Nationalities and Peoples Region
WHO	-	World Health Organization

Abstract

Background: Malaria remains endemic in 106 countries while parasite-based diagnosis increasing. Most suspected cases of malaria still not properly confirmed, resulting in over-use of anti-malarial drugs and poor disease monitoring. Malaria rapid diagnostic tests (RDTs) are alternative diagnostic methods which have enabled reliable biological diagnostic testing in all situations where previously only clinical diagnosis was available. The diagnostic accuracy of RDTs can vary significantly across different geographical regions. This variable quality of malaria RDTs, and consequently their diagnostic performance, have made it difficult for policy makers to determine which tests are the most suitable.

Objective: The aim of the study was to evaluate the diagnostic performance of CareStart™ Malaria Pf/Pv Combo RDT by comparing with light microscope for malaria diagnosis in Arbaminch Hospital and Health center, Southern Ethiopia.

Methods: A cross-sectional study design was conducted from October 1, 2015 up to October 15, 2016. A total of 160 patients were included in the study. Socio demographic data collection was done using structured questionnaire. Finger prick blood sample was obtained from those study subjects for Care Start™ Malaria Pf/Pv combo test, thin and thick film was prepared immediately for microscopic examination. Collected data from this study was entered using Epi Data version 3.1 and analyzed using SPSS version 20.0 and Microsoft Excel 2013. Sensitivity, specificity, PPV and NPV was determined in comparison with the gold standard Microscopy method.

Result: In this study, it was found that Care start ™ combo test had an overall sensitivity, specificity, PPV, and NPV of 97.44%, 93.67%, 93.83%, and 97.37% respectively, with Kappa value of 0.877 to detect the presence or absence of malaria. Sensitivity and specificity of the kit for p. falciparum detection was 63.27 % and 94.3% and for p. vivax detection was 86.96 % and 95.62 % respectively, which is below WHO standard. Agreement between Light microscopy and Care Start ™ malaria RDT for specific identification of malaria species is moderate with kappa value of 0.568. It showed better performance at Arbaminch health center than Arbaminch Hospital with sensitivity, specificity, PPV, and NPV of 96.07%, 80%, 92.45% and 88.88% respectively.

Conclusion and recommendations: The kit evaluated has good overall performance in detection of malaria cases which is almost comparable with the Gold standard microscopy

method. But, since it has still drawback in correctly identifying malaria species, further studies should be carried out to use the CareStart™ Malaria Pf/Pv Combo Test instead of microscopy for the diagnosis of malaria.

1. Introduction

1.1 Background of the study

Malaria is a parasitic disease which is transmitted to humans by mosquitoes of the genus *Anopheles*. Until very recently, the etiologic agents of human malaria were believed to belong to four species: *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. However, recent studies have revealed that a fifth malaria parasite of non-human primates, *P. knowlesi*, can also naturally infect humans (1).

Malaria is one of a few diseases which has a quick, simple and accurate biological diagnostic method even in a low-technology setting (2). Clinical diagnosis is most of the time becoming unreliable due to the non-specific nature of signs and symptoms of malaria (3), so that it results in a waste of already scarce resources and impacts negatively on the prompt treatment of malaria (2). Even though various techniques are available for malaria diagnosis, Light microscopy remains preferred and standard for laboratory diagnosis of malaria (3). However, blood film microscopy is time-consuming, needs significant technical skills, good-quality reagents and trained personnel, thus its accuracy is of highly variable quality in sub-Saharan African hospitals (4).

RDTs are an alternative diagnostic method which enabled reliable biological diagnostic testing in all situations where previously only clinical diagnosis was available (5). The first commercial RDT was marketed in 1994, and more than 200 devices from more than 60 manufacturers are now available in the world market (6). It has been shown that RDT is easy to use, heat stable and have the ability to detect low parasite load (7). Thus RDT is an ideal diagnostic tool for malaria diagnosis in settings that are resource constrained. The main advantages of the malaria RDTs are that they are easy to use, do not require electricity or complex equipment and the results are available in 15–30 minutes after finger-prick blood collection (5).

The principle of a RDT is to capture malaria antigen from peripheral blood flowing across a membrane containing specific anti-malaria antibodies. Thus, there are different types of RDTs depending on the antigen they target: those targeted to histidine-rich protein-2 (HRP-2) only detect *P. falciparum*, those which target the parasite enzyme lactate dehydrogenase (LDH) and aldolase can detect non- falciparum from mixed infection. LDH test uses either monoclonal

antibodies which react with LDH of all species including *P. falciparum* (or known as Pan-LDH), or antibodies specific for *P. falciparum* LDH (8).

A distinction between the HRP-2 and LDH based tests is that HRP-2 may persist in the blood stream for days or weeks after treatment, whereas LDH is only detected if live parasites are present (9). HRP-2 based *P. falciparum*-specific tests generally have greater sensitivity (over 90%) than the pLDH-based tests when compared with microscopy in clinical cases, whereas sensitivity of pLDH tests for non-*P. falciparum* species was low (9). In addition to variation in antigen detected, the tests are available in many formats including plastic cassettes, cards or dipsticks (10).

Malaria Rapid Diagnostic Tests (RDTs) were introduced in the nineties and have undergone many improvements (11). Using single species RDT (only for *P. falciparum*) as an alternative diagnostic testing device for malaria has been started in Ethiopia since 2005 at health posts by health extension workers (3). Currently multispecies RDTs, like CareStart Malaria Pf/Pv Combo Test, which is capable of specifically detecting both P. falciparum and P. vivax species, are being supplied by the Ethiopian Ministry of Health to health posts (12).

1.2 Statement of problem

Globally, an estimated 3.2 billion people in 95 countries and territories are at risk of being infected with malaria and developing disease. According to the World Malaria Report 2015, there were 214 million cases of malaria globally in 2015 and 438 000 malaria deaths. The burden was heaviest in the WHO African Region, where an estimated 90% of all malaria deaths occurred, and in children aged under 5 years, who accounted for more than two thirds of all deaths (13).

In Ethiopia, malaria is the leading cause of morbidity and mortality. Almost 75% of the country is malarious and an estimated 51 million people (68% of the population) live in areas at risk of malaria (7, 14). *Plasmodium falciparum* and *Plasmodium vivax* are common plasmodium species in the country and account for about 60% and 40% of infections, respectively, during the peak transmission period which is from September to December after heavy summer rains (9).

The diagnostic accuracy of RDTs can vary significantly across different geographical regions (15). This variable quality of malaria RDTs, and consequently their diagnostic performance, have made it difficult for policy makers to determine which tests are the most suitable (5). Furthermore, specially RDTs for malaria which relay on the detection of histidine- rich protein 2 (HRP-2), produced only by *P. falciparum* may be misleading in areas of high transmission because they remain positive for a number of days or weeks after an infection, even if treated, thus a positive result with a history of a recently treated infection is difficult to interpret (2).

Like other biological tests, malaria RDTs are prone to deterioration through exposure to heat and humidity, and through manufacturing faults. The apparent accuracy of any RDT in detecting malaria parasites will depend on various factors, including the concentration of the target antigen in host blood, the mechanics of antigen and antibody flow along the nitrocellulose strip, the physical condition of the RDT, including the integrity of antibodies and conjugate, the availability of target epitopes to bind antibodies in the tests (that is, variation in antigen structure); the quality of test preparation and interpretation; and the accuracy of the reference standard. Though they are potentially useful, their adoption needs to be guided by local test sensitivity (16, 17).

According to World Health Organization (WHO) recommendation, RDTs should be implemented with a comprehensive quality control strategy. First, RDTs should be purchased

from a manufacturer that follows good manufacturing practices (GMP). Second, each lot of RDTs should be tested on arrival in the country of use to ensure that the tests weren't exposed to extreme temperatures or other conditions that may affect RDT performance. WHO also recommends post deployment testing at the health facility level, but these recommendations are less developed (18).

In Ethiopia different studies have been carried out to evaluate the performance different types of malaria RDT's (3, 8, 9, 14, 19, 20). Even though Arbaminch city and the surrounding hot rural areas are one of the most highly malarious places in Ethiopia and using malaria RDTS as alternative diagnostic tool, up to my knowledge there are no published studies carried out in the study area to evaluate their performance. The study area hot climate condition is not suitable for the malaria CareStart^{TM} RDT storage (which is from 4^0c -30^0c) to maintain its integrity, so that frequent evaluation of its performance evaluation should be done.

The aim of this study is therefore to evaluate the diagnostic performance of CareStart^{TM} Malaria by determining its sensitivity and specificity using microscopy as gold standard and expert malaria microscopist reading as a reference standard.

