

**ST. PAUL'S HOSPITAL MILLENNIUM MEDICAL COLLEGE DEPARTMENT OF
PUBLIC HEALTH**



**ETHIOPIAN FIELD EPIDEMIOLOGY AND LABORATORY TRAINING PROGRAM
(EFELTP)**

COMPARISON OF CARE START™ HRP2/pLDH COMBO RAPID MALARIA TEST WITH
LIGHT MICROSCOPY AMONG GOVERNMENTAL HEALTH INSTITUTIONS IN METEKEL
ZONE, BENISHANGUL- GUMUZ REGION, ETHIOPIA, 2018.

BY

CHEMIREW EJIGU (B.Sc.)

**THESIS SUBMITTED TO THE DEPARTMENT OF PUBLIC HEALTH IN PARTIAL
FULFILLMENT FOR THE DEGREE OF MASTER OF PUBLIC HEALTH IN FIELD
EPIDEMIOLOGY AND LABORATORY TRAINING PROGRAM
(EFELTP)**

Advisors:

- 1. Mr. TEMESGEN GELETA(MPH)**
- 2. Mr. GETACHEW TILAHUN (MPH)**

May, 2018

Addis Ababa, Ethiopia

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Acronyms and Abbreviations

AIDS	Acquired Immuno-Deficiency Disease
ASL	Above Sea Level
BGR	Benishangul- Gumuz Region
B.Sc.	Bachelor of Sciences
CDC	Centers for Disease Control and Prevention
EFELTP	Ethiopian Field Epidemiology and Laboratory Training Program
EHNRI	Ethiopian Health and Nutrition Research Institute
EPHI	Ethiopian Public Health Institute
EQA	External Quality Assurance
ERB	Ethical Review Board
FMOH	Federal Ministry of Health
HEWs	Health Extension Workers
HIV	Human Immuno-Deficiency Virus
HRP-2	Histidine Rich Protein-2
IQC	Internal Quality Control
IRS	Indoor Residual Spray
K.M	Kilo Meter
LLINs	Long Lasting Insecticide Treated nets

LM	Light Microscopy
MDGs	Millennium Development goals
MPH	Master of Public Health
NPV	Negative Predictive Value
OPDs	Out Patient Departments
P	Plasmodium
pLDH	Plasmodium Lactate Dehydrogenase
PPV	Positive Predictive Value
R&D	Research and Development
RDTs	Rapid Diagnostic Tests
SNNPR	South Nations, Nationalities and People's Region
SPHMMC	St. Pawl's Hospital Millennium Medical College
SPSS	Statistical Package for Social Sciences
SSA	Sub-Saharan Africa
WHA	World Health Assembly
WHO	World Health Organization

Abstract

Background: In 2010, WHO recommended that, where possible all cases should be confirmed by parasitological diagnosis before treatment with anti-malarial medicines. Severe malaria disease needed to be confirmed by parasitological examination of the presence of falciparum malaria by either thick blood smear or RDTs although their sensitivity depends on parasitic density. The use of antigen-detecting rapid diagnostic tests (RDTs) provides the possibility of parasite-based diagnosis in areas where good quality microscopy cannot be maintained.

Objective: The purpose of the Research is to compare the performance of CareStart™ rapid malaria test in reference to the gold standard Light Microscopy among governmental health Institutions in Metekel Zone of Benishangul-Gumuz region.

Methods and Materials: A Cross-Sectional Study was conducted in Benishangul-Gumuz region to compare the performance of malaria diagnostic tests among acute febrile patients suspected for malaria who were visiting the Out patients (OPDs) of three Health institutions. A total of 2677 patients were included in the study. Data was collected using standardized questionnaire and laboratory request and entered, cleaned and analyzed using Epi-Info 7 and SPSS version 23 statistical software packages. The Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, Percent agreement and kappa Value was calculated.

Result: The overall sensitivity of CareStart Malaria RDT was 97.1% (95–98%, 95% CI) and its specificity was 99.5% (99.5–99.8%, 95% CI). Moreover, the overall Positive Predictive value (PPV) of the test kit was 96.8% (94–98%, 95% CI) and its Negative Predictive Value (NPV) was 99.5% (99.3–99.8%, 95% CI). The calculated sensitivity of the CareStart RDT for Plasmodium falciparum or mixed infection was 97.6% (95.5–99.7%, 95%CI) and the specificity of the kit for the same species was 99.5% (95.2–99.8%, 95%CI). In addition, a sensitivity of 95.2% (81.5–98.9%, 95%CI) and a specificity of 99.5% (99.2–99.8%, 95%CI) for Plasmodium vivax was found. There was an excellent agreement between the two test methods with a kappa value of 0.965 (0.927–1.0, 95%CI).

Conclusion: The CareStart RDT test showed high sensitivity and specificity with an excellent agreement to the reference light microscopy. Hence, the use of the RDT should be strengthened in place where use of light microscopy is limited.

Key Words: Blood Film, Malaria, Metekel, Malaria rapid diagnostic test, Microscopy.

1. Introduction

1.1. Background

The name malaria is derived from two Latin words mal; aria meaning “bad air”. This is due to the mistaken belief that it was caused by bad air following the observation that it occurred more frequently around damp places and marshy areas. Almost all races of human beings have at one time or another suffered from malaria [1, 2].

Malaria is an entirely preventable and treatable disease. It is caused by a microscopic protozoan parasite belonging to the genus *Plasmodium* (*P*), with four different species specifically infecting humans (*P. falciparum*, *P. vivax*, *P. Ovale* and *P.malariae*) and parasites of non-human primates, such as *Plasmodium knowlesi*, infecting humans occasionally. The four species of plasmodium have their respective morphology, biological and clinical characteristics. The *P. Ovale*, *P. vivax* and *P. malaria* are classified as relapsing malaria because they form resting stages in the liver as hypnozoites and can cause a secondary or persisting stage of their life cycle. It spreads from one person to another by the bite of certain sorts of *Anopheles* mosquitoes. There are more than 400 different species of *Anopheles* mosquito, but only 30 are malaria vectors of major importance [3].

Among the four species, *P. falciparum* is the most dangerous malaria parasite, the most prevalent on the African continent and responsible for most malaria related deaths. *P. vivax* has a wider distribution than *P. falciparum*, and predominates in many countries outside of Africa. Although *P. vivax* can occur in Africa, the high frequency of a genetic trait known as Duffy negativity among African populations makes many individuals resistant to infection with *P. vivax* [4].

As the malaria parasites enter the blood stream they infect and destroy red blood cells. Destruction of these essential cells leads to fever and flu-like symptoms, such as chills, headache, muscle aches, tiredness, nausea, vomiting and diarrhea. These initial symptoms are non-specific: in other words, they are self-reported symptoms that do not indicate a specific disease process [5].

Malaria remains the most important parasitic disease, being a major threat in the world and leading to some 600,000 deaths per year. These deaths are predominantly caused by *P. falciparum* in African

children younger than five years old, widespread morbidity, and an under-recognized burden of disease related to *P.vivax* that puts 2.3 billion people at risk, particularly in Asia [6, 7].

Of the approximately 3.2 billion people living in malarious countries, 1.2 billion are at high risk; the World Health Organization (WHO) states that there were 214 million (range 149 to 303 million) cases of symptomatic malaria in 2015 [8, 9].

Some population groups are at considerably higher risk of contracting malaria, and developing severe disease, than others. These include infants, children under 5 years of age, pregnant women and patients with HIV/AIDS, as well as non-immune migrants, mobile populations and travelers. World Health Organization recommends protection for all people at risk of malaria with effective malaria vector control. Two forms of vector control namely long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) are effective in a wide range of circumstances [5].

In addition to the ethical reasons, it is also the huge economic burden that calls for global action to reduce and eliminate this intolerable burden for the global benefit. Malaria is a disease of poverty, and malaria control and elimination is a contribution to effective and sustained poverty alleviation [10].

Diagnosis of malaria based on clinical signs and symptoms alone is not specific and usually leads to excessive use of ant malarial drugs [11]. As a result, the World Health Organization recommends that malaria should be confirmed by parasite based diagnosis before treatment is given. Severe malaria disease needs to be confirmed by parasitological examination of the presence of falciparum malaria by either thick blood smear or rapid diagnostic tests (RDTs) [12,13] although their sensitivity depends on parasitic density [14]. Thick blood smear microscopic examination is the gold standard diagnostic tool for both severe and uncomplicated malaria in many areas of sub-Saharan Africa, but its accessibility is limited [15] In areas where laboratory-based diagnostic service is not available, clinical signs and symptoms and use of RDTs are alternative approaches that could be adopted until a time when microscopic diagnostic services expand [11].

1.2. Statement of the Problem

Globally, an estimated 3.3 billion people, nearly half of the world's population, in 97 countries and territories are at risk of being infected with malaria, and 1.2 billion are at high risk i.e. greater than one case of malaria per 1000 population each year [16]. Each year, one million people die from malaria, and the number has been increasing over the past two decades [17]. Each year, about 19 to

25 million women in malaria endemic areas of Africa become pregnant and are at risk of malaria during pregnancy. It is estimated that at least 1.5 million pregnant women in sub-Saharan Africa (SSA) suffered from malaria and its adverse consequences [18].

In many malaria endemic countries, patients are only clinically diagnosed and a small proportion of malaria cases are tested, owing to a lack of diagnostic capabilities. Therefore, there is considerable uncertainty surrounding the estimates of the number of cases and deaths, mainly in the African region, and any attempt to establish the number of malaria cases globally is subject to argument [19]. At the clinic level, treatments are often given presumptively to patients presenting with fever or other symptoms compatible with malaria. The emergence and spread of resistance to chloroquine and other anti-malarial drugs have had a dramatic impact on the evolution of malaria mortality [20], especially in Africa [21] and have prompted to change to more expensive therapeutic combinations.

In the prevention and control of malaria, prompt and accurate diagnosis is the key to effective disease management [22]. However, in many countries malaria is being diagnosed clinically, an unreliable method leading to over-diagnosis and over-treatment [23]. Similarly, in Ethiopia, clinical diagnosis and empirical treatment has been the mainstay of malaria management in areas where laboratory facilities are not available. Due to the non-specific nature of signs and symptoms of malaria, clinical diagnosis is unreliable [22, 24, 25].

In Ethiopia, previous reports on malaria surveillance were based on microscopic results. The majority of these studies were conducted either with little or no information or with few technical resources [26-28]. LM remains preferred and standard for laboratory diagnosis of malaria and has been shown to detect about 75% of malaria infections in high transmission areas. However, it has weaknesses of missing up to 88% of infections in low transmission areas [29]. Moreover, the detection of low numbers of parasites, the labor-intensive procedure, its inaccessibility and lack of affordability in most peripheral health facilities in the country remain a hurdle in differentiating *Plasmodium* species by microscopy [30].