1.3 Significance of the study

Regular evaluation of performance of RDT test kits is very important for improving patient correct treatment as well as decreasing malaria drug resistance. Therefore, the study finding will have a great contribution for the local malaria eradication program by presenting the actual performance of currently used malaria RDT (Care Start^{TM} RDT) in the study area by measuring its sensitivity, specificity, PPV, and NPV. It also provides base line information for further studies in the study area about the performance, advantages as well as the limitations of mRDTs.

2. Literature review

2.1 Global Experience

In different parts of our world various researches has been carried out to evaluate a performance of CareStart™ kits as well as other types of RDTs. A study conducted between China-Myanmar endemic borders to evaluate the diagnostic performance of CareStart™ RDT malaria HRP2/pLDH (Pf/pan) combo test showed that CareStart™ RDT kit's sensitivity and specificity for the diagnosis of malaria 89.68% and 98.26% respectively, compared to standard microscopy, whereas the sensitivity and specificity for *falciparum* malaria were 88.52% and 98.26%, and for *vivax* malaria: 90.77% and 100%. The CareStart positive predictive values were 98.26% compared to 100% for PCR-corrected, and the negative predictive values of 89.68% were the same in microscopy as PCR-corrected (21).

Another retrospective study in a reference laboratory on a panel of stored blood samples obtained from inter- national travelers suspected of malaria evaluated the CareStart Malaria HRP-2/pLDH (Pf/pan) Combo Test. The overall sensitivity for the detection of *P. falciparum* was 88.8%, increasing to 94.3% and 99.3% at parasite densities above 100 and 1,000 per micro/l respectively. For *P. vivax*, *P. ovale* and *P. malariae*, overall sensitivities were 77.6%, 18.4% and 30.4% respectively. For *P. vivax* sensitivity reached 90.2% for parasite densities above 500/micro/l (11).

A study conducted in Honduras also evaluated the ability of a newly developed rapid malaria diagnostic test (OptiMAL) to detect *Plasmodium vivax* and Plasmodium falciparum malaria during an outbreak. The results demonstrated that the OptiMAL test had sensitivities of 94 and 88% and specificities of 100 and 99%, respectively, when compared to traditional blood films for the detection of *P. vivax* and *P. falciparum* malaria (22).

Some studies were done to evaluate multiple types of RDTs at the same time. For example, a study which was carried out in Korea evaluated 4 rapid malaria diagnostic kits (RDTs): Optimal test, SD BIOLINE Malaria Ag P.f/Pan test, Humasis Malaria P.f/Pan antigen test and CareStart Malaria Pf/Pv Combo test in comparison with the reference standard, the Giemsa-stained traditional microscopic diagnosis. Compared with the reference standard, the sensitivity and specificity for *Plasmodium vivax* were 92.7 and 100% for SD BIOLINE Malaria Ag P.f/Pan; 94.6% and 100% for

OptiMAL; 95.5% and 100% for both Humasis Malaria P.f/Pan antigen test and CareStart Malaria Pf/Pv Combo test (23).

A study conducted in Colombia also evaluated the diagnostic capacity of three malaria rapid diagnostic tests (RDTs), NOW-Malaria-ICT, OptiMAL-IT, and Paracheck-Pf, against expert microscopy. According to the research finding, Paracheck-Pf and NOW-malaria-ICT were more accurate in detecting *P. falciparum* (sensitivities 90.8% and 90.1%, respectively) in comparison with Optimal-IT (83.6%). But, For *P. vivax* diagnosis, Optimal-IT had a higher sensitivity than NOW (91.0% and 81.4%, respectively) (24).

2.2 African Experience

In Africa where more than 90% of the total death due to malaria is occurring (7), studies are still continuing to evaluate the performance of malaria RDTs. The diagnostic performance of histidine-rich protein 2 (HRP-2)-based malaria rapid diagnostic test (RDT) was evaluated in Nigeria, by comparing RDT results with expert microscopy. Overall, 11.9% (35/295) tested positive with RDT compared with 10.5% (31/295) by microscopy: sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 100%, 98.5%, 88.6%, and 100%, respectively (25). SD Bioline P.f /P.v RDT kit was also evaluated at Gadarif Hospital, Eastern Sudan, but in this case by comparing with PCR. The sensitivity and specificity of the microscopy was 94.4% and 100%, respectively. The corresponding values for RDT evaluation were 83.3% and 92.0%, as compared with PCR as the gold standard (4).

A similar study was conducted in nine health facilities in Rufiji District, Tanzania, to assess sensitivity and specificity of a histidine- rich protein II (HRP2)-based RDT (Paracheck) in routine use at rural health facilities, mean operational sensitivity of RDTs based on reference microscopy was 64.8%, but varied greatly by health facility, range 18.8–85.9%. Sensitivity of RDTs increased with increasing parasite density. Specificity remained high at 87.8% despite relatively poor slide quality (26).

Another study which is conducted in Mashonaland East Province, Zimbabwe evaluated the performance of two diagnostic assays in malaria diagnosis, Paracheck and SD Bioline RDTs. Both of them performed fairly equally well and above WHO targets in Mudzi (high malaria burden), while in Murewa (low malaria burden) Paracheck and SD Bioline RDTs had sensitivities of 86.7% and 86.7%; specificities of 97.2% and 90.7%; test efficiencies of 95.9% and 90.2%; PPVs of 73.3%

and 45.2%; and NPVs of 98.8% and 98.7% respectively. Sensitivities for Paracheck and SD Bioline RDTs reduced from 99.2% at parasitemias above 1000/µl each to 33.3% and 50.0% respectively at parasitemias below 1000/µl with variations not statistically significant (27).

Evaluation of three tests and expert microscopy of samples from suspected malaria patients were carried out in Madagascar. Compared with microscopy, the sensitivity of the three tests to detect *Plasmodium falciparum* malaria was 97% for the CareStart™ Malaria test, 89.4% for the SD Malaria Antigen Bioline™ test, and 92.6% for the OptiMAL-IT™ test. The three tests were less sensitive in detecting non-*P.falciparum* infections, and the sensitivity decreased at levels of parasitemia 500 parasites/L for *P. falciparum* and 5,000 parasites/L for other *Plasmodium* species (28).

A study conducted among febrile children in Sokoto, Nigeria, for evaluation of Biotec Malaria pf Rapid Device. The RDT had a sensitivity of 90.2% and specificity of 95.4%; with positive and negative predictive values of 93.0% and 93.4% respectively. Test sensitivity is reduced by low parasite density (100% at > 1600/µl Vs 69.2% at <800/µl) (17).

2.3 Ethiopian Experience

In Ethiopia there are a number of published researches done on evaluation of performance of malaria RDTs (3, 8, 9, 14, 19, 20, 29), most of them on CareStart^{TM} Malaria Pf/Pv combo test type of RDT. According to the study conducted in Wondo Genet, southern Ethiopia, CareStart Malaria Pf/Pv Combo test for the diagnosis of *Plasmodium falciparum* has sensitivity of 99.4%, specificity of 98%, and PPV of 94.4% and NPV of 99.8%. Sensitivity, specificity, PPV and NPV of the test for the diagnosis of *P.vivax* were 99.4%, 98.2%, 94.5% and 99.8%, respectively (29).

CareStart^{TM} Malaria Pf/Pv combo test was also evaluated compared to microscopy in Butajira area, south-central Ethiopia. RDT showed agreement with microscopy in detecting 79 positives in household surveys (n=796) and 375 positives in health center survey (n=1,598). RDT performance varied in both survey settings, lowest PPV (64.3%) for *Plasmodium vivax* and *P. falciparum* (77.2%) in health centers; and *Plasmodium vivax* (76.7%) and P. falciparum (87.5%) in household surveys. NPV was low in P. vivax in health centers (77.2%) and household (87.5%) surveys (8).

According to a study conducted in north-west Ethiopia, the overall sensitivity and specificity of CareStartTM RDT was found to be 95% and 94.2%, respectively when compared with light

microscopy. The sensitivity of the CareStartTM RDT for *Plasmodium falciparum* or mixed infection was calculated to be 92.9% while a sensitivity of 90.9% was found for non-falciparum species. The specificity for *P. falciparum* or mixed infections was found to be 95.4% while it was 97.3% for non-falciparum species (3).

Another study which was carried out at Serbo health center in Jimma zone, southwestern Ethiopia, evaluated the diagnostic performance of CareStart Malaria Pf/Pv Combo test relative to microscopy. The validity of CareStart Malaria Pf/Pv Combo test for the diagnosis of *Plasmodium* was very good with a sensitivity of 95.8%, specificity of 100%, and positive predictive value of 100% and negative predictive value of 96% (14).