Additionally, microscopy is time consuming, requires trained personnel and needs careful preparation and application of reagents to ensure quality results [25, 31].

According to National Malaria Strategic plan 2014-2020, by 2017 and beyond, 100% of suspected malaria cases are diagnosed using RDTs or microscopy within 24 hours of fever onset. However,

there has been a problem in diagnosing malaria at health facility laboratories. Misdiagnosis of malaria parasite is common. Parasite species identification is not done properly in some laboratories. This problem needs to be addressed through establishing a laboratory diagnosis quality assessment system [32].

1.3. Significance of the Study

Generally, in Ethiopia and particularly in Beishangul-Gumuz Region, Hospitals and health centers diagnose malaria by LM, whereas the health posts employ RDT for diagnosis of *P. falciparum* and *P. vivax* [33]. The studies conducted in different regions of the country had generated the sensitivity, specificity and predictive values of the RDTs for both *P. falciparum* and *P. vivax* [33-35]. Moreover, the performance of these RDTs requires continuous monitoring and evaluation.

However, the study conducted in one of the health centers of the region generated the sensitivity, specificity and predictive values of the RDTs for only *P. falciparum* [36]. Hence, the performance of the RDT especially for detecting *P. vivax* in the region is not evaluated yet. Therefore, this study will help to generate the sensitivity, specificity and predictive values of the RDTs for both *P. falciparum* and *P. vivax*. in health facilities of Metekel zone as well as for the region moreover it will help to reveal current performance of the KIT in the Region being as one way of laboratory diagnosis quality assessment system.

2. Literature Review

In 2010, WHO recommended that, where possible all cases should be confirmed by parasitological diagnosis before treatment with anti-malarial medicines [37]. Severe malaria disease needs to be confirmed by parasitological examination for the presence or absence of falciparum malaria by either thick blood smear or RDTs although their sensitivity depends on parasitic density [38-41]. For more than a century, use of microscopy has been considered the ‘gold standard’ for malaria diagnosis, species identification, and to quantify parasitaemia [42] and it is the gold standard diagnostic tool for both severe and uncomplicated malaria in many areas of SSA, but its accessibility is limited [25]. In areas where laboratory-based diagnostic service is not available, clinical signs and symptoms and use of RDTs are alternative approaches that could be adopted until a time when microscopic diagnostic services expand [24].

A useful and effective RDT must have sufficiently high sensitivity to be able to accurately identify as many ‘true’ malaria cases as possible, especially in areas where reliable microscopy is not available or used infrequently. This is particularly important so that infections can be effectively and specifically treated based on parasite species. The screening sensitivity of an RDT can be influenced by the epidemiological characteristics and infection dynamics in the target population. As parasite antigen concentrations in the blood and parasitaemia levels can vary due to multiple host and parasite factors, the performance level of an RDT can be similarly affected depending on the malaria-endemic population involved in the product assessment [43].

The performance of disease screening test is described by the diagnostic strength of the association between the predictor variable (RDT result) and outcome variable (disease) as measured against a ‘gold standard’ test. The most common and useful complementary measures for evaluating a test are sensitivity (the proportion of true diseased persons in a population who are test positive – the true positive rate), specificity (the proportion of truly non-diseased persons who are so identified by the test – the true negative rate), and the positive and negative predictive values (the probability that the disease is present or not when the test is positive or negative, respectively) [44-45]. Another measure, test consistency describes diagnostic reliability (precision) and includes a measure of agreement (Kappa statistic) between one test and another (e.g., new test versus a ‘standard’) using dichotomous variables (malaria positive or negative). A Kappa score of 1 indicates perfect agreement, while 0 indicates the equivalent of chance having produced the apparent agreement [46, 47].

The use of antigen-detecting rapid diagnostic tests (RDTs) provides the possibility of parasite-based diagnosis in areas where good quality microscopy cannot be maintained [42]. There are numerous malaria RDTs that are commercially available in many formats, including plastic cassettes, cards or dipsticks, and quality depends on manufacturer as well as storage conditions [31]. The variation depends on malaria antigen targeted for detection in blood flowing along a membrane containing specific anti-malaria antibodies. Previous studies from clinical trials have found that 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, whilst sensitivity of pLDH tests for non-*P. falciparum* species was low [48,49]. Specificity of both types of tests was reported to be good (>85%).

The CareStart™ P.f/P.v combo test (detecting histidine-rich protein (pHRP-2) and *P. vivax*-specific lactate dehydrogenase (pLDH) has been evaluated in a few field settings with report of higher sensitivities for *P. falciparum* (99.4%) with parasite densities above 100/μl, and low sensitivities at parasite densities <100/μl (60.0%) in Ethiopia [37]. Moreover, increasing sensitivity at higher parasite densities (100% at >500/μl) was reported in Madagascar [50]. On other hand, sensitivities for detection of *P. vivax* were significantly higher than those found in our country with pLDH (pan) (91.0%), but similar results for the CareStart™ malaria pLDH (pan/Pf) reported in Myanmar [51]. The increase of sensitivities at higher parasite densities is a well-known phenomenon with break points around 100/μl (*P. falciparum*) and 500/μl (*P. vivax*) [38, 48, 51, 52].

The CareStart™ P.f/P.v combo test has been used by health extension workers (HEWs) at health posts in Ethiopia since 2005 [37-40]. It is a new tool for the rapid qualitative determination of malaria HRP-2 and pLDH in human blood, aiding the diagnosis of malaria infection.

According to the National Malaria Indicator Survey 2015, Overall, malaria prevalence among all age groups living in malarious and areas >2,000m and ≤2,500m Above Sea Level (ASL) by Rapid Diagnostic Test (RDT) and Light Microscopy (LM) was very low. The prevalence was 1.2% by RDT and 0.5% by microscopy. However, by region, Gambella (6%) and Benshangul-Gumuz Region (BGR) (3%) have the highest prevalence by microscopy [53].

The aim of the study is to evaluate the performance of CareStart™ malaria Pf/pan rapid test kit in reference to the gold standard LM in north-west Ethiopia.

3. Objectives

3.1. General objective

The purpose of the Research is to compare the performance of CareStart™ rapid malaria test in reference to the gold standard LM among governmental health facilities present in Metekel Zone, Benishangul-Gumuz Region.

3.2. Specific objectives

- ❖ To determine the sensitivity of CareStart™ rapid malaria test.
- ❖ To determine the specificity of CareStart™ rapid malaria test.
- ❖ To calculate the positive predictive value of CareStart™ rapid malaria test.
- ❖ To calculate negative predictive value of CareStart™ rapid malaria test.
- ❖ To evaluate the diagnostic agreement between CareStart™ rapid malaria test and LM.

4. Methods and Materials

4.1. Study Area

Metekel Zone is one of the three Zones found in Benishangul_Gumuz Region. The Zone's capital Gilgel Beles is at a distance of 547kms from Addis Ababa and 350 Kms away from the Region's capital City Assosa. It is bounded by North Gondar in the North, Kamash Zone in the South, Awi Zone in East and Sudan in the West direction. The area has an elevation between 1,000 and 1,050 m above sea level. The annual temperature of the area ranges from 28°C to 43°C, with average annual rainfalls of 1,050 ml. The Zone has total population of 375,890, of which 81,677 are urban residents and 294,213 are rural settlers. And 188,700 (50.2%) are males and 60,819 under five years, 45,019 (15-49 females), 1,535 pregnant women are found. The Zone has one General Hospital, 18 health centers and 167 health posts. It has sub-tropical weather condition with some low land areas and Malaria is the leading cause of morbidity among top ten diseases, followed by Pneumonia and Helminthiasis in adults and diarrhea leading in under five. Although malaria transmission is seasonal in most areas of Ethiopia, in selected study areas, malaria transmission takes place throughout the year.

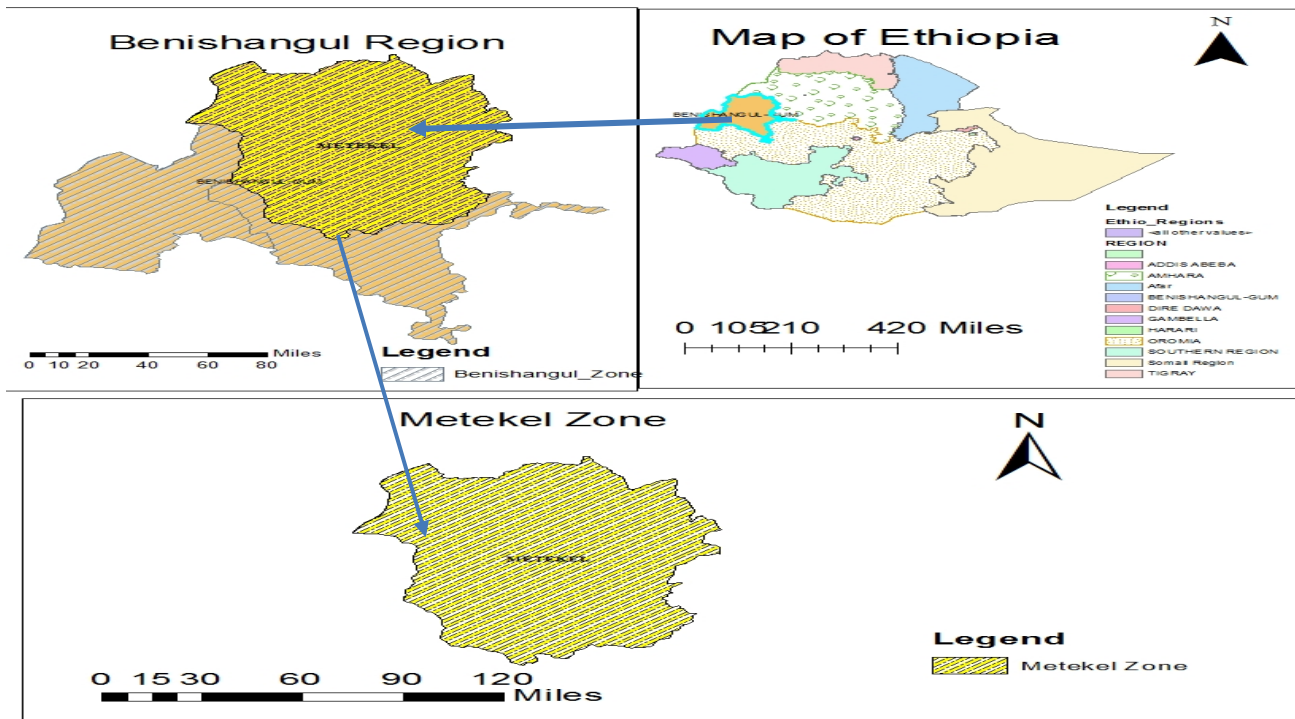


Figure 1: Demographic map of Metekel Zone, Benishangul-Gumuz Region, 2017.