Performance of CareStart™ Malaria Pf/Pv Combo test for the diagnosis of *Plasmodium falciparum* and *Plasmodium vivax* infections were also evaluated in the Afar Region, North East Ethiopia. The sensitivity and specificity of the test were 98.5% and 98.0% respectively, with a positive predictive value (PPV) of 91.7% and a negative predictive value (NPV) of 99.7% for the diagnosis of *P. falciparum* infection. The corresponding sensitivity and specificity for the diagnosis of *P. vivax* infection were 100% and 99.6% respectively, with PPV and NPV of 86.2% and 100%, respectively (20).

The ability of CareStart Malaria Pf/Pv Combo Test to diagnose *Plasmodium* malaria was very good, with 99.8% sensitivity and 97.7% specificity, according to the study which was conducted on acute febrile patients visiting the Felegeselam Health Center. The sensitivity and specificity of the CareStart Test was comparable with the thick blood smear in diagnosing malaria (19).

Performance of three multi-species RDTs (CareStart, ParaScreen and ICT Combo) was compared with 'gold standard' microscopy at three health centers in Jimma zone, Oromia Regional State. All three RDTs were equally sensitive in detecting *P. falciparum* or mixed infection: 85.6%. RDT specificity was similar for detection of *P. falciparum* or mixed infection at around 92%. For detecting *P. vivax* infection, all three RDTs had similar sensitivity in the range of 82.5 to 85.0%. CareStart had higher specificity in detecting P. vivax (97.2%) than both ParaScreen and ICT Combo (30).

3. Objectives of the study

3.1 General objective

The general objective of this study is to evaluate the performance of currently used rapid malaria diagnostic test (Care start ^{TM} RDT) in comparison with Gold standard light microscopy method in Arbaminch General Hospital and Arbaminch health center, South Ethiopia.

3.2 Specific objectives

- To evaluate the performance of CareStart^{TM} RDT by calculating the sensitivity, specificity, PPV and NPV.
- To calculate parasite load and evaluate its relationship with the sensitivity and specificity of CareStart^{TM} RDT.
- To compare the performance of CareStart^{TM} RDT at Arbaminch General Hospital and Arbaminch health center.

4. Methods and materials

4.1 Study area

The study was conducted in Arbaminch General Hospital and Arbaminch health center which provides general health care service and higher levels of clinical care for catchment area populations. Arbaminch Health Center is one of the health centers in Arbaminch where the highest number people with early symptoms suggestive of malaria obtain services for diagnosis and treatment of malaria. Arbaminch town is located in Gamo Gofa zone of the southern nations, nationalities and people's region about 500 kilometers south of Addis Ababa. It is found at an altitude of 1200–1300 meters above sea level with an average annual temperature of 29.7°C and rain fall of 700 mm (31). Based on the 2007 Census conducted by the CSA, the town has a total population of 74,879, of whom 39,208 are men and 35,671 women (32).

4.2 Study design and Period

Cross-sectional analytic study design was employed from October 1, 2015 up to October 15, 2016.

4.3 Study participants

The study participants were all individuals who came to Arbaminch hospital and Arbaminch health center being suspected of malaria and have a request order for malaria diagnosis.

4.3.1 Inclusion criteria

All age group patients with a clinical signs and symptoms of malaria who presented to the outpatients and MCH department of Arbaminch hospital as well as Arbaminch health center and sent to the laboratory department for confirmation during the study period were eligible for enrolment into the study.

4.3.2 Exclusion criteria

Patients who do not fulfill the inclusion criteria and refused participation was excluded from the study.

4.4 Variables

4.4.1 Dependent variables

- Performance of CareStart^{TM} RDT

4.4.2 Independent variables

- Age
- Sex

- parasite load
- Malaria symptoms

4.5 Sample size and Sampling Technique

Assuming confidence level of 95% so that an error risk of 1.96, an expected minimum sensitivity and specificity of all RDTs 95%, compared with microscopy according to World Health Organization (33), a margin of error of 5% plus an additional 10% of the sample to account for invalid and unclear results, a minimum sample of 80 participants were recruited. According to this assumption, a total of 160 study subjects (80 malaria positive and 80 malaria negative) were included in this study. A convenient sampling technique was employed to obtain those study subjects.

$n = \frac{(Z)^2\, p\,(1-p)}{d^2} = 80$ (including 10% to account for invalid and unclear results)

Where n = sample size, Z = 95% confidence interval (1.96), D = Margin of error (5%), P = Sensitivity or Specificity (95%).

4.6 Data Gathering Procedures

Questionnaire and format were developed for the purpose socio-demographic data collection and for reporting the laboratory result of malaria status by both light microscope and RDT as well as percent parasite density of participants. They were prepared originally in English after reviewing relevant literatures and then translated to Amharic. Pretest of questionnaire for the clarity and consistency of questions was done and necessary correction was made based on the findings of the pretest.

4.7 Sample collection and processing

A blood sample was obtained from eligible study subjects visiting Arbaminch general hospital and health center in the specified study period, having signs and symptoms of malaria and sent to laboratory diagnosis. From each study participants, a finger prick blood sample was collected for malaria testing with both CareStart^{TM} RDT and Microscope. Two blood films were prepared, the first blood film was processed either at hospital or health center laboratory setup as routine way and the result was recorded separately for the research purpose according to the pre-labeled identification number. The second blood film was dried and fixed (the thin film) and examined at Arbaminch College of health science teaching laboratory by the principal investigator for quality

assurance purpose. The CareStart^{TM} RDT test was done at site of sample collection by different laboratory personnel so that the one who did the microscopy do not know the result of the RDT.

4.8 Laboratory procedures

Rapid diagnostic testing for malaria

The available RDT kit was used in this study according to the manufacturer's instructions. It is used for the qualitative detection of antigens produced by *P. falciparum* and *p. vivax*. These antigens are the Histidine Rich Proteine-2 (PfHRP2) and lactate dehydrogenase (LDH). Briefly a drop of whole blood (20 µl) was added to the card pad followed by three drops of lysis reagent. The RDT result was read within 10 minutes and the results recorded immediately.

Microscopy

Blood smears were stained with 10% Giemsa and examined under 100*X* oil immersion objective lens of a light microscope by two independent laboratory technologists (one at the hospital/health center and the other at the teaching laboratory (AMCHS)) that are blinded to each other's results.

Parasite density

Parasite density estimation was done by measuring the level of infection in red blood cells by examination of a thin blood film which is confirmed to be more accurate (34). To quantify malaria parasites against RBCs, parasitized RBCs among 500-2,000 RBCs on the thin smear were counted and result expressed as % parasitemia.

4.9 Methods of data analysis

Data collected from this study were entered using Epi Data version 3.1 and analyzed using SPSS version 20.0 and Microsoft Excel. The data include participant demographic information, clinical sign and symptom, parasite type, previous treatment history and RDT result. Sensitivity, specificity, Positive predictive values (PPVs), and Negative predictive values (NPVs), of the CareStart^{TM} Malaria RDT were calculated. Kappa value was calculated to determine the agreement between the results of microscopy and the diagnostic test kit.

4.10. Data quality management

Fresh blood samples were transferred directly to the sample pad by the provided sample applicator. All CareStart^{TM} malaria test kits were labeled with patient ID number and the procedure as well as result recording period was according to manufacturer's instruction. In all cases, the results of the

CareStart test were determined earlier than microscopic results, with strict blinding to microscopic examination of the thick and thin blood smears. To eliminate observer bias, quality control was done by the principal investigator at Arbaminch College of health science teaching laboratory blindly repeating all test results at the facility level. Discordant slides between the primary microscopic reading and secondary reading was re-analyzed for the third time by the most experienced laboratory technician from Arbaminch health center. All laboratory procedures were conducted based on the standard operating procedures

Finally, slide reading for discordant thick blood smears with that of RDT results were done at Adama regional laboratory blinded to initial microscopy and RDT results.

4.11 Ethical consideration

Ethical permission to perform the study was obtained from Department of Research and Ethical Review Committee (DRERC) of Medical Laboratory Science, School of Allied Health Science, College of Health Science, Addis Ababa University. Similarly, by showing the letter from Addis Ababa University, permission from both Arbaminch general hospital and Gamo Gofa zone health department was obtained. Then step by step, the laboratory head as well as other staffs was communicated about the study. Informed written consent was obtained from all study participants or care givers/parents if in case the study participant is children (age less than 18 years old). All malaria positive study participants especially who are infected with *P. falciparum* malaria were treated with Coartoum drug which was donated from Gamo Gofa zone health department.