4.2. Study Design and period

A Cross-Sectional Study was conducted from December 18/2017-January 8/ 2018.

4.3. Source Population

All acute febrile patients who visited the OPDs of the health centers and the Hospital during the study period were source population for the study.

4.4. Study Population

All malaria suspected acute febrile illness patients who visited the OPDs of Mambuk Health Center, Gelgel-Beles Health Center and Pawe Hospital during the study period were selected as study population.

Inclusion and Exclusion Criteria:

Eligible study subjects were those patients with acute febrile illnesses (body temperature $> 37.5^{\circ}\text{C}$) or history of fever during the last 2 weeks and suspected for malaria by the clinician at the date of data collection.

Exclusion Criteria:

Individuals who took anti-malarial drugs within the last 2 weeks before the data collection date or already known malaria patients whose test was requested for checking treatment effect were excluded from the study.

4.5. Sample Size Determination

According to Ethiopia's national Malaria Indicator 2015, BGR has 3% prevalence of Malaria using blood film microscopy [53]. Sample size was calculated to compare performance of the RDT to microscopy in detecting both *P. falciparum* and *P. vivax* at each site, assuming RDT sensitivity of $\geq 95\%$ and specificity of $\geq 80\%$, with 80% power and a 5% significance level [54, 55].

Sample size (n) based on sensitivity = $\frac{Z_{1-\alpha/2}^2 \times S_N \times (1 - S_N)}{L^2 \times Prevalence}$, and

sample size (n) based on specificity = $\frac{Z_{1-\alpha/2}^2 \times S_P \times (1 - S_P)}{L^2 \times (1 - Prevalence)}$,

where n = required sample size,

S_N = anticipated sensitivity,

S_P = anticipated specificity,

α = size of the critical region ($1 - \alpha$ is the confidence level),

$z_{1-\alpha/2}$ = standard normal deviate corresponding to the specified size of the critical region (α), and

L = absolute precision desired on either side (half-width of the confidence interval) of sensitivity or specificity.

Using the above formula, the required maximum sample size for Sensitivity is 2433 and 10% non-respondent 244. Therefore, total 2677 acute febrile cases suspected for malaria were included in the study.

4.6. Sampling Technique

Two health Centers and one General Hospital present in 10 to 20 km radius and equally endemic for malaria were conveniently selected. Among the OPDs in health facilities, all OPDs in each health center and two randomly selected OPDs in the hospital were included. Among acute febrile patients who visit the included OPDs, all patients suspected for malaria by the clinicians were sent to the laboratory and tested for malaria using both test methods until the desired sample size reached.

4.7. Study Variables

Dependent variable: Malaria Disease status by RDT and LM, Malaria Species by RDT and/or LM.

Independent variables: Age, sex, Residence, Ethnicity, Occupation, Religion, Marital Status, Education, clinical signs and symptoms.

4.8. Operational definition of terms

Sensitivity: the ability of malaria RDT to identify true cases; is the proportion of people with malaria who have a positive RDT for the disease.

Specificity: the ability of malaria RDT to identify true Negatives; is the proportion of people without malaria who have a negative RDT.

Negative Predictive Value (NPV): is the probability of not having malaria when the RDT result is negative (normal).

Positive Predictive Value (PPV): is the probability of the presence of malaria in a person with a positive (abnormal) RDT result.

-The proportion of malaria cases identified by RDT that is true

Malaria cases

Kappa Value (K): The fraction of the observed agreement not due to chance in relation to the maximum non-chance agreement when using a categorical classification of a variable.

4.9. Data Collection Method and Procedure

4.9.1. Data Collection Tool

The socio-demographic characteristics and clinical data of the participants were collected using a structured and pre-tested questionnaire which was adopted from WHO and FMOH.

Finger-prick samples were collected and placed in a grease-free, clean, glass slide. The same finger-prick blood sample was used to carry out the RDT in parallel, following manufacturer's instructions.

4.9.2. Data collection Procedure

The Nurses in the OPDs collected the socio-demographic characteristics and clinical data of the participants using a structured and pre-tested questionnaire.

The technologists in the laboratory collected and performed malaria diagnosis using both the RDT and LM methods. After Cleaning the area with an alcohol swab, Squeezing the end of the fingertip, piercing with a sterile lancet, and wiping away the first drop of blood, 5µl of whole blood was added into the sample well (small well) and two drops (60µl) of assay buffer was added into the buffer well. Then, blood was lyzed with buffer to release more Ag. Parasite antigen bound to the labeled antibody, buffer flushed labeled Ag-Ab complex along the test strip, some labeled Ag-Ab complex

was trapped on the test line, and excess labeled Ab was trapped on the control line. Finally, the test result was read within 20 min.

The same finger-prick samples were collected and placed in a grease-free, clean, glass slide. In a single slide, both thick and thin films were prepared. The thin films were fixed with Absolute methanol after air-drying, the slides were stained in 10% Giemsa solution for 15 min. Thin and thick films were read in the health centers and the Hospital laboratories by an experienced laboratory technician/technologist and the result was considered negative if no parasites seen after examination of at least 200 fields at 1,000x magnification. The technician/technologist was blinded to the results of the RDT.

All blood films of discordant results, all positive slides and 10% of negative slides were re-checked for the second time by an experienced Technologist at Benishangul-Gumuz Regional State Public Health Research Laboratory Institute who was also blinded to initial microscopy and RDT results. In cases of discordant results happened, a third expert lab technologist in BGR Public Health Research Laboratory Institute checked the slide reading. The results of the third expert reader were accepted final results.

4.10. Data Quality

The selected health center laboratories are currently involving in external quality assurance (EQA) at Benishangul-Gumuz Regional State Public Health Research Laboratory Institute and the Hospital's Laboratory is involving in EQA program at Ethiopian Public Health Institute (EPHI).

Additionally: The Questionnaire was pre-tested prior to use, Data collectors were trained on the test methods, the room Temperature of the laboratories was monitored daily, Buffer Tablet was used to maintain the pH of the Geimsa working solution every 24 hours, the quality of Geimsa working solution was checked using known positive and negative samples every week, all the test procedures and the interpretation of results were accomplished following the manufacturer's instructions and the principal investigator and Experienced technologists at the regional Laboratory analyzed all the discordant results, all positive slides and 10% of negative slides.

Moreover, prior to entering the data into the computer, the missing variables and consistency of filling of questionnaires and completeness of data was checked out consciously.

4.11. Data Analysis

Data that was gained from questionnaire and test result was checked for its completeness, consistency and entered in a computer then analyzed using Epi-Info and Statistical Software Packages version 23 (SPSS) for descriptive cross-tabulation. The Sensitivity, Specificity, Predictive Values and kappa Value were calculated.

4.12. Ethical issues

This work was realized based on the ethical clearance of the Ethical Review board(ERB) of St. Paul's Hospital Millennium Medical College (SPHMMC) and BGR Health Bureau. We had taken permission from the health facilities to use their laboratories. Written informed consent was signed by the participants to solicit their consent to participate in the study. Children ≤ 15 yrs were recruited in the study after obtaining informed assent from their parents or care-givers and we maintained the confidentiality of participants.

5. RESULT

Two thousand six hundred seventy-seven acute febrile cases were participated in the study. Among the participants 77.3 %(N=2068) were urban residents. Regarding their religion, 56.9% (N=1523) participants were Orthodox, 19.9% (N=533) were Muslims, 1.9% were Catholics and the 12% were other followers. Among those 2677 participants, 54.43% (N=1457) were females and the age range was 1 month–70years.

Among 2677 patients tested by blood smear microscopy (the Gold standard), (N=347) 12.96% of patients tested were positive for malaria parasites (Table 1).

Table 1: The positivity Rate of Malaria by CareStart malaria RDT test and BF, among Acute Febrile patients visiting three health facilities, Metekel Zone, Benishangul-Gumuz Region, 2018.

		Result by BF		Total
		Positive (N)	Negative (N)	
Result by RDT	Positive	337	11	348
	Negative	10	2319	2329
	Total	347	2330	2677

Among 347 positive patients tested by blood film, (N=208) 59.94% were infected with *P. falciparum*, (N=126) 36.31% were infected with *P. vivax* and (N=13) 3.75% were infected with both *P. falciparum* and *P. vivax*. (Table2).

Similarly, by the CareStart malaria RDT test, (N=348)13.0% of 2677 patients tested for malaria were positive for malaria parasites. Among 348 people with malaria, 60.34% of the cases were infected with *P. falciparum*, 37.36% were infected with *P. vivax* and the remaining2.3% were infected with mixed infection (*P. falciparum* and *P. vivax*) (Table 2).

Table 2: The Performance of CareStart Malaria RDT against Gold Standard BF, Metekel Zone, Benishangul-Gumuz Region, 2018.

		Result by BF				Total
		P.f	P.v	Mixed	Negative	
Result by RDT	P.f	203	0	2	5	210
	P.v	0	120	4	6	130
	Mixed	0	1	7	0	8
	Negative	5	5	0	2319	2329
	Total	208	126	13	2330	2677

There were sixty-eight (2.54%) discordant results obtained between the blood film and CareStart RDT test. 17 results detected as P.v by RDT were reported as P.f, 12 results detected as negative by RDT were reported as P.f, 1 result detected as mixed (P.f & P.v) by RDT was reported as P.v, 5 results detected as negative by RDT were reported as P.v, 2 results detected as P.f by RDT were reported as mixed (P.f & P.v) and 31 results detected as P.v by RDT were reported as mixed (P.f & P.v) by health facility Microscopistes. All those discordant results were re-examined by experts at Benishangul-Gumuz Public Health Laboratory Research Institution and 51 of them were corrected.

Taking the blood film as a gold standard test for malaria, the sensitivity and specificity of CareStart RDT were 97.12% (95% confidence interval [95% CI] = 95.36–98.88%) and 99.53% [95% CI] = 99.23–99.80%), respectively (Table 3).

The PPV and the NPV of CareStart were 97.8% (95% CI = 94.8–99.1%) and 99.7% (95% CI = 97.6–100%), respectively (Table 3).

There was also an excellent agreement between blood smears and CareStart RDT Test results, with a kappa-value of 0.97 (95% CI = 0.93–1.0) (Table 3).

Table 3: Overall Performance of CareStart Malaria RDT to detect Malaria, Metekel Zone, Benishangul-Gumuz Region, 1018.