5. Result

5.1 Demographic characteristics of the study population

In this study a total of 160 study participants suspected of malaria examined for malaria parasites by thick/thin blood smear microscopy and Care Start ™ combo test. Of the total participants, 89 (55.6%) were males and 71 (44.4%) were females with ages ranging from 1 year to 70 years old with a mean age of 24 and median 21. Malaria prevalence is higher in males which is 62.5% (50 of 80) than that of females (37.5%).

The age distribution of the patients as depicted in Table 1, the majority (45 %) of the patients were within 16 -30 years age range, followed by 6-15 years (19.4%). Age groups of 16-30 years of age were found to be the most affected accounting for 46.25% (37 of 80) of the total confirmed cases.

Table 1: Age group distribution of study participants, Arbaminch 2015.

Age group	Frequency	Percent	Microscopy positive result
< 5 years	16	10	9
6-15 years	31	19.4	24
16-30 years	72	45	37
31-45 years	25	15.6	9
> 45 years	16	10	1
Total	160	100	80

5.2. Clinical characteristics of the study population

The mean duration of malaria symptoms of the subjects at presentation were 4.98 days with a minimum of 1 day and maximum of 31 days. As indicated in table 2, study subjects were presented with symptom of fever (86.2%), headache (84.9%), sweating (80.5 %), chills (78.6%), and nausea (65.4%). From the total percipients who have had any or a combination of the malaria symptoms 49.7 % were reported to be microscopically confirmed positive for malaria.

Table 2: Distribution of malaria symptoms, Arbaminch 2015.

Malaria symptoms at presentation	Yes	No
Fever	137 (86.2%)	22 (13.8%)
Chills	125 (78.6%)	34 (21.4%)
Sweating	128 (80.5%)	31 (19.5%)
Nausea	104 (65.4%)	55 (34.6%)
Headache	135 (84.9%)	24 (15.1%)

Parasite positivity was 62.5% in men and 37.5% in women. The majority (78.75 %) of study participants reported that they didn't take any anti-malarial therapy and the rest thirty-four subjects (21.25%) have had anti-malarial drugs in the past two months. Coartoum (10.63%), Chloroquine (8.75%), Coartoum and Chloroquine together (1.88 %) are the anti-malarial therapies which were used by 21.25 % of the study participants.

5.3. Comparison of standard microscopy with care start ™ combo RDT

5.3.1 Microscopy results

Of the total of 160 blood smear slides eligible for analysis, microscopy detected malaria parasites overall in 80 (50%) blood smear samples. From the total microscopically examined blood smear samples of study participants, 51 (63.75%) were infected with P. falciparum, 23 (28.75%) were infected with *P. vivax*, and the rest six (7.50 %) were accounted for mixed infection with *P. falciparum* and *P. vivax*. Malaria species prevalence for the microscopically confirmed positive cases of study participants, were indicated in the figure below.

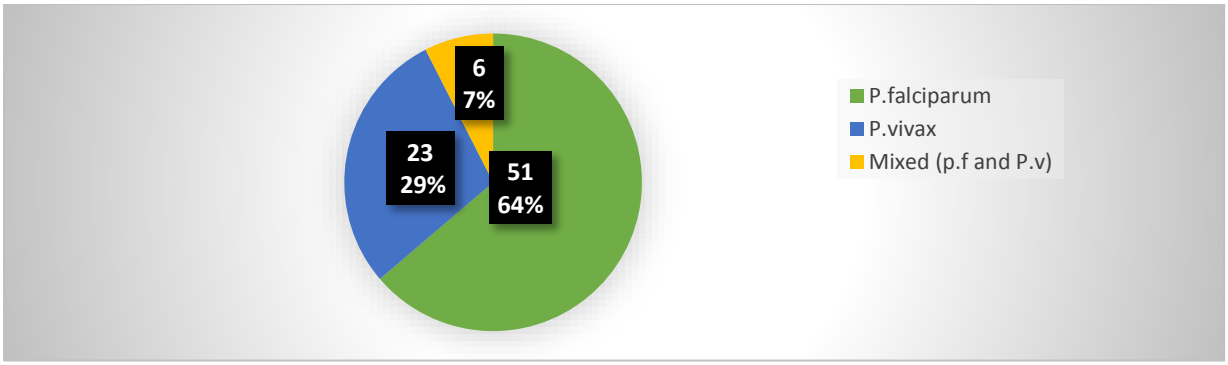
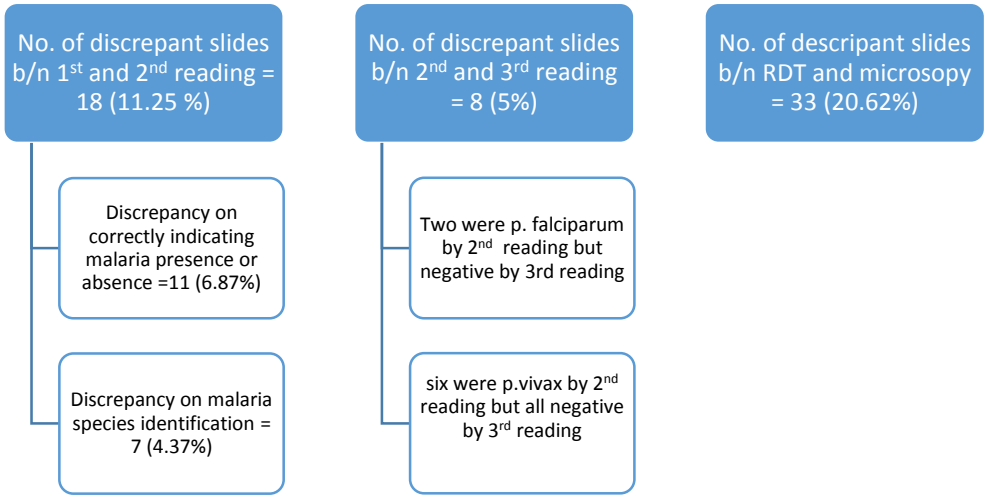


Figure 1: Malaria species prevalence among microscopically confirmed positive cases, Arbaminch 2015.

Figure 2: variation between readings at facility level and teaching laboratory



Generally there was less discrepancy between the 2nd reading and 3rd reading with 10 slides giving similar result than between the 1st reading and 3rd reading with only four slides giving similar negative result. Of those 10 agreed slides of the 2nd reading and 3rd reading, three were similar *P.vivax*, one was similar *P.falciparum* and the rest six were similar negative reading. From those eight slides of 2nd reading which didn't agree with the 3rd reading two were *P. falciparum* by the 2nd reading but negative, six were *P.vivax* but negative by the 3rd reading. From those discrepant 14 slides of the 1st reading, 10 were *P. falciparum* but nine negative and one *P.vivax* result, two were *P.vivax* but negative and *P. falciparum,* one mixed infection but *P.vivax* and one negative but *P.vivax* according to the 3rd reading.

Similarly, a total of 33(20.62%) discrepant reading between RDT and microscopy were re-analyzed by the WHO certified malaria microscopist at Adama malaria center who is blinded to initial microscopy and RDT results to avoid discordant results and the reading of the microscopist was taken as final.

5.3.2 RDT results

Care Start TM malaria RDT detected malaria in 81(50.6%) patients, detected no infection in 76(47.5%) patients and there were 3(1.9%) invalid test results. According to the Care Start TM malaria RDT, among 81(50.6%) of people with malaria, 37(45.7%) were infected with P. falciparum, 26(32.1%) with *P. vivax* and the remaining 18(22.2%) were found to be infected with both P. falciparum and *P. vivax* (Table 3).

Table 3: Malaria species detected by RDT, Arbaminch 2015.

		Malaria species detected by RDT			
		P. Falciparum	*P. vivax*	Mixed	Total
RDT positive result	Count	37	26	18	81
	%RDT result	45.7%	32.1%	22.2%	100.0%

Generally, microscopy and RDT gave the same positive result for a total of 76(47.5%) study participants, the same negative result for a total of 74(46.25%) study participants. Five (3.12%) study participants were positive by RDT, but the result of microscopy didn't agree. Similarly, two (1.25%) study participants were positive by microscopy but negative by RDT (Table 4).

Table 4: Comparison of Care Start ™ malaria RDT to microscopy, Arbaminch 2015.

		Microscopy result		
		Positive	Negative	Total
Care Start ™ RDT result	Positive	76	5	81
	Negative	2	74	76
	Invalid	2	1	3
	Total	80	80	160

In this study, microscopy and Care Start ™ malaria RDT gave similar *P. falciparum* test result for 31 study participants, *P. vivax* test result for 20 study participants and mixed infection with P. falciparum and *P. vivax* for only 5 study participants. As presented below in table 5, surprisingly RDT gave mixed infection for 13 study participants which are only *P. falciparum* by microscopy.

Table 5: Comparison of Care Start ™ malaria RDT to microscopy for identification of malaria species, Arbaminch 2015.

		Malaria species seen by microscopy			
		P. falciparum	*P. vivax*	Mixed	Total
Malaria species detected by RDT	*P. falciparum*	31	3	0	34
	P. vivax	3	20	1	24
	Mixed	13	0	5	18
	Total	47	23	6	76

5.4. Sensitivity, Specificity, PPV and NPV of Care Start ™RDTs

Taking a thick blood smear as a gold standard test for malaria, the overall sensitivity and specificity of Care Start ™RDTs were found to be 97.44% (95% CI = 91.04% to 99.69%) and 93.67% (95% CI = 85.84% to 97.91%) respectively. The PPV and the NPV of the device was found to be 93.83% (95% CI = 86.18% to 97.97%) and 97.37% (95% CI = 90.82% to 99.68%), respectively (Table 6). There was a very good agreement between the light microscopy and Care Start ™ RDT to detect the presence or absence of malaria parasite with a Kappa value of 0.877, P<0.01 which is statistically significant .

Table 6: Overall performance characteristics of Care Start ™ in comparison to the standard light microscopy, Arbaminch 2015.

Performance characteristics	Care start™ malaria
Sensitivity (95% CI)	97.44% (91.04% to 99.69%)
Specificity (95% CI)	93.67% (85.84% to 97.91%)
Positive predictive value (95% CI)	93.83% (86.18% to 97.97%)
Negative predictive value (95% CI)	97.37% (90.82% to 99.68%)

The CareStart ™ test kit was 63.27% (95% CI = 48.29% to 76.58%) sensitive and 94.50% (95% CI = 88.40% to 97.95%) specific to diagnose *P. falciparum* malaria. The PPV and NPV of CareStart to diagnose *P. falciparum* were 83.78% and 85.12% respectively. The corresponding sensitivity and specificity of Care Start for the diagnosis *P. vivax* malaria were 86.96% (95% CI = 66.41% to 97.22%) and 95.62% (95% CI = 90.71% to 98.38%), respectively, with 76.92% PPV and 97.76% NPV. The sensitivity, specificity, PPV, and NPV of Care start™ for the diagnosis of mixed infection (*P. Falciparum* and *P. vivax)* was 83.33%, 91.56%, 27.78%, and 99.30% respectively (Table 7). Accordingly, the overall agreement between Light microscopy and Care Start ™ malaria RDT for specific identification of malaria species is moderate with kappa value of 0.568, P<0.01.

Table 7: Performance characteristics of Care Start ™ by species identified in comparison to the standard light microscopy, Arbaminch 2015.

	Sensitivity % CI	Specificity % CI	PPV % CI	NPV % CI
For *P. falciparum*	63.27% [48.29 -76.58]	94.50% [88.40 -97.95]	83.78% [67.99 - 93.81]	85.12% [77.51- 90.94%]
For *P. vivax*	86.96% [66.41- 97.22]	95.62% [90.71- 98.38]	76.92% [56.35- 91.03]	97.76% [93.60- 99.54]
For mixed infection	83.33% [35.88-99.58%]	91.56% [86.00 - to 95.43]	27.78% [9.69 - 53.48]	99.30% [96.14- 99.98]
Overall performance	97.44% [91.04 - 99.69]	93.67% [85.84- 97.91]	93.83% [86.18- 97.97]	97.37% [90.82-99.68]

5.5. Parasite density

Parasite density was done by measuring the level of infection in red blood cells by examination of a thin blood film. To quantify malaria parasites against RBCs, parasitized RBCs among 2,000 RBCs on the thin smear were counted and result expressed as Percent parasitemia. In this study the percent Parasitimia ranged from 0.05 % up to 2.85%. On analysis of the diagnostic performance of Care start ™ RDT by percent parasitemia, generally the sensitivity of the RDT increases when the percent parasitemia increase in the individual study subjects. Assessment of sensitivities at percent parasitemia thresholds below 0.049 % were not displayed due to there is no positive test result by microscopy within that percent parasitemia range. Exceptionally, at percent parasitemia from "0.05%- 0.50 %", the RDT showed better performance with sensitivity of 97.44 % than that with range from "0.51% - 1.00%" sensitivity of 96.15%. Starting from 1.01% parasitemia up to the maximum percent parasitemia (2.85 %), the care start RDT showed a 100 % sensitivity. Association of percent parasite load with RDT and Microscopy result is presented in table 9 below.

Table 8: Association between sensitivity of Care start ™ and percent parasitemia, Arbaminch, 2015.

Range of Percent parasitemia	Sensitivity % CI
< 0.049 %	ND
0.05 % - 0.50 %	97.44 % [86.52 - 99.94]
0.51 % - 1.00 %	96.15 % [80.36 - 99.90]
1.01 % - 1.50 %	100 % [59.04 - 100]
1.51 % - 2.00 %	100 % [15.81 - 100]
>2.01 %	100 % [39.76 - 100]

ND – Not displayed

Table 9. Association of percent parasite load with RDT and Microscopy result, Arbaminch 2015.

Parasite load percent			Microscopy result		Total
			Positive	Negative	
<0.049 %	RDT result	Positive		5	5
		Negative		74	74
		Invalid		1	1
	Total			80	80
0.05% -0.5 %	RDT result	Positive	38		38
		Negative	1		1
		Invalid	1		1
	Total		40		40
0.51% -1.0 %	RDT result	Positive	25		25
		Negative	1		1
		Invalid	1		1
	Total		27		27
1.01 % -1.5%	RDT result	Positive	7		7
	Total		7		7
1.51% - 2.0%	RDT result	Positive	2		2
	Total		2		2
>2.01%	RDT result	Positive	4		4
	Total		4		4
Total	RDT result	Positive	76	5	81
		Negative	2	74	76
		Invalid	2	1	3
	Total		80	80	160

Care Start ™ combo test for the detection of *P. falciparum* had a sensitivity of 100% for study participants with percent parasitemia > 1.51%. As indicated below (Table 10), the sensitivity of the

test kit declined from 80.77% (for P.f) and 90.91 % (for P.v) to 46.67 % and 75.00 % respectively, even though the percent parasitemia increased**.**

Table 10: Sensitivity and Specificity of Care Start ™ Malaria Pf/Pv Combo test for the diagnosis of malaria species infections at different levels of percent parasitimia, Arbaminch 2015.

	Sensitivity		**Specificity**	
Range of Percent parasitemia	***P. falciparum***	***P. vivax***	***P. falciparum***	***P. vivax***
< 0.049 %	ND	ND	ND	ND
0.05 % - 0.50 %	80.77 %	90.91 %	92.31%	88.89 %
0.51 % - 1.00 %	46.67 %	75.00 %	81.82 %	94.74 %
1.01 % - 1.50 %	0 %	100 %	100 %	100 %
1.51 % - 2.00 %	100 %	ND	ND	ND
>2.01 %	100 %	100 %	100 %	100 %

ND – Not displayed

5.6. Performance at Arbaminch Hospital and Arbaminch health center

During the study period study participants were included from both Arbaminch Hospital and Arbaminch health center. More participants 95 (59.4%) were recruited from Arbaminch Hospital and the rest 65 (40.6%) were from Arbaminch Health Centre. The RDT evaluated not presented the same overall performance at Arbaminch Hospital and Arbaminch health center. It showed better performance at Arbaminch health center with sensitivity, specificity, PPV, and NPV of 96.07%, 80%, 92.45% and 88.88% respectively. The corresponding sensitivity, specificity, PPV, and NPV at Arbaminch Hospital was 83.78%, 92.75%, 86.11% and 91.42%.

6. Discussion

Using rapid diagnostic testing devices to test the presence or absence of malaria parasite has many advantages. Specially in countries like Ethiopia where the majority of the population living in rural part of the country where access to electricity, infrastructures are limited and very little well trained malaria microscopists using RDTs as a diagnostic testing methods as well as treatment monitoring method has great advantage. But, the most important point not to be forgotten is since the sensitivity as well as specificity of these diagnostic testing methods may vary from population to population even in the same country, there diagnostic performance should be tested periodically to counter check wither they are with the WHO minimal sensitivity and specificity for any RDTs.

In this study, Care start ^{TM} combo test showed an overall sensitivity, specificity, PPV, and NPV of 97.44%, 93.67%, 93.83%, and 97.37% respectively. The sensitivity of the devise evaluated is better when compared with other studies (3, 4, 17, 21, 27) carried out in different parts the world. This may be due to the superior reference method (PCR) used (4, 21) and variation in the type of RDT kit evaluated(17, 27). Variation occur among malaria RDT products even though the principle are similar. However some other studies (19, 25) demonstrated better sensitivity than ours. The specificity of the devise evaluated is reduced when compared with other studies (17, 19, 21, 25, 27).