Test Method	Sensitivity	Specificity	PPV	NPV	% Agreement	Kappa
CareStart	0.9712	0.9953	0.9684	0.9957	99.22	0.9655
RDT	(0.95-0.98)	(0.99-0.99)	(0.94-0.98)	(0.993-0.998)	(98.88-99.55)	(0.9277-1.0)

The Sensitivity of CareStart Test to diagnose P.f was 97.6% (95% CI = 95.52-99.68%) and the corresponding specificity for the species was 99.53% (95% CI = 99.23-99.80%). The PPV and NPV of CareStart to diagnose P. falciparum were also 96.8% (95% CI = 94-98%) and 99.57% (95% CI = 99.3-99.8%), respectively.

The Sensitivity of CareStart RDT test to diagnosis P. vivax malaria was 95.24% (95% CI = 91.52-98.96%) whereas the specificity was 99.53% (95% CI = 99.23-99.8%), with 92.31% (95% CI = 87-96%) PPV and 99.57% (95% CI = 99.3-99.8%) NPV (Table 4).

Moreover, the sensitivity and specificity of CareStart RDT test to diagnosis mixed malaria infection (P.f&P.v) were 53.84% (95% CI = 26.76-80.95%) and 99.53% (95% CI = 99.23-99.8%), respectively, with 87.5% (95% CI = 64.59-100%) PPV and 99.57% (95% CI = 99.3-99.8%) NPV (Table 4).

Table 4: Performance of CareStart Malaria RDT to detect Malaria Species, Metekel Zone, Benishangul-Gumuz Region, 1018.

Test Method	Malaria Species	Sensitivity	Specificity	PPV	NPV
CareStart	P.f	0.976	0.9953	0.9684	0.9957
RDT		(0.9552-0.9968)	(0.9923-0.998)	(0.94-0.98)	(0.993-0.998)
	P.v	0.9524	0.9953	0.9231	0.9957
		(0.8152-0.9896)	(0.9923-0.998)	(0.87-0.96)	(0.993-0.998)
	Mixed	0.5384	0.9953	0.875	0.9957
		(0.2676-0.8095)	(0.9923-0.998)	(0.6459-1.0)	(0.993-0.998)

6. DISCUSSION

The performance of the CareStart Malaria Pf/Pv Combo Test to diagnose Plasmodium infection showed high level of overall sensitivity (97.1%) and specificity (99.5%). The overall high level of sensitivity and specificity of the RDT in this study was in line with other studies from south-west Ethiopia Sensitivity (99.4%), Specificity (97.8%) [38], North-West Ethiopia sensitivity (99.8%), Specificity (97.7%) [36] and Madagascar sensitivity (99.8%), Specificity (97.7%) [50]. Moreover, the CareStart Malaria Pf/Pv Combo Test fulfilled the performance criteria set for the rapid diagnosis of malaria [13, 42].

However, it revealed slightly higher sensitivity(97.1%) and specificity(99.5%) than results from North-West Ethiopia sensitivity (95%), Specificity (94.2%) [34] and South-west Ethiopia sensitivity (95.7%) [35]. In addition, the overall specificity (99.53%) is far high from studies done in Butajira (82.7%) [35] and 73% in Ghana [56].The differences in the specificity and specificity of the RDT could be due to observer variation or host factors [57].

This study had also evaluated the performance of the CareStart RDT in detecting different species of malaria parasite.

The sensitivities of the CareStart Malaria Test obtained presently 97.6% for diagnosing *P. falciparum* and 95.2% for *P. vivax* were also comparable with the findings in previous studies 99.8% for *P. falciparum* and 99.9% for *P. vivax* in Pawe [36], 99.4% for *P. falciparum* and 99.9% for *P. vivax* in Wondogenet [38] ,99.4% for *P. falciparum* and 99.9% for *P. vivax* in Koladba [34], 96.2% for *P. falciparum* in Karachi [58], and 96% for *P. falciparum* in Sierra Leone [59]. But relatively higher than the finding 85.7% for *P. falciparum*, 85% for *P. vivax* in Oromiya[33],85.7% for *P. falciparum*, 88.2% for *P. vivax* in Uganda [49] and 88.5% for *P. falciparum* and 90.8% for *P. vivax* China-Myanmar [51].

The specificity of the CareStart Malaria Test obtained presently for diagnosing both *P. falciparum* and *P. vivax*(99.53%) was also comparable with the findings in previous studies 97.8% for *P. falciparum* and 99.9% for *P. vivax* in Pawe [36], 97.8% for *P. falciparum* and 99.9% for *P. vivax* in Wondogenet [38] ,95.4% for *P. falciparum* and 97.3% for *P. vivax* in Kola-Dbba [34], 96.2% for *P. falciparum* in Karachi [58], 96% for *P. falciparum* in Sierra Leone [59] and 94.7% for *P. falciparum*, 93.6% for *P. vivax* in London[60]. However, the result of this study was relatively higher than the findings 88.5% for *P. falciparum*, 90.8% for *P. vivax* in China-Myanmar. [51].

Such variations within the same test could be explained by the presence of persistence HRP2 in patients who had been treated 28 days before in case of *P. falciparum* [31] and the sequestration of the parasites in the deep organs to reduce circulating parasites [61]. Sequestration may reduce the number of circulating parasites to below the microscope threshold detection level [62] or due to observer variation and/or host factors [57].

The RDT had high PPV (96.8%) meaning that patients will be correctly diagnosed as positive for malaria and avoids unnecessary treatment. Similarly, higher the NPV (96.8%) means that it was reliable in ruling out malaria to avoid unnecessary Malaria treatment.

RDT-based diagnosis is vital to make treatment decisions at all health facility levels in general and the peripheral areas in particular, where there is no microscopy. Such parasite-based treatment reduces unnecessary consequences resulting from treatments given based on clinical symptoms, including the development of drug resistance. Therefore, the CareStart Malaria Pf/Pv Combo Test is a promising device for diagnosis of *P. falciparum* and *P. vivax* particularly in Benishangul-Gumuz Region and in general where the two species are co-endemic.

7. CONCLUSION

The CareStart RDT Test showed nice sensitivity and specificity, with an excellent agreement with the reference blood film microscopy in the identification of both *P. falciparum* and *P. vivax* species. Hence, the use of CareStart Malaria Pf/Pv Combo Test should be strengthen for the diagnosis of malaria where microscopy is limited and malaria caused by *P. vivax* and *P. falciparum* is co-endemic, particularly in Benishangul-Gumuz Region.

8. RECOMMENDATION

We strongly recommend Benishangul-gumuz Region Health Bureau to strengthen use of RDT for the diagnosis of malaria for early initiation of treatment in set ups where LM cannot be used routinely.

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ANNEX 1: Consent Form

Title: Comparison of CARE START™ HRP2/PLDH COMBO Rapid Malaria Test with Light Microscopy in Metekel Zone, Benishangul- Gumuz Region, Ethiopia, 2017.

Introduction: Hello, my name is Chemirew Ejigu. I am Field Epidemiology Resident at St. Paul's Hospital Millennium Medical college department of Public Health. Thank you for your willingness to participate in my study.

Purpose: To compare the performance of Rapid Malaria Test with Light Microscopy in Metekel Zone, Benishangul- Gumuz Region, Ethiopia, 2017.

Procedure: If you agree to take part, this will take about 30 minutes of your time. There are two parts. First, we will ask you about demographic information of you and your family, ant-Malaria treatment intake in the past one week.

Second, I will ask you for blood samples to test for malaria using RDT and Blood film. I need only small amount of blood. All information collected during this study will be kept private and will only be known by the principal Investigator.

Benefits: This project will help you and other people living in Benishangul-Gumuz Region.

Risks: There may be mild pain during collecting the blood sample.

Privacy: I will keep information about you private. I will not collect your name. Only the investigator will have access to the data and only for investigation purpose. I will not use any information that might identify you when I present or publish the study's results.

Payment: there is no cost to you for being part of the project. The approximate time that this study will take is 30 minutes. There will be no involvement past today.

Participant Agreement: The project has been explained for me. I have been given a chance to ask questions. I feel that all my questions have been answered. Being in this study is my choice. I may change my mind and leave the study any time during the study.

Participant Signature_____

Date_____

Name of persons obtaining consent_____

Signature of persons obtaining consent_____

Date_____

Thank you For Your Cooperation!

ANNEX 2: Questionnaire for Demographic and Clinical Data

1. ID. Number _____
2. Name: _____ MRN _____
3. Address: Woreda _____ Kebele _____
4. Residence: Urban _____ Rural _____
5. Ethnicity 1. Amhara 2. Gumuz 3. Shinasha 4. Oromo 5. Other(Specify) _____
6. Age _____ Year _____ Month _____
7. Sex 1. Male 2. Female
8. Occupation 1. Farmer 2. Merchant 3. Student 4. House wife 5. Other _____
9. What is your religion 1. Orthodox 2. Muslim 3. Protestant 4. Catholic
5. other _____
10. What is your marital status? 1. Single 2. Married 3. Divorced 4. Widowed 5. NA
11. Level of Education 1. Illiterate 2. Read and writing only 3. Primary school
4. Secondary School 5. Tertiary School (college+)
12. Which Signs and Symptoms do you have?
1. Fever 2. Headache 3. Fatigue 4. Sweating/chills/rigors 5. Vomiting
6. Splenomegaly 7. Myalgia and arthralgia 8. Anemia 9. Hypoglycemia
13. Record of Body Temperature _____

Thank You!!!

ANNEX 3: Laboratory Request & Result Form for Malaria Diagnosis

Patient Name _____ Age _____ Sex _____ MRN _____

Address _____ OPD/Ward _____

Requested by _____ Sign _____ Date requested _____

Clinical Dx _____ Specimen from _____

Date of Specimen Collection _____ Time _____ Collected by _____

Lab Serial No _____ Reception No _____

Test Type	Result	
RDT	Negative	
	Positive	P.f
		P.v
		P.Malare
		P.ovale
		Mixed
B. F	Negative	
	Positive	P.f
		P.v
		P.malare
		P.ovale
		Mixed

RDT doneby: _____ Date: _____ Time: _____

B. F doneby: _____ Date: _____ Time: _____

Reviewed/Approved by: _____ Date: _____ Time: _____

ANNEX 4: Room Temperature Monitoring Chart

DAY								Supervisor
							Acceptable	
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31								

Note: If room temperature is outside the range, remove the items stored in room temperature

Reviewed By: _____ Signature: _____ Date: _____

ANNEX 5: Malaria Staining IQC Chart

S. no	Date IQC slide prepared	Date ICQ Performed	IQC ID	Negative Control	Positive control	QC result Passed/Failed	Action taken if failed	Initial	R E

Approved by: _____ Signature: _____ Date: _____ Time: _____