It showed a better performance when compared with the study conducted between China-Myanmar endemic borders with sensitivity and specificity for the diagnosis of malaria 89.68% and 98.26% respectively (21). This better performance may be due to the better reference method they used to counter check the standard microscopy. In contrast when compared with the study conducted on acute febrile patients visiting Felegeselam Health Center with 99.8% sensitivity and 97.7% specificity (19), our device (CareStart^{TM} RDT) showed poor overall performance. Our device has showed almost comparable overall performance with studies carried out in north-west Ethiopia with overall sensitivity of 95% and specificity of 94.2% (3) and at Serbo health center in Jimma zone, south-western Ethiopia with overall sensitivity 95.8% and specificity of 100% (35). This comparable performance may be due to almost similar method used for sampling as well as research procedure.

This study had also tried to evaluate the performance of the CareStart^{TM} RDT in detecting different species of malaria parasite. In this study, the sensitivity and specificity of Care Start ^{TM} combo test for the diagnosis of *P. falciparum* is 63.27 % and 94.3% respectively which is very poor

performance when compared to other studies with sensitivity and specificity of 99.4 % and 98 % in Wondo genet (29), 98.5 % and 98.0 % in Afar region (20), and 85.6 % and 92 % in Oromia Regional State (30), respectively. Variations in test sensitivity between these studies may be due to variations in epidemiologic characteristics of the study population, level of parasitemia, test methodology and skill of microscopists (10). In addition it may also be due to the chance of malaria RDT kits to give mixed infection for study participants who have only *P. falciparum* is very high, according to the explanation given by the malaria microscopist and it needs further study for scientific explanation. In our study 13 (8.12%) study participants which are *P. falciparum* by the standard microscopy and senior malaria microscopist analysis were found to be mixed infection by the Care Start ™ combo test.

Even though the sensitivity and specificity of Care Start ™ combo test for the diagnosis of *P. vivax* is better than for that of *P.falciparum*, with 86.96 % and 95.62 % respectively, it has almost comparable performance for the diagnosis of *P. vivax* with the study carried out at three health centers in Jimma zone, Oromia Regional State (30). It has showed a better sensitivity for *P. vivax* diagnosis when compared with a study finding at USA on a panel of stored blood samples (11) and a study conducted in Colombia (24). This may be due to the superior reference method (PCR) used and variation in the type of RDT kit evaluated in those studies. It showed poor performance when compared with other studies in Wondo Genet (99.4%, 98.2%) (29), Afar (100%, 99.6%) (20) and China-Myanmar (90.77%, 100%) (21).

Although the manufacturer's instructions were strictly followed, the poor performance of this RDT kit in the current study could be due to the high false positive results possibly because of the persistent nature of HRP-2 (36).

Accordingly, even though there was a very good agreement between the light microscopy and Care Start ™ RDT to detect the presence or absence of malaria parasite with a Kappa value of 0.877, the overall agreement between Light microscopy and Care Start ™ malaria RDT for specific identification of malaria species is very poor with kappa value of 0.568.

The performance of Care Start ™ RDT was noted to be significantly influenced by level of percent parasitemia. In this study the percent Parasitimia ranged from 0.05 % up to 2.85%. On analysis of the diagnostic performance of care start ™ malaria RDT by percent parasitimia, as expected the sensitivity and specificity of the RDT increased when the percent parasitimia increase in the

individual study subjects. Similar scenario was observed in other studies (11, 17, 20, 24, 26-28) except the method of parasite density estimation they used which is parasite count against WBC in thick blood film. It was observed that four study participants with lower percent parasitemia with in the range of "0.05% -0.5 %" and "0.51% -1.0 %" were positive with Microscopy but the result of RDT was two negative and two invalid result. This shows how much RDT kits possibly will be affected by parasite density.

It was noticed that the RDT kit used has demonstrated a better performance at Arbaminch health center. This better performance at Arbaminch health center with sensitivity and specificity of 96.07% and 80% respectively than at Arbaminch Hospital may be due to the fact that health center staffs routinely practice malaria microscopic examination than those hospital staffs. As it is observed during data collection period, malaria microscopy is the major routinely practiced activity in Arbaminch health center than Arbaminch Hospital, which may have its own contribution for the variation in the performance of the kit evaluated in this different setups. The other reason for poor performance at Hospital may be most patients visit hospital after being chronically diseased and even after taking anti-malaria drugs, which has direct effect on the diagnostic accuracy of either the RDT kit or microscopy. For example, 14 (21.53%) of study participants from Arbaminch health center came to the facility during 1st day of onset of malaria symptom but only four (4.21%) participants came Arbaminch Hospital during 1st day of onset of malaria symptom, which may be the reason for the poor performance.

7. Strength and limitation of the study

The strength of this study was all the laboratory procedures were performed strictly following the standard operating procedures and all discordant results obtained during quality control between malaria slide readings at health facility and at college laboratory was analyzed by well experienced expert microscopist who is blinded both for the slide readings as well as the RDT results. The main limitation of the study was not using PCR as a reference method, since it is known that microscopy is imperfect and its sensitivity decrease with decreased parasite density.

8. Conclusion and Recommendation

In this study, the RDT kit showed good performance in detection of malaria which is almost comparable performance with the Gold standard microscopy method. So that it can be used in areas where there are no trained personnel's, electricity, necessary equipment's, and reagents which are necessary for malaria microscopy. Since the performance of RDT may vary with various factors such as loss of integrity of kits during transportation, deferent climate condition and /or in appropriate storage condition, frequent local sensitivity testing should be carried out.

Specially, as the performance of currently used RDT was poor for accurate diagnosis of *P.falciparum* or *P.vivax*, Gamo Gofa zone health department should select and purchase the right type malaria RDT which display good performance by conducting area specific performance evaluation tests. According to our finding, the kit has still drawback in correctly identifying malaria species, further studies should be carried out to use the CareStart Malaria Pf/Pv Combo Test instead of microscopy for the diagnosis of malaria in areas where microscopy is limited and malaria caused by P. vivax and P. falciparum is co endemic, like Arbaminch.

Parasite load was estimated based on percent parasitemia of infected RBCs and direct relationship was observed with the performance CareStart Malaria Pf/Pv Combo Test. As expected and similar to other study findings, the device we used revealed that when the percent of infected RBCs increase the sensitivity and specificity of the device increased. Similarly it was observed that with lower parasite density, the ability of the kit evaluated presented poor performance, so that I recommend the manufacturer as well as other concerned bodies for further studies in the fields of malaria RDTs.

Better performance of the kit was observed at Arbaminch Health center than Arbaminch Hospital from where more study participants 95 (59.4%) were recruited, with sensitivity, specificity, PPV, and NPV of 96.07%, 80%, 92.45% and 88.88% respectively. The corresponding sensitivity, specificity, PPV, and NPV at Arbaminch Hospital was 83.78%, 92.75%, 86.11% and 91.42%. Different level of performance of the RDT among Arbaminch Hospital and Arbaminch health center observed may be due to variation in the competence of laboratory technicians on malaria diagnosis by both RDT and microscopy, so that the health department should provide refreshing training on malaria diagnosis frequently. The other reason may be due to the fact that most study participants

came to Arbaminch health center during the early symptoms not having anti-malarial drugs which has contributing factor for good performance the kit evaluated. So that my recommendation also goes to Gamo Gofa health department to create public awareness that the community should visit health facilities during early symptoms of malaria.

9. References

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Annexes

Annex 1 | Informed consent form

A | PURPOSE OF THE STUDY

To diagnose malaria precisely, we need to do laboratory tests. Rapid tests to diagnose malaria within 30 minutes (RDTs) are now available but we do not know if they are accurate or reliable. The main purpose of this study is to evaluate the performance of CareStart^{TM} combo test RDT for the diagnosis of malaria. We would like to compare the result of this rapid test with the results of a laboratory-based Geimsa stained slide microscope test to see if they are as accurate as microscope result.

B | STUDY PROCEDURES

If you agree to participate in the study, you was assigned a study ID number. We will prick your finger and take a drop of blood from you. Your name will not appear on any specimens or study forms.

C | VOLUNTARY PARTICIPATION

Your decision not to participate or to withdraw from participation will not affect the care you will receive at the clinic in any way. Even if you do agree to become a study participant, you can withdraw from the study at any time (verbally).

D | DISCOMFORT AND RISKS

You might feel a small amount of discomfort or have a small amount of bruising on your finger where the blood will be taken.

E | BENEFITS

There will be no immediate benefits from your participation in the study. When the study results are known and if the rapid tests are acceptable in terms of accuracy, everyone who comes to the clinic could benefit from having this test available to diagnose malaria and receive the right treatment the same day.

F | COMPENSATION

There will be no monetary compensation for this study, but routine medical consultation and appropriate referral services are available.

G | CONFIDENTIALITY STATEMENT

The records concerning your participation are to be used only for the purpose of this research project. Your name will not be used on any study forms or labels on laboratory specimens or in any

report resulting from this study. At the beginning of the study, we will give you a study identification number and this number will be used on the forms and on the laboratory specimens. Any information obtained in connection with this study will be kept strictly confidential. Only members of the study team will have access to information linking your name with your study number.

H | QUESTIONS AND FREEDOM TO WITHDRAW FROM THE STUDY

You can withdraw from the study at any time without affecting your present or future medical care at the clinic. You can contact any of the study personnel if you have questions about the research.

I, RESULTS PUBLICATION

When the researchers have analyzed the data, the results and the explanation of its implications will be posted at the hospital for everyone's information.

J | PARTICIPANT STATEMENT

I have been informed verbally and in writing about this study and understand what is involved. I also know whom to contact if I need more information. I understand that confidentiality will be preserved. I understand that I am free to withdraw from the study at any time without affecting the care I normally receive at the clinic. I agree to participate in this study as a volunteer subject and will be given a copy of this information sheet to keep.

_____ _____

Date Name of participant

_____

Signature (or thumb print or cross) of participant

_____ _____

Date Name of guardian

_____

Signature (or thumbprint or cross) of guardian and relationship to participant

K | INVESTIGATOR'S STATEMENT

I, the undersigned, have defined and explained to the volunteer in a language he/she understands, the procedures of this study, its aims and the risks and benefits associated with his/her participation. I have informed the volunteer that confidentiality will be preserved, that he/she is free to withdraw from the trial at any time without affecting the care he/she will receive at the clinic. Following my definitions and explanations the volunteer agrees to participate in this study.

_____ _____

Date Name of investigator who gave the information about the study

Signature: _____

Annex 2 | Outline of data collection form

Addis Ababa University

Department of clinical laboratory management and quality assurance

Interview Questionnaire on "Performance evaluation of CareStart™ Malaria Pf/Pv Combo Test in Arbaminch General Hospital and Arbaminch health center, Gamo Gofa zone, South Ethiopia."

ID No. _____ Date: ___/___/__ __ __ __ Time: ----------

dd /mm/ yyyy

1. PARTICIPANT DETAILS

AGE _____ (in yrs.)

SEX M F

Address _____

Duration in the specified address _____

2. CURRENT SYMPTOMS

Duration of current illness _____days

Symptoms *(current*)

Fever [1] Yes___ [2] No___

Chills [1] Yes___ [2] No___

Sweating [1] Yes___ [2] No___

Headache [1] Yes___ [2] No___

Others [1] Yes___ [2] No___ Specify_____

3. TREATMENT

(*In the two weeks, what type of medicines have you taken?*)

Couartum [1] Yes___ [2] No___

Chloroquine [1] Yes___ [2] No___

Sulfadoxine/Pyrimethamine [1] Yes___ [2] No___

Primaquine [1] Yes___ [2] No___

Others [1] Yes___ [2] No___ Specify_____

(*Do you keep any malaria medicine at home?*)

Access to medication [1] Yes___ [2] No___

RDT RESULTS (*On separate form*)

ID No. _____ Date: ___/___/__ __ __ __ Time: ----------

dd /mm/ yyyy

Time blood drawn: ----------

Line 1	Line 2	Control line	Result

0 (no band), 1+ (weak band), 2+ (medium band), 3+ (strong band)

Comments _____

(E.g. flow problems, background staining)

---- --------- -------- --- - -- - - - - - - - -- -- - - - -- - - - - - - - -- - - - - - - - - - - - - - - -- - - - --- - - - --

Microscopy result

1. Hemoparasite (malaria species) seen: - [1] Yes___ [2] No___ if yes,

Plasmodium falciparum	Plasmodium vivax	Mixed infection	Other

Annex 3 (Amharic version)

በጥናቱ ለመሳተፍ ፍቃደኝነት መጠየቂያ ቅጽ

ሀ. የጥናቱ አላማ / እቅድ

የወባ በሽታን በትክክሉ መርምሮ ለማወቅ ማለትም በአንድ ሰው ላይ መኖር አለመኖሩን ለማረጋገጥ የግድ የቤተ-ሙከራ ፍተሻ ማድረግ ያስፈልጋል፡፡ ፈጣን የወባ መመርመሪያ መሳሪያዎች አሁን በብዛት ይገኛሉ፡፡ይሁን እንጂ በትክክሉ ወባ መኖር አለመኖሩን ያረጋግጡ እያረጋገጡ በተጨባጭ በተለያዩ የመልካ-ምድር ና የአየር ሁኔታ መረጋገጥ አለባቸው፡፡የዚህ ጥናትም ዋነኛ ዓላማ "CareStart™" የተባለውን የፈጣን ወባ መመርመሪያ መሳሪያ ከማይክሮስኮፕ ውጤት ጋር በማወዳደር በአርባምንጭ ከተማና በአካባቢዋ የሚፈለገውን ውጤት እያመጣ መሆን አለመሆኑን መፈተሽ ነው፡፡

ለ. የጥናቱ ሂደት

በጥናቱ ለመሳተፍ ፈቃደኛ ከሆንክ/ሽ፣የግል የመለያ ቅጥር ይሰጥሃል/ሻል ከዛም በመቀጠል ከጣትህ/ሽ ላይ ትናንሽ የደም ናሙና ይወሰዳል፡፡ስምህ/ሽ በማናቸውም ሁኔታ በናሙናው ላይም ሆነ በጥናት ወረቀቱ ላይ አይኖርም፡፡

ሐ. በፍቃደኝነት ላይ የተመረኮዘ ተሳትፎ ስለመሆኑ

በጥናቱ ላይ ያለመሳተፍ ውሳኔህ/ሽ ወይም በማንኛውም ሰዓት ከተሳትፎ ራስህን/ሽን ማግለል፣በህክምና ተቋሙ የምታገኘውን/ኚውን የህክምና አልግሎት በምንም አይነት እንዲቀንስ አያደርገውም፡፡ በጥናቱ ለመሳተፍ ተስማምተህ/ሽ ከሆነ እንኳን በማንኛውም ሰዓት ከጥናቱ ራስህን/ሽን ማግለል ይቻላል፡፡

መ. አለመመቸት ወይም አስጊ ሁኔታ

በተወሰነ መልኩ ትንሽ ያለመመቸት/ ህመም ሊኖር ይችላል፡፡ያም የሚሆነው የደም ናሙና ከጣትሽ ላይ ሲወሰድ ነው፡፡ከዚህ በዘለለ ምንም ዓይነት አስጊ ሁኔታ አይኖርም፡፡

ሠ. ጥቅም

በጥናቱ መሳተፍህ/ሽ ወዲያውኑ የምታገኘው/ኚው ጥቅም ቢኖር ወባ ከተገኘ አስፈላጊው ህክምና በነጻ እንድታገኝ/ኚ ይደረጋል፡፡ በዘላቂነትም የጥናቱ ውጤት ሲታወቅ እና **CareStart™ Malaria Pf/Pv Combo Test** የተባለው ፈጣን የወባ መመርመሪያ መሣሪያ ተቀባይነት ያለው፣በትክክል ወባን መመርመር የሚያስችል መሳሪያ መሆኑ ሲረጋገጥ ማንኛውም ሰው ወደ ጤና ተቋሙ በመምጣት ጥራቱን የጠበቀ አጭር ጊዜ የሚፈጅ መመርመሪያን ተጠቅሞ ትክክለኛውን መድሃኒት እንዲወስድ ያስችላል፡፡

ረ. ማካካሻ

በጥናቱ በመሳተፍህ/ሽ ምንም አይነት የገንዘብ ማካካሻ አይኖርም ነገርግን ዘወትር የሕክምና ምክር አገልግሎት እንደሁም የተሻለ የጤና አገልግሎት በማግኘት ተጠቃሚ እንዲሆኑ ያስችላል፡፡

ሰ. የመረጃህ/ሽ ደህንነት

ካንተ/ቺ የተወሰዱት መረጃዎች ለዚህ ጥናት ብቻ ነው አገልግሎት ላይ የሚውሉት፡፡ስምህ/ሽ በማንኛውም ሁኔታ በጥናቱ ውስጥ እንዲሁም በቤተሙከራ ናሙና መያዣ ላይ አይለጠፍም፡፡በጥናቱ መሳተፍህ/ሽ የሚስጥር ቁጥር ይሰጥሃል/ጥሻል፡፡ያየሚስጥር ቁጥር በመረጃ መሰብሰቢያ ቅጽ እንደሁም በናሙናው ላይ ይኖራል፡፡ከጥናት ቡድኑ አባላት በስተቀር ይህንን ጥናት አስመልክቶ ማንኛውም መረጃ በሚስጢር ይቀመጣል፡፡

ሸ. <u>ጥያቄዎች እና ከጥናቱ ራስን የማግለል ነፃነት</u>

በማንኛውም ሰዓት ራስን ከጥናቱ ማግለል ይቻላል፡፡ይህም በምንም አይነት መልኩ የአሁን ወይም የወደፊት የህክምና አገልግሎት ተጠቃሚነትን አያስተጓጉልም፡፡ስለ ጥናትና ምርምሩ ምንም አይነት ጥያቄ ካለዎት የጥናት አባላትን መጠየቅይ የቻላል፡፡

ቀ. <u>የጥናት ውጤት ማሳወቅ</u>

ተመራማሪዎቹ መረጃውን ካመኑ በኋላ የጥናቱ ውጤት እና መግለጫዎች ሁሉም ተገልጋይ እንዲያዩ በሆስፒታሉ የሚለጠፉ ይሆናል፡፡

በ. <u>የጥናቱ ተሳታፊ አስተያየት</u>

ስለ ጥናቱ አላማ እንዲሁም ከኔ ምን አንደሚጠበቅ በቃልም ሆነ በጽሁፍ የቀረበውን በሚገባ ተረድቻለሁ፡፡ተጨማሪ መረጃም ከፈለጉ ማንን እንደማናግርም አውቂያለሁ፡፡የሰጠውት መረጃም በአግባቡ እንደሚጠበቅ ተረድቻለሁ፡፡በማንኛውም ሁኔታ ከጥናቱ ራሴን ማግለል እንደምችል ይህም በምጠቀመው የጤና አገልግሎት ላይ ምንም አሉታዊ አስተዋጽኦ እንደሌለው ተረድቻለሁ፡፡በገዛ ፈቃዴ በዚህ ጥናት ለመሳተፍ ተስማምቻለሁ፡፡

_____ _____

ቀን የጥናቱ ተሳታፊ ስም

_____

ፊርማ /የተሳታፊ/

_____ _____

ቀን የአሳዳጊ /ጠባቂ/

_____

ፊርማ የአሳዳጊ /ጠባቂ/ ስም

ተ. <u>የተመራማሪው አስተያየት</u>

እኔ ከታች የፈረምኩት፤ ስለጥናቱ ሂደት፣ ዓላማውን፣ጥቅሙን እንደሁም ሊያስከትል የሚችለውን መጠነኛ ህመም /ጣታቸው ሲወጋ/ የጥናቱ ፈቃደኛ ተሳታፊዎች በሚገባቸው ቋንቋ ገለፃ አድርጊያለሁ፡፡የሰጡት መረጃ በሚስጢር እንደሚጠበቅ እንዲሁም በማንኛውም ሰዓት ከጥናቱ ራሳቸውን ሊያገሉ እንደሚችሉ ተነግሮዓቸዋል፡፡ገለፃውን ተከትሎ በጐፈቃደኛው/ዋ በጥናቱ ለመሳተፍ ፈቃደኛ ሆነዋል፡፡

_____ _____

ቀን የጥናቱ ተመራማሪ ስም

_____

ፊርማ /የ ተመራማሪ/

<u>የመረጃ መሰብሰቢያ ቅጽ</u>

Addis Ababa University

Department of clinical laboratory management and quality assurance

"Performance evaluation of CareStart™ Malaria Pf/Pv Combo Test in Arbaminch General Hospital and Arbaminch health center, Gamo Gofa zone, South Ethiopia."

የቃለ መጠይቅ መጠየቂያ ቅጽ

የመለያ ቁጥር _____ ቀን፣ _____/_____/____ ሰዓት_____

ቀን ወር ዓ.ም.

1. **<u>የተሳታፊ መረጃ</u>**

   እድሜ ____

   ፆታ፡ ወንድ☐ ሴት☐

   በአሁኑ ሰዓት የሚሰማ የሕመም ስሜት

   ህመሙ ከጀመረ አንስቶ የፈጀው ጊዜ ____ ቀን፡፡

2. **<u>ምልክቶች</u>**

   ትኩሳት አለ☐ የለም☐

   ብርድ ብርድ ማለት አለ☐ የለም☐

   ማላብ አለ☐ የለም☐

   ራስምታት አለ☐ የለም☐

   ሌላ አለ☐ የለም☐ ግለጽ _____

3. **<u>የወሰድከው/ሽው መድሃኒት</u>**

   በባለፉት ሁለት ሳምንት ምን አይነት መድሃኒት ወስደሃል/ሻል?

   Courtum አዎ☐ አይ☐

   Chloroquine አዎ☐ አይ☐

   Sulpodoxine አዎ☐ አይ☐

Primaquine አዎ ☐ አይ☐

ሌላ አዎ ☐ አይ ☐ ግለጽ _____

የፈጣን የወባ *መመርመሪያ* መሳሪያ ውጤት

የመለያ ቁጥር _____ _____/_____/____ ሰዓት_____

ቀን ወር ዓ.ም.

ደም የተቀዳበት ሰዓት -------

መስመር አንድ	መስመር ሁለት	መቆጣጠሪያ መስመር	ውጤት

0 (መስመር የለም) 1+ (ደካማ መስመር) 2+ (መካከለኛ ድምቀት ያለው መስመር) 3+ (ጠንካራ ድምቀት ያለው መስመር)

አስተያየት _____

_____

Microscopy

Plasmodium falciparum	Plasmodium vivax	ቅልቅል /ሁለቱም/	ሌላ

አስተያየት _____

Annex 4: Malaria light microscopy procedure

- Clean the finger with cotton wool dampened with alcohol.
- Dry the finger with a clean cotton cloth, using firm strokes to stimulate blood circulation.
- Using a sterile lancet and a quick rolling action, puncture the ball of the finger or toe.
- Apply gentle pressure to the finger or toe and express the first drop of blood; wipe it away with dry cotton wool.
- Apply gentle pressure to the finger and collect a single small drop of blood medium sized on the middle of the slide for the thin film.
- Apply further gentle pressure to express more blood, and collect two or three larger drops on the slide, about 1 cm away from the drop intended for the thin film.
- Wipe the remaining blood off the finger with cotton wool.
- Using another clean slide as a spreader and with the slide with the blood resting on a flat, firm surface, touch the small drop of blood with the edge of the spreader, allowing the blood to run right along the edge.
- Handling the slides by the edges or a corner, make the blood film by using the corner of the spreader to join the drops of blood, and spread them to make an even, thick film.
- Fix the thin film by dabbing it with a pad of cotton wool dampened with methanol or by briefly dipping the film into methanol.
- Avoid contact between the thick film and methanol, as methanol and its vapors quickly fix the thick film, and it does not stain well.
- Make up a 10% solution of Giemsa in the buffered water by mixing three drops of Giemsa from the stock solution, using the Pasteur pipette, with 1 ml of buffered water.
- Pour the stain gently under the staining tray until each slide is covered with stain, or gently pour the stain onto the slides lying face upwards on the plate or rack.
- Stain the films for 8–10 min.
- Gently wash the stain from the slide by adding drops of clean water.
- Place the slides in the drying rack, film side downwards, to drain and dry.
- Place the slide on the microscope stage, with the blood film uppermost.
- Place one or two drops of immersion oil on the area of the blood film to be examined.
- Examine with the x100 oil immersion objective both thick film and thin film.

Annex 5: CareStart^{TM} RDT procedure

1. Collect the blood sample (5$\mu\ell$) using a pipette provided or micropipette
2. Add 5 $\mu\ell$ of whole blood into the 'S' well.
3. Add 60 $\mu\ell$ assay buffer solution (3 drops for vial type or 2 drops for bottle type) into the "A" well.
4. Start a timer
5. Read result in 20 minutes.

Annex 4: Declaration

I the undersigned, declare that this is my original work and has not been presented for a degree in this or any other university and all sources of materials used for this thesis have been acknowledged.

Name: Temesgen Eticha

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia.

Date of submission _____

This proposal has been submitted with my approval as University Advisor

Name _____

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia.

Name _____

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia.