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COMPARATIVE ASSESSMENT OF MICROSCOPY, MALARIA RAPID DIAGNOSTIC TEST AND POLYMERASE CHAIN REACTION AS DIAGNOSTIC TEST TOOLS IN ADAMA WOREDA, EAST SHOA ZONE OF ETHIOPIA

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL BIOCHEMISTRY.

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This is to certify that the thesis prepared by **Getaneh Tegegne** entitled on: **Comparative assessment of microscopy, malaria rapid diagnostic test and polymerase chain reaction as malaria diagnostic tools in Adama Woreda, East Shoa zone of Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in medical biochemistry complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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DECLARATION

I declare that this research paper entitled on: **Comparative assessment of microscopy, malaria rapid diagnostic tests and polymerase chain reaction as malaria diagnostic tools in Adama Woreda, East Shoa Zone of Ethiopia**, is my original work and has not been presented for any degree in any other university, and that all sources of materials used for the research has duly been acknowledged.

Getaneh Tegegne

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Date____________________

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LIST OF ABBREVIATIONS AND ACRONYMS

AHRI: -Armauer Hansen Research Institute

DBS :-Dried Blood Spots

DNA: - Deoxyribonucleic Acid

HRP-2: -Histidine-Rich Protein 2

IRS: --Indoor Residual Spraying

LM: -Light Microscope

NAATs: - Nucleic Acid Amplification-Based Tests

NPV: -Negative Predictive Value

PBS: -Phosphate Buffer Saline

PCR - Polymerase Chain Reaction

PLDH: - Plasmodium Lactate Dehydrogenase

PPV: -Positive Predictive Value

RDTs: - -Rapid Diagnostic Tests

RNA: -Ribonucleic Acid

TBE: -Tris Borate EDTA

WHO: - World Health Organization

OPERATIONAL DEFINITION

True Positive (TP): - the response is positive, and the prediction is also positive or Persons who have disease and are test positive.

True Negative (TN): - the response is negative, and the prediction is also negative or persons who do not have disease and are test negative.

False Positive (FP): - a negative response is falsely predicted as Positive or Persons without disease but with positive test.

False Negative (FN): - a positive response is falsely predicted as Negative or Person with disease but with negative test.

Sensitivity: the ability of a test to correctly identify individuals who have a given disease or disorder.

Specificity: the ability of a test to correctly exclude individuals who do not have a given disease or disorder.

Positive Predictive Value (PPV): - the probability that an individual with a positive screening result has the disease.

Negative Predictive Value (NPV): -the probability that an individual with a negative screening result is without the disease.

Water body: the existence of different water sources: -river, pond, swamp, lake and stagnant water having a potential source of being either temporary or permanent mosquito breeding sites near/around/ the house of participants.

ABSTRACT

Background: One of the main challenges in controlling morbidity and mortality caused by malaria is limited access to effective diagnosis in areas where malaria is endemic. This study was designed to compare the performance of Carestart^{TM}pf/pan RDT, Giemsa microscopy and 18S nested PCR for the diagnosis of malaria.

Methods: Health facility and community based cross-sectional study was conducted from December ,2016 to February, 2017 in villages of Batodegama kebele and at Adama malaria control center located in Adama woreda, East Shoa Zone of Oromia Regional State. A total of 330 residents (202 suspected malaria cases and 128 healthy individuals without any symptom) were enrolled in this study. Finger prick blood samples were taken from each participant, for Carestart^{TM} pf/pan RDTtest, Giemsa microscopy and Dry Blood Spot (DBS) for 18S nested PCR assay.

Result: From 128 asymptomatic, participants, 20.3 %, 6.3 % and 3.9 % were positive with nested 18S PCR, Giemsa microscopy and RDT respectively. Similarlly from 202 symptomatic participants malaria parasite were detected in 27.2 %, 13.9 % and 12.9 % by 18S nested PCR, Giemsa microscope and RDT respectively.

As compaired to Giemsa microscopy; Carestart^{TM}pf/pan RDT perform equivalent 100.0 % for all parameters (sensitivity, specificity, positive predictive value (PPV) and a negative predictive value (NPV)) for the diagnosis of symptomatic malaria infections, where as in detecting asymptomatic cases, it had a lower sensitivity (62.5 %). While comparing Giemsa microscopy and Carestart^{TM}pf/pan RDT with 18S nested PCR to diagnose symptomatic malaria infections both presented an equivalent sensitivity of 50.0 %, specificity of 100.0 %, positive predictive value (PPV) of 100.0 % and negative predictive(NPV) value of 84.6 % and in detecting asymptomatic malaria infections, the Carestart^{TM}pf /pan RDT presented a sensitivity of 15.4 %, a specificity of 98.0 %, a positive predictive value (PPV) of 66.7 % and a negative predictive value (NPV) of 82.0 %. Giemsa microscopy presented a sensitivity of 19.2 %, a specificity of 97.1 %, a positive predictive value (PPV) of 62.5 % and a negative predictive value (NPV) of 82.5 %.

Conclusion: The performance of CareStart ^{TM} pf/pan RDT and Giemsa microscopy was comparable and both of them had significantly lower sensitivity compaired to 18S nested PCR.

1. INTRODUCTION

1.1 Background of the study

Malaria is the most prevalent mosquito borne disease posing a potential health risk to almost half of the world's population (Derua *et al.* (2012). The disease is transmitted by Anopheles mosquito and caused by infectious disease agents *Plasmodium vivax (Pv), P. falciparum (Pf), P. ovale (Po), P. malaria (Pm),* and the recently emerging *P. knowlesi (Pk)* (Birhanie *et al*., 2014). Between 2010 and 2015, malaria burdens have declined significantly in most endemic countries, incidence rates decreased by 21 % globally (the same figure in Africa) and mortality rate fell by an estimated 29 % globally and by 31 %, in African region (WHO, 2016). This emphasizes the need for an accurate diagnosis and treatment of all malarial infection (asymptomatic and symptomatic cases) in addition to other vector control strategies (Mawili-Mboumba *et al*., 2013). Currently malaria is diagnosed by light microscopy (thick or thin blood smears), rapid diagnostic tests (RDTs) such as ParaScreen, SD Bioline and CareStart™ *Pf/pan* comb test (Tangpukdee *et al*., 2009) and using different nucleic acid amplification-based tests (NAATs) such as polymerase chain reaction (PCR) (Kemleu *et al.,* 2016).

Microscopy has been the most commonly used method of malaria diagnosis, prognosis and monitoring of the patient (Lima *et al*., 2011). However, this technique is time-consuming, needs significant technical skills, good-quality reagents and trained personnel. Additionally, the technique is not sufficiently sensitive for use in cases of low density parasite carriage (as in asymptomatic carriers) and mixed species infections given its detection limit of approximately 100 parasites / µL for most mid-level microscopists and 10 to 50 parasites / µL for expert microscopists (Mawili-Mboumba *et al*., 2013, Kemleu *et al.*, 2016). Furthermore, the requirement of equipment and electricity in microscopy based diagnosis and its labor-intensiveness, necessitates other simple, quick, accurate, and cost-effective diagnostic tests (WHO *et al*., 1999).

Of great interest in this regard is the recent introduction of RDTs, which may offer valid alternative or complementary to microscope particularly in rural seating where microscopy services are not accessible or affordable (Ayele *et al*., 2012a). Malaria RDTs are easy to use and can detect specific Plasmodium parasite antigens in blood flowing along a membrane containing specific anti-malaria antibodies (Kashif *et al*., 2013). .

They commonly come in three different formats: those that detect Histidine-Rich Protein 2 (HRP-2) specific to *P. falciparum*, RDTs that detect plasmodium Lactate Dehydrogenase (pLDH) and available as *P. falciparum*-specific, pan-specific, and *P. vivax* specific and those

RDTs that detect Aldolase (pan-specific) (Chong *et al*., 2014). Most of the currently deployed RDTs are designed to detect both HRP-2 and pLDH antigens (Chong et al., 2014). Plasmodium lactate dehydrogenase based RDTs has an overall detection limit of 100-200 p / μL for *P. falciparum* and 200-500 p / μL for *P. vivax*. Rapid diagnostic tests based on HRP2 antigen have a detection limit of 50-100 p /μL (Wu *et al.*, 2015).

Moreover, the performance of routine malaria diagnostic techniques (RDTs and microscopy) in the case of low parasitaemia and mixed infection is limited. Thus, new laboratory diagnostic techniques that display high sensitivity and high specificity, without subjective variation, are urgently needed (Tangpukdee *et al.*, 2009).

Recently various Deoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA)-based molecular approaches have been developed for the laboratory diagnosis of malaria, including conventional PCR, nested PCR, real-time PCR and Loop-Mediated Isothermal Amplification (LAMP) that allow the differentiation of all four species of *Plasmodium (Johnston et al., 2006).* The nested PCR approach identifies the species-specific Plasmodium DNA by amplifying the 18s ribosomal RNA region of the parasite (Mekonnen *et al*., 2014). Their capacity to detect low-grade and asymptomatic infections from different sample types, including Dried Blood Spots (DBS), makes these techniques ideal diagnostic tool for malaria elimination efforts (Oriero *et al.,* 2015).

Although different diagnostic methods are employed heterogeneous results of these diagnostic methods have been documented in different studies and settings (Fançony *et al*., 2013). So far, in Ethiopia, no studies have comprehensively evaluated the concordance across PCR, RDT and microscopy detection methods simultaneously in asymptomatic and symptomatic populations. This study therefore aimed to determine and compare the performance of CareStart Malaria Pf /pan RDT available in Ethiopia with Giemsa microscopy and 18S nested PCR using Giemsa microscopy /18S nested PCR / as gold standard in asymptomatic and symptomatic populations groups.

1.2. Statement of the problem

Malaria has been major cause of illness and death for many years (Ayele *et al*., 2012b). Approximately 30% of the overall disability-adjusted life years lost ,12% of outpatient consultations and 10% of admissions to Ethiopian health facility is caused by malaria (Alemu *et al*., 2013).

One of the main challenges in controlling morbidity and mortality caused by malaria is limited access to effective diagnosis in areas where malaria is endemic(Aydin Schmidt, 2014). According to world health organization (WHO) estimation only 62% of patients with suspected malaria received a diagnostic test in 2013 (WHO, 2014).

Although microscopy is the commonly used diagnostic technique, its accuracy under operational conditions in Africa is often low and the risk of false negative results increases with decreasing parasite densities and mixed infections (Reyburn *et al.*, 2007). Sensitivity of microscopy is highly dependent on the quality of smear, staining, skills of microscopes, species of the parasite and geographical location (Nicastri *et al.*, 2009). A study in Ethiopia reported microscopy sensitivity that varied between 44% and 96%, and a specificity of greater than 90% (Endeshaw *et al.*, 2012). It is also time consuming and labor intensive, cannot detect sequestered *P. falciparum parasites* (Maltha *et al.*, 2013). In low transmission areas (PCR prevalence of <10%) on average 88% of infections are not detected by microscopy (Okell *et al.*, 2009). Moreover the role of microscopic examination as the gold standard for malaria diagnosis has been questioned due to false negative results at low parasitaemia levels (less than 20–30 parasites/µl of blood) and frequent errors in species identification in mixed infections (Kang *et al*., 2017).

While RDTs are an alternative diagnostic tests for microscopy, their diagnostic performance vary significantly across different geographical regions making it difficult for policymakers to determine which tests are the most suitable (Eticha, 2016). Additionally the apparent accuracy of any RDT to detect malaria parasites depends on various factors including, concentration of the antigen in the blood, physical condition of the RDT, quality of test preparation and interpretation; accuracy of the reference standard, Lot or Bach of RDTs ,transmission intensity and target population leading to varying estimates of agreement between tests (Woyessa *et al*., 2013). In most settings microscopy and RDTs miss more than 30%-50% of asymptomatic infections when compared to NAATs (Okell *et al*., 2009).

Moreover the occurrence of heterogenous results for microscopy and malaria rapid diagnostic tests in different studies and settings poses dilemma to clearly select each of these techniques for accurate malaria diagnosis (Bendezu et al., 2010). Thus the performance of these diagnostic tests needs to be extensively evaluated using highly sensitive molecular techniques in addition to microscopy to ensure that they have acceptable diagnostic value (Maltha *et al.*, 2013).

2. LITERATURE REVIEW

2.1. Global experience

While various researches carried out in different parts of the world to evaluate performance of CareStartTM kits as well as other types of RDTs, most of these were conducted with reference to Giemsa microscopy.

A study conducted in north-west Angola, compared the performance of microscopy and Paracheck-Pf® in the detection of *P. falciparum* infections, using PCR as the gold standard. Compared to microscopy, Paracheck-Pf® had significantly higher sensitivity (72.8 % *versus* 60.0 %), specificity (94.3 % *versus* 92.5 %), positive predictive value (PPV) of (70.7 % *versus* 60.0 %) and negative predictive value (NPV) (94.8 % *versus* 92.5 %) (Fançony *et al.*, 2013).

The diagnostic performances of rapid anti-gen capture assay (NOW Malaria Test) versus traditional thick and thin blood smears for malaria diagnosis was compaired using PCR as a standard in United States. Overall, in all species of malaria, the RDT was superior to traditional blood smear examination with a better sensitivity of 97.0 % (92 of 95 specimens), compared with 85.0 % (81 of 95) for traditional Giemsa blood smear and a better NPV (99.6 % vs 98.2 %). The largest discrepancy in performance was for the diagnosis of P. *falciparum*, with 100% sensitivity (74 of 74 specimens) for the RDT, compared with only 88.0 % (65 of 74) for blood smear. For non P. *falciparum*, malaria the sensitivity of the RDT was lower, at 86.0 % (18 of 21 specimens) which was comparable to the 76.0 % sensitivity (16 of 21) for blood smear (Stauffer *et al*., 2009).

A study conducted between China-Myanmar endemic borders to evaluate the diagnostic performance of CareStartTM RDT malaria HRP2/pLDH (Pf/pan) combo test. Their result showed that CareStartTM RDT kit's sensitivity and specificity for the diagnosis of malaria 89.7 % and 98.3 % respectively, compared to standard microscopy whereas the sensitivity and specificity for *P. falciparum* malaria were 88.5% and 98.3 %, and for *P. vivax* malaria 90.77% and 100.0 %. The CareStart PPV were 98.3 % compared to 100.0 % for PCR-corrected, and the NPV of 89.7 % were the same in microscopy as PCR-corrected (Xiaodong et al., 2013).

Diagnostic performance of microscopic examination and PCR was evaluated to establish optimal malaria diagnosis method in Myanmar. Evaluation of the 1125 samples by species-specific nested PCR analysis revealed that the agreement between microscopic examination and nested PCR was 87.3 % (261/299). Nested PCR successfully detected 38 *Plasmodium falciparum* or *Plasmodium vivax* infections, which were missed in microscopic examination.

Microscopic examinations also either misdiagnosed the infected *Plasmodium* species, or did not detect mixed infections with different *Plasmodium* species in 31 cases (Kang *et al*., 2017).

Diagnostic performance of four RDTs (CareStartTM, SDBioline, NanoSign and Asan Easy tests) for the detection of *Plasmodium Vivax* Malaria was evaluated against the standard of nested-PCR and microscopic examination among171 patients diagnosed with *P. vivax* malaria at local health centers, and 82 asymptomatic and aparasitemic healthy volunteers in South Korea. The CareStartTM and SD Bioline had higher test sensitivities (99.4 % and 98.8 %, respectively) compared with the NanoSign and Asan Easy tests (93.0 % and 94.7 %, respectively). Specificity was 100.0 % for all of the RDTs. The CareStartTM and SD Bioline tests detect *P. vivax* in samples with parasite densities ,150/mL, which was a slightly better performance than the other two RDTs (Kim *et al*., 2013).

The diagnostic ability of three RDT kits (CareStart Pv/Pf Combo kit, First Response Malaria Antigen pLDH/HRP2 Combo test and CareStart™ Malaria Combo test) for the diagnosis of mixed species malaria infections was evaluated and the results were compared with the findings of microscopy against nested PCR in Southeastern Iran. The sensitivity of light microscopy for the detection of mixed-species malaria infections was 16.6 %. Nested PCR revealed 12 patients with mixed-species infection. The CareStart Pv/Pf Combo kit detected 58.0 % of the mixed-species infections, which were determined by nested PCR (sensitivity = 58.3 %). For identifying *P. falciparum, P. vivax*, and mixed-species infections, the concordance rates (kappa statistics) of microscopy and CareStart Pv/Pf Combo kit with nested PCR were 0.76 and 0.79, respectively. The diagnosis of *P. vivax* malaria was confirmed in 61/69, 63/69, and 64/69 patients in CareStart1, First Response, and CareStart Pv/Pf Combo kits, respectively. The diagnosis of *P. falciparum* malaria was confirmed in 18/19 patients in CareStart1 kit, but all of the *P. falciparum* infection were detected by First Response and CareStart Pv/Pf Combo kits (Ehtesham *et al*., 2015).

A household-based, cross-sectional malaria survey was conducted in Mawza District, a malaria-endemic area in Yemen to evaluate light microscope (LM) and an RDT, combining both *P. falciparum* HRP2and pLDH, for *P. falciparum* malaria diagnosis and survey in a malaria-endemic area against PCR as the reference method. The sensitivity, specificity, PPV and NPV of the RDT were 96.0 % ,56.0 % ,76.3 % and 90.4 % respectively. On the other hand, LM showed sensitivity of 37.6 %, specificity of 97.6 %, PPV of 95.9 % and NPV of 51.3 %. The sensitivity of LM dropped to 8.5 % for detecting asymptomatic malaria. Among asymptomatic malaria individuals, LM and RDT-based prevalence rates were 1.6 and 25.6 %, respectively. However, rates of 88.2 and 94.1 % of infection with *P. falciparum* were found among patients who reported fever in the 48 h prior to the survey by LM and HRP-2/pLDH RDT, respectively (Alareqi *et al*., 2016).

The CareStart™ Malaria HRP-2/pLDH (Pf/pan) Combo Test was evaluated on a collection of samples obtained in returned international travelers using microscopy corrected by PCR as the reference method. Overall sensitivity for the detection of *P. falciparum* was 88.8 %. For *P. vivax, P. ovale* and *P. malariae*, overall sensitivities were 77.6 %, 18.4 % and 30.4 % respectively. Incorrect species identification occurred in 11/495 samples (2.2 %), including 8/320 (2.5 %) P. falciparum samples which generated only the pan-pLDH line (Gillet *et al*., 2009).

The performance of RDTs, SDBioline® Malaria Ag P.f./Pan test for diagnosis of malaria in Health center (symptomatic cases) and community (asymptomatic cases) against light microscopy was assessed a stable transmission zone of Kinshasa. Rapid diagnostic tests and microscopy were concordant in 84.3 % and 83.4 % children in the health center and at the community level, respectively. The sensitivity was high (>95.0 %), but the specificity was too low and lower in the community (66.9 %) compared to the health center (79.4 %). Positive predictive value was higher in the health center (60.0 %). The rate of false positives was 21.1 % in the health center compared to 40.0 % in the community. Negative predictive value was high in both settings: 98.3 % versus 96.5 % in the community and health center respectively (Ilombe *et al*., 2014).

The performance of Blood Smear, Antigen Detection (ICT and OptiMAL), and Nested-PCR Methods for malaria Screening among Refugees were compared against PCR in Canada. Using PCR as the "gold standard," both microscopy (sensitivity, 50.0 %; specificity, 100.0 %) and antigen detection (ICT sensitivity, 37.5 %; ICT specificity, 100.0 %; OptiMAL sensitivity, 29.1%; OptiMAL specificity, 95.6 %) performed poorly (Ndao *et al*., 2004).

A study was also conducted to assess the performance of Makromed Dipstick Assay (P. falciparum-specific HRP2) compared to PCR and microscopy for the diagnosis of Plasmodium falciparum malaria in 200 febrile returned travelers. Compared to PCR as the reference standard, the dipstick assay had a sensitivity of 97.0 % and a specificity of 96.0 %.When microscopy was used as the reference, the corresponding sensitivity and specificity of the dipstick assay were 98.9 % and 92.5 %, respectively (Richardson *et al*., 2002).

2.2. African experience

Microscopy and RDT (ParaHIT*f* dipstick test) were done locally and the accuracy evaluated by qualitative PCR for *Plasmodium spp*. in Two Tanzanian Hospitals. For microscopy diagnosis sensitivity, specificity, NPV and PPV were 69.2 %, 95.5 %, 97.4 % and 56.3 % respectively. Correspondingly for RDT diagnosis sensitivity, specificity, NPV and PPV were 69.2 %, 100.0 % 97.5% and 100.0 %. Microscopy and RDT accuracies were 93.5 % and 97.6 %, respectively (Nicastri *et al*., 2009).

A study in Gadarif Hospital, Eastern Sudan, evaluated the performance of SD Bioline P. f /P. v RDT kit and microscopy against PCR among the febrile pregnant women. The result documented that the prevalence of 17 (11.0 %), 26 (16.7 %) and 18 (11.5 %) positive cases of *P. falciparum* by microscopy, RDT, and PCR, respectively. The sensitivity and specificity of the microscopy was 94.4 % and100%, respectively. The corresponding values for RDT evaluation were 83.3 % and 92.0 %, as compared with PCR as the gold standard (Kashif *et al*., 2013).

The diagnostic performance of routine diagnostic tests microscopy and rapid diagnostic tests RDTs (Premier Medical Corporation, Gujarat, India) detecting HRP2 for diagnosis of malaria against PCR were evaluated in four hospitals in the Volta region of Ghana. By comparison, the sensitivities and specificities of RDT results when compared to PCR were slightly higher than microscopy compared to PCR. These were 56.4 % versus 41.7 % and 90.0 % versus 81.9 %, respectively, but generally lower than expected (Dinko *et al*., 2016).

The diagnostic performance of RDT (SD Bioline P.f ∕ P.v), microscopy and molecular techniques for the diagnosis and identification of malaria parasites were evaluated in Kassala, eastern Sudan. When microscopy was compared with PCR, an agreement of 96.1% and k = 0.88 (sensitivity 85.7 % and specificity 100.0 %) was found. However, when RDT was compared with PCR, an agreement of only 81.2 and k = 0.48 (sensitivity 69.0 % and specificity 84.0 %) was found. The sensitivity and specificity of the RDT were 77.8% and 84.9%, respectively, when compared with Microscopy (Osman *et al*., 2010).

The performance of different malaria RDT kits (First Response (FR), CareStart (CS), SD Bioline (SD), and Binax Now (BN)) was evaluated against microscopy/ PCR using 500 samples from febrile patients in western Kenya. Compared to microscopy, the sensitivity of eight RDTs to detect malaria parasites was 90.3–94.8 %, the specificity was 73.3–79.3 %, the PPV was 62.2–68.8 %, and NPV was 94.3–96.8 %. Compared to PCR, the sensitivity of the RDTs to detect malaria parasites was 71.1–75.4 %, the specificity was 80.3–84.4 %, the PPV was 80.3–83.3 %, and the NPV was 73.7–76.1 %. The RDTs had a moderate measure of agreement with both microscopy (>80.1 %) and PCR (>77.6 %) with a κ > 0.6. CareStart had the higher agreement value of 78.8 % while First Response had the lower agreement of 77.6 % (Wanja *et al*., 2016).

2.3. Ethiopian experience

The PCR-based parasite prevalence of sub-microscopic *P. falciparum* infections was compared against microscopy and RDT (SD BIOLINE Malaria Ag P.f/ P.v test kit) in West Arsi Zone, Shalla district, Ethiopia. The sensitivity of PCR relative to microscopy and RDT was, therefore, 90.7 %and 80.0 %, respectively. The sensitivity of microscopy and RDT relative to PCR was 16.5 % (49/299) and 24.2 % (80/330), respectively. The overall PCR-based prevalence of P. falciparum infection was 5.6- and 3.3-fold higher than that determined by microscopy and RDT, respectively (Golassa *et al*., 2013).

CareStartTM Malaria Pf/Pv combo test was evaluated compared to microscopy in north-west Ethiopia. According to this study the overall sensitivity and specificity of CareStartTM RDT was found to be 95 % and 94.2 %, respectively when compared with light. 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-*P. falciparum* species. The specificity for *P. falciparum* or mixed infections was found to be 95.4 % while it was 97.3 % for non-*P.falciparum* species (Moges *et al*., 2012).

A study conducted to assess *P. ovale species* and the ability of microscopists to detect and identify *Plasmodium species* in north-west Ethiopia. By species-specific PCR 233 Plasmodium spp were detected and the majority 155 (66.5 %) were *P. falciparum* followed by P. vivax 69 (29.6 %) and 9 (3.9 %) samples were positive for *P. ovale*. During microscopic examination, there were high 16.3 % (49/300) false negative reports and all mixed infections and P. ovale cases were missed or misclassified (Alemu *et al*., 2013).

The performance of Giemsa microscopy for the diagnosis of malaria was compaired with nested PCR in north-west Ethiopia**.** According to this study 61.6 % (183/297) patients tested positive for malaria by Giemsa microscopy and 73.1 % (217/297) by nested PCR. Among microscopy-negative samples, 13.1% (39/297) samples turned malaria-positive in nested PCR. In nested PCR, the rate of mixed *Plasmodium* infections was 4.7 % (14/297) and 3.0 % (9/297) were positive for *Plasmodium ovale*. Using nested PCR as reference the sensitivity, PPV and NPV of Giemsa microscopy were 82.0 %, 93.8 %, 97.3 % and 65.8 %, respectively, with a good agreement (κ = 0.67) to nested PCR. The sensitivity and specificity of Giemsa microscopy in identifying *P. falciparium* infections were 74.0 % and 87.4 % and 63.2 % and 96.5 % for *P. vivax* infections, respectively (Alemu *et al*., 2014).

The performance of RDT, microscopy and PCR were also evaluated in study that was undertaken to assess the presence and prevalence of asymptomatic *P. falciparum* and *P. vivax* infections in West Arsi Zone of the Oromia Region of Ethiopia. Here the prevalence of asymptomatic *Plasmodium* carriage *(P. falciparum, P. vivax and* mixed species) was 5.0 % (55/1,094) as determined by microscopy, while the prevalence as determined using RDT was 8.2 % (90/1,094). Polymerase chain reaction was done on 47 of 55 microscopy-confirmed and on 79 of 90 RDT-confirmed samples and detected parasite DNA in 89.4 % (42/47) of the microscopy-positive samples and in 77.2 % (61/79) of the RDT-positive samples. Thus, the true positivity rate for microscopy and RDT relative to PCR were 89.4 % and 77.2 %, respectively (Golassa *et al*., 2015).

3. SIGNIFICANCE OF THE STUDY

Early and adequate diagnosis and prompt treatment is one of the main strategies in malaria prevention, control and effective disease management. Reduction of malaria morbidity and drug resistance intensity plus the associated economic loss of these two factors require urgent scaling up of the quality of parasite-based diagnostic methods. Differences in quality performance score had been reported for the routinely used diagnostic methods for malaria at different settings. For sustainable establishment of these accurate approaches in operational research to strengthen malaria control and elimination efforts, simple and affordable methods, with parsimonious reagent and equipment requirements are essential. Data on the sensitivity and specificity of each test methods is important information to help guide test selection by national malaria control programmers. The analysis presented in these study, forms a baseline to evaluate further how diagnostic methods can be improved to inform malaria control and elimination strategies.

4.OBJECTIVES

4.1. General objectives

- The aim of this study was to compare the performance of CareStart^{TM} pf/pan RDT, Giemsa microscopy and nested polymerase chain reaction for the diagnosis of malaria using Giemsa microscopy/nested polymerase chain reaction /as a standard.

4.2. Specific objectives

- To determine the diagnostic performance of CareStart^{TM} Pf/Pan RDT test, Giemsa microscopy and nested polymerase chain reaction among different population groups.
- To describe the levels of malaria infection among different population groups.
- To evaluate diagnostic performance of CareStart^{TM} Pf/Pan RDT Giemsa microscopy and nested polymerase chain reaction between plasmodium species.

5. METHODOLOGY

5.1. Study area and study design

A cross-sectional study was conducted from December 2016 to February 2017 in villages of Batodegaga kebele and at Adama malaria center located in Adama woreda, East Shoa Zone of Oromia Regional State. Adama town is located at 100 km east of Addis Ababa at an altitude of 1,664 meters above sea level and 8.54°N 39.27°E. According to the central statistics agency, Adama woreda has a total population of 180,710 (CSA July 2012) in its 35 rural villages, 5 suburban kebeles and 15 sugar factory camps. Geographically the altitude varies from 1590-1700 meters above sea level. The annual average rainfall is about 838mm. The annual mean temperature of the city ranges from 14 to 30^{o}C with mean temperature of 22 ^{o}C (Tesfaye, 2014). Ninety five percent of the villages in the woreda are located in the lowland area of the Great Rift Valley and 90% of the population is at risk of malaria. Morbidity data from health facility indicates that malaria stands the first among the ten top diseases (Sissay *et al.*, 2013).

Large scale irrigation by Wonji Sugar Factory and Koka hydroelectric dam on the Awash River has created suitable environment for malaria vector breeding. In addition, the warm and moist air condition, annual rainfall, coexistence of parasite (*P. falciparum* and *P. vivax*) and vector species in the area are contributing /risk factors for the transmission of malaria infection in the study area (Bogale, 2007).

5.2. Study population

A total of 330 individuals, 202 self-presenting individuals with clinical symptoms and 128 healthy community members without any malaria symptom were enrolled in this study. Individuals having history of antimalarial medication within 15 days of initiation of study and children younger than 6 months of age were excluded from the study. In both cases (patients and healthy community members), convenient sampling strategy was implemented to recruit consenting participants.

5.3. Study variable

5.3.1. Independent variable

- ✓ Species of the malaria parasite
- ✓ Socio-demographic factors
- ✓ Population groups/asymptomatic vs symptomatic/

5.3.2. Dependent variable

- ✓ Parasite positivity
- ✓ Performance indicators of the test (sensitivity, specify, PPV and NPV and Level of agreement /kappa value/)

5.4. Ethical consideration

This study was undertaken as part of a bigger study that was undergoing at Armauer Hansen Research Institute (AHRI) with a prior approval from the Ethics Review Committee of the AHRI (PO52/14). The specific study protocol was reviewed and approved by the Ethics Review Committee of the department of Biochemistry at Addis Ababa University (DDRERC 1/17). Before sample collection all study participants were informed in their language (Oromifa/Amharic) about the purpose of the study, the reason why blood was taken, the risks associated with finger pricking, and the reason why they were asked to give information on each questionnaire. Written informed consent /assent was obtained from participants or guardians /parents of the participant in case of minors. Individuals who were tested positive for each *plasmodium* species were linked to the nearest health institution for appropriate action as per the national malaria treatment guideline (FMOH,2004). Accordingly, *Plasmodium vivax* positives were treated with chloroquine, 25 mg/kg for three days (10 mg base per kg on days 1 and 2, and 5 mg base per kg on day 3). Artemether-lumefantrine (20 mg artemether plus 120 mg lumefantrine in a fixed dose combination) was administered, based on body weight, two times a day for three days to *Plasmodium falciparum*-positive patients.

5.5. Sample collection and processing

Socio-demographic data was collected by trained health professionals using structured and pre-tested questionnaires prepared in Oromifa and Amharic, that was translated to English (for latter data entry purpose). Once clinical evaluation has been taken by health professionals, finger prick blood sample (0.3ml) was collected from each participant and used for malaria examination using RDT (Carestart^{TM} pf/pan comb test), thick and thin smear Giemsa microscopy and to prepare DBS on 3 MM Whatman filter paper (Whatman, Maidstone, UK) (figure-1) that were air dried and stored in -20^{o}C freezer with desiccants (Geejay Chemicals Ltd) until being processed for PCR.

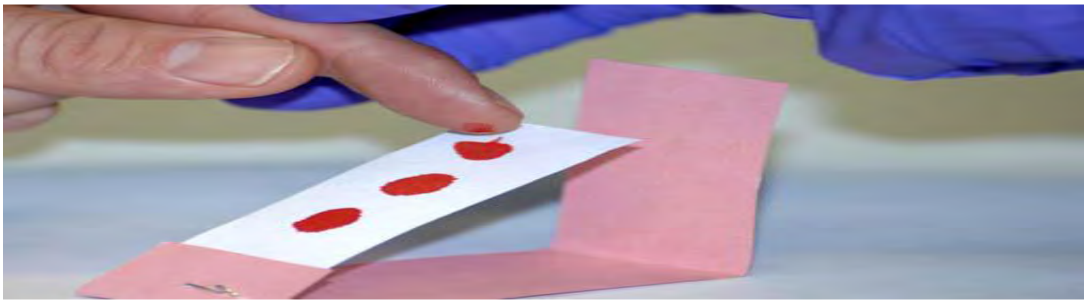

Figure-1:-DBS preparation on Whatman 3 MM filter paper
(Edelbroek *et al.*, 2009).

5.6. Microscopic examinations

Thick and thin blood smears were prepared directly from finger prick blood on the same slide. The thin blood smears were fixed with methanol for 30 seconds. After being air-dried in a horizontal position, it was stained with 10% Giemsa solution for 20 minutes. Following the standard protocols, the stained smears were investigated with a light microscope at 100× magnification to confirm the presence of malaria parasites and the results were classified qualitatively as either negative, *P. falciparum*-positive, *P. vivax*-positive, or mixed infection. Hundred high power fields were examined on a thick film before a negative result was declared. All these activities were carried out by expert microscopists, who are certified on malaria diagnosis and species identification, under supervision of the MSc student.

5.7. CarestartTM pf/pan RDT test

The CareStart TM Malaria Pf/Pan Combo test contains a membrane strip, which is pre-coated with two monoclonal antibodies at two separate lines across a test strip. One monoclonal antibody (test line 2) is pan-specific to pLDH of the *Plasmodium* species (*P. falciparum, P. vivax, Plasmodium malariae* and *Plasmodium ovale*) and the other line (test line 1) consists of a monoclonal antibody specific to HRP2 of the *P. falciparum* specie. The kit is designed for the differential diagnosis of *P. falciparum* and other pan-specific species (Xiaodong *et a*l., 2013).

The test was performed according to the instruction of the manufacturer (Access Bio, Inc., New Jersey, USA). Briefly, the kit was labeled with respective sample code and 5 µl of blood specimen was added into sample well of the test device. Two drops (60 µL) of lysis buffer were added into the buffer well to lyse the cells, release the antigen and facilitate antibody recognition.

The RDT test results were read after 20 min following the manufacturer's instructions and interpreted as follow: -two bands (one band in the control area and another band in the Pf area (test line 1) indicates a positive result for *P. falciparum* ;two bands (one band in the control area and another band in the pan area (line2)) indicating infections due to *Plasmodium species*; visualization of three bands i.e.(bands in the control area ,in the *P*f area(line1) and pan area(line2) indicates a mixed infection and only one band in the control area within the result window indicates a negative result; in case the control line did not appear, the result was interpreted as invalid (Woyessa *et a*l., 2013).

For suspected malaria cases (symptomatic participants) all test procedures and interpretation of results for RDT were performed by WHO certified malaria microscopists from Adama malaria control centers and for asymptomatic individuals it was conducted by MSc student and senior laboratory technologists from AHARI.

5.8. DNA extraction and Polymerase chain reaction analysis

The molecular diagnosis of malaria infection in all subjects was carried out using nested PCR amplification of the small subunit of 18S rRNA gene. For this, the genomic DNA on filter paper was extracted using Saponin/Chelex-100 dual extraction method (Baidjoe *et al*., 2013). In brief, 6 mm diameter of disc cut with puncher and transferred into a 2mL Eppendorf tube and incubated in 200µL of a 0.5% Saponin solution (SIGMA-ALDRICH, Germany) in sterile phosphate buffer solution (PBS) (SIGMA-ALDRICH, UK) at room temperature overnight on a shaker. The supernatant was transferred to storage deep well plates. The white filter paper discs were washed with PBS. After removing the remaining fluid with a vacuum system 150µl of a 6% Chelex in DNase/ RNase free water solution was added to each tube and finally heated at 95°C for to 30 minutes while it was span dawn in between (4x on ice). After the last incubation tubes were spun down at maximum speed for 5 minutes to allow the Chelex to settle. 80µl of the DNA containing solution was transferred to new clean tubes in small volume aliquots. Samples were stored at −20°C until further analyses.

Extracted samples were analyzed using a nested PCR. In the initial amplification reaction (Nest1), Genus-specific oligonucleotide primers pairs (rPLU5 and rPLU6) were used and in the second amplification reaction (Nest 2) species-specific primers (rFAL1 and rFAL2) for *P. falciparum* and (rVIV1 and rVIV2) for *P.vivax* were used (Table-1) (Snounou, 1995).

Amplification for the first reaction (nest 1) was performed in a 25-µL reaction mixture containing 1X PCR reaction buffer, 2mM MgCl2, 0.25 mM of each the dNTPs, 0 .25 µM each primer, 1U/reaction of Taq DNA polymerase and diluent of MLQ (nucleic acid free water) (in the form of master mix) and 5 ng/ µL genomic DNA of the parasite. Similarly the second (nest 2) reaction was performed in a total of 25 µL reaction mixture containing the same reagents but the template in this case was the product of first (nest 1) reaction.

Both positive control (previously confirmed sample DNA and cultured Malaria DNA) and negative controls tube containing MLQ (nucleic acid free water) were included in every PCR. PCR cycling conditions included an initial denaturation at 95°C for 10 min; followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1.5 min; and a final extension at 72°C for 10 min for nest1and PCR conditions for nest2 were identical to those reported above except for the number of cycles, (30cycle instead of 35cycle) and 1minute instead of 1.5 minute for the first extension 72°C.

Following PCR amplification, the nested PCR products along with a 100 bp DNA ladder (Invitrogen, Karlsruhe, Germany) were subjected to electrophoresis on a 2% agarose gel on 0.5X Tris-Borate EDTA (TBE) agarose gel buffer containing ethidium bromide (5mg/ml) to visualize the DNA. The gel was run for about 45-60 minutes in 0.5X TBE at 120 volts and visualized with UV illumination (Zhou *et al.*, 2006). Depending on the specific primer pairs, expected product size of *P. falciparum* (205 bp) and *P. viax* (120 bp) were determined by comparing with known molecular size marker.

All test procedures and interpretation of results from DNA extraction up to post PCR analysis was performed by MSc student and seiner laboratory technologists from AHARI.

Table-1:-Oligonucleotide primers used for 18S nested PCR

Specificity /reaction	Name	Sequence	Product Size
Plasmodium Nested1	rPLU6 rPLU5,	5'-CTT GTT GTT GCC TTA AAC TTC-3' 5'-TTA AAA TTG TTG CAG TTA AAA CG-3	1200 bp
P. falciparum Nested 2	rFAL1 rFAL2	5'-TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT-3' 5'-ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC-3'	205 bp
P. vivax Nested 2	rVIV1 rVIV2	5'-CGC TTC TAG CTT AAT CCA CAT AAC TGA TAC-3' 5'-ACT TCC AAG CCG AAG CAA AGA AAG TCC TTA-3'	120 bp

(Snounou, 1996).

5.9. Quality control

All the test procedures and the interpretation of results were accomplished based on standard operating procedures (SOP). During sample collection, processing, DNA extraction, DNA template preparation from samples and their storage, expire date of PCR regents and working solutions, cleanses of PCR working environment and equipment, positive and negative controls to each analyzed sample were assessed as quality control. Manufacturers protocol on each test kit was kept as indicated (Snounou *et al.*, 2002).

5.10. Data analysis

Collected study data were checked for completeness and any incomplete information was excluded from the entry. The data obtained was first entered and managed using REDCap a web based data capturing tools from data management unit of AHRI. Data were then analyzed using STATA11 (StataCorp, College Station Texas, USA). The figures for sensitivity, specificity, and predictive values were calculated using the 'diagt' command in Stata (Singh *et al.*, 2010). Logistic regression analysis was used to examine the association between each potential risk factor and PCR positivity. The results of carestart^{TM} pf/pan RDTs and 18S nested PCR were first compared against Giemsa microscopy as the reference test. In subsequent analysis, the Carestart^{TM}pf/pan RDT and Giemsa microscopy were compared against 18S nested PCR (all samples of asymptomatic and symptomatic malaria cases) as the reference test, for any species (overall) and against the species identified by 18S nested PCR. The level of agreement between various diagnostic technique was assessed using Kappa (K) value. A K value of 0.21–0.60 is moderate, a K value of 0.61–0.80 is good, and a K value over 0.80 is an almost perfect agreement (Osman *et al.*, 2010). For each measure listed above 95% confidence intervals (CI_{95}) were calculated. Within all comparisons, differences in which $p < 0.05$ were considered statistically significant (Tseroni *et al*., 2015).In all data analysis procedures the data was analyzed with MSc student and AHRI data management unit members.

6.RESULTS

6.1. Study population

In this study, a total of 330 participants (128 Asymptomatic and 202 Symptomatic individuals) were examined using Giemsa microscopy, Carestart TM pf/pan RDT and 18S nested PCR to detect and identify malaria parasites. Of these participants, 63.9 % (211) were males. The median age of the participants was 23 years (IQR:12-35). 40 % of participants reported having slept under a long-lasting insect side treated net the night before. Of all, participants, only 8.2 % have insecticide sprayed houses, and 323 (97.9 %) of participants had water bodies (favorable mosquito breeding sites) in their neighborhood. For the majority of participants, the houses were mud plastered wall (44.9 %), earth type floor (73.6 %), Grass thatch roof (67.6 %) and 44.9 % houses had opening on the eaves. Of all participants, only 21 (6.4 %) had malaria in the last three weeks. Having electricity, radio and television was reported by 140 (42.4 %), 115 (34.9 %) and 140 (42.4 %) of study participants respectively. A detailed description of socio-demographic characteristics and positivity rate by18S nested PCR is given in (Table-2).

6.2. proportion of malaria infected individuals detected by RDTs, microscopy and PCR

From a total of 202 symptomatic individual participants, 27.2 % (55/202) individuals were positive using 18S nested PCR. Likewise, from 128 asymptomatic individual participants 20.3 % (26 /128) were positive with 18S nested PCR. Over all 24.6 % (81/330) of individuals were 18S nested PCR positive**,** of these 69.1% (56/81) were infected with *P. vivax,*23.5 % (19/81) were infected with *P. falciparum* and 7.4 % (6/81) were infected with mixed infections. The representative species-specific 18Snested PCR amplification results for the *Plasmodium* species are shown in Fig-2 and 3.

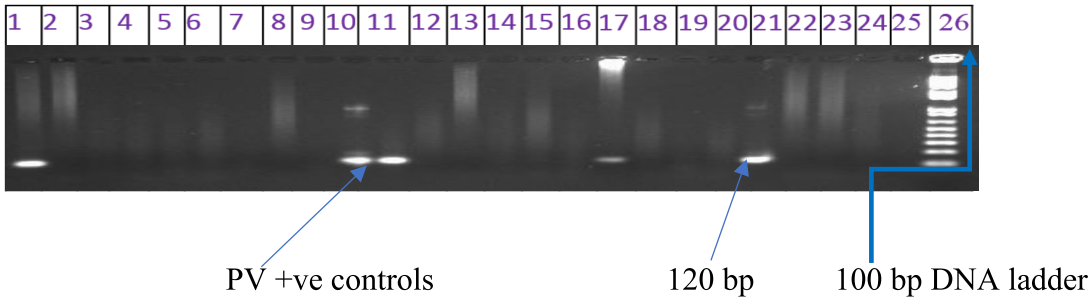

Figure-2:-Identification of *Plasmodium vivax* by 18S species-specific nested PCR.

Lane: 10,11=*Plasmodium vivax* positive controls: Lanes 1,17and 21 *Plasmodium vivax* positive samples (120 bp amplicon): Lane 26:100 bp DNA ladder; lane 25: negative control and Lane;2,3,4,5,6,7,8,9,12,13,14.15,16,18,19,20,22,23 and 24= negative samples.

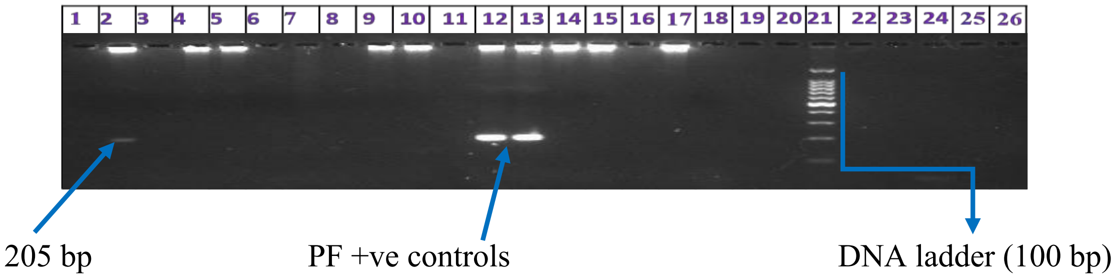

Figure-3:-Identification of *Plasmodium falciparum* by 18S species-specific nested PCR. Lane:2 *Plasmodium falciparum* positive samples (205 bp amplicon); Lane:12 and 13-plasmodium *falciparum* positive control; Lane: 17 negative control, Lane 21:100 bp DNA ladder; lane 11,19,20,22,23,24,25 and 26: empty well. Lane;1,3,4,5,6,7,8,9,10=negative results.

Correspondingly, the CareStart TM Pf/Pv RDT identified malaria infection in 9.4 % (31/330) patients of these, 77.4 % (24/31) had *P. vivax* infection, 15.6% (5/31) had *P. falciparum* infection and 6.5 % (2/31) had mixed infections. The Giemsa microscopy detected malaria infection in 10.9 % (36/330) patients: 72.2 % (26/36) had *P. vivax* infection, 22.2 % (8/36) had *P. falciparum* infection, and 5.6 % (2/36) had mixed (*P. falciparum* plus *P. vivax*) infection (Table-3).

Table-2:-Results of 18S nested PCR, Giemsa microscopy and CareStart TM Pf/Pv RDT for the diagnosis of malaria in 330 participants, in Adama woreda East Shewa zone of Ethiopia, December 2016 to February 2017.

Species	18S nested PCR			Gimesa microscopy			CareStart TM Pf/Pv RDT		
	Asymptomatic cases Number (%)	Symptomatic cases Number (%)	Total Number (%)	Asymptomatic cases Number (%)	Symptomatic cases Number (%)	Total Number (%)	Asymptomatic cases Number (%)	Symptomatic cases Number (%)	Total Number (%)
P. Falciparium	7(26.9%)	12(21.8%)	19(23.5%)	5(62.5%)	3(10.7%)	8(22.2%)	4(80%)	1(3.9%)	5(20.8%)
P. vivax	17(65.4%)	39(70.9%)	56(69.1%)	3(37.5%)	23(82.1%)	26(72.2%)	1(20%)	23(88.5%)	24(77.4%)
Mixed infection	2(7.7%)	4(7.27%)	6(7.4%)	0(0%)	2(7.1%)	2(5.6%)	0(0%)	2(7.7%)	2(6.5%)
TOTAL +VE	26(20.3%)	55(27.2%)	81(24.6%)	8(6.3%)	28(13.7%)	36(10.2%)	5(4.1%)	26(12.9%)	31(9.4%)
Invalid								7(3.5%)	7(2.1%)
Negative	102(79.7%)	147(72.8%)	249(75.5%)	120(93.75%)	174(86.1%)	294(89.1%)	123(96.1%)	169(83.7%)	292(88.5%)
Total tested	128	202	330	128	202	330	128	202	330

Out of 81 nested18S PCR confirmed cases 71.6 %) (58) were males and 28.4 % (23) were females. When we see the percentage of malaria infected individuals (PCR confirmed cases) with different age categories among asymptomatic and symptomatic participants, in asymptomatic participants it was 11.54 % for <5 years,26.9 % for 5-9 years, 26.9 % for 10-14 years and 34.6 % for ≥ 15 years. Similarlly in symptomatic participants percentage of malaria infected individuals were 0 % for <5 years,10.9 % for 5-9 years, 9.1 % for 10-14 years and 80 % for ≥ 15 years (Table-3).

Table-3:-Proportion of malaria infection in asymptomatic and symptomatic participants by age and sex groups as diagnosed by rapid diagnostic test, Giemsa microscopy and 18 S nested PCR Adama woreda East Shewa zone of Ethiopia, December 2016 to February 2017.

		Proportion of infection in asymptomatic participants				Proportion of infection in symptomatic participants		
Age group		Nested PCR	Microscopy	RDT		Nested PCR	Microscopy	RDT
	Tested	N (%)	N (%)	N (%)	Tested	N (%)	N (%)	N (%)
<5 years	8	3(11.5%)	0(0%)	1(20%)	1(0.5%0	0(0%0	0(0%)	0(0%)
5-9 years	30	7(26.9%)	1(12.5%)	0(0%)	11(5.5%)	6(10.91%)	1(3.57%)	1(3.9%)
10-14 years	40	7(26.9%)	2(25%)	1(20%)	11(5.5%)	5(9.09%)	1(3.57%)	1(3.9%)
>15 years	50	9(34.6%)	5(62.5%)	3(60%)	179(88.6%)	44(80%)	26(92.9%)	24(92.3%)
Total	128	26(100%)	8(100%)	5(100%)	202(100%)	55(100%)	28(100%)	26(100%)
Male	58(43.3%)	12(46.2%)	4(50%)	3(60%)	153(75.7%)	46(83.6%)	23(82.1%)	21(80.8%)
Female	70(56.69%)	14(53.9%)	4(50%)	2(40%)	49(24.3%)	9(16.4%)	5(17.9%)	5(19.3%)
Total	128	26(100%)	8(100%)	5(100%)	202(100%)	55(100%)	28(100%)	26(100%)

Univariate analysis, indicated that participants who had previous malaria infections had more chance of being infected (infected participants: 52.4 % vs. non-infected participants 21.9 %, ($p = 0.003$) (Table-4). Those who were living in a house with cement-plastered wall had significantly lower (16.4%) PCR positivity than those who were living in a house with wooden walls plastered with Clay or Mud (27.7 %) ($p=0.03$). There were no significant differences in the proportion of malaria infections between different age groups ($p \geq 0.51$) and between male and female participants ($p=0.15$). In addition, bed net use in the previous night ($p \geq 0.30$), IRS in the previous 12 months ($p \geq 0.74$), existence of water body around the house ($p=0.11$), availability of electricity ($p=0.69$), radio ($P=0.54$), television ($p=0.69$), house construction types: type of roof ($p \geq 0.23$), type of floor ($p \geq 0.42$) and holes on the eave ($p \geq 0.15$) were not significant predictors of 18S nested PCR positivity. Five variables from univariate analysis (sex, previous malaria infections, wall type of individuals house, holes on the eave and existence of water body around the house) having $P< 0.2$ were again analyzed using multivariate analysis. In this case previous malaria infections ($p =0.001$) and house wall with cement-plastering ($p=0.05$) were significant predictors of 18S nested PCR positivity but sex of participants ($p=0.07$), holes on the eave($p=0.19$) and existence of water body around the house ($p=0.9$) was not significant predictors of 18S nested PCR positivity.

Table-4:-Parasite positivity as detected by 18S nested PCR among different socio demographic factors of participants using logistic regression analysis, Adama woreda East Shewa zone of Ethiopia, December 2016 to February 2017.

Socio-demographic factors			Univariate analysis			Multivariate analysis
	No. tested%	No. positive%	Unadjusted odds ratio	95%CI	P-value	Adjusted odds ratio (P value)
Sex						
Male	211 (63.9 %)	58 (71.6 %)	1.51	Reference		
Female	119 (36.1 %)	23 (28.4 %)		0.86- 2.64	0.152	0.56(0.07)
Age in years						
<5 years	9 (2.7 %)	3 (33.3 %)		Reference		
5-9 years	41(12.4%)	12 (29.3 %)	0.83	0.18-3.86	0.810	
10-14 years	51(15.5%)	12 (29.3 %)	0.62	0.13-2.84	0.534	
>15 years	229 (69.4 %)	54 (23.6 %)	0.62	0.15 - 2.55	0.505	
previous malaria infections						
yes	21 (6.4 %)	11 (52.4 %)		Reference		
No	297 (90.0 %)	65 (21.9 %)	0.25	0.10-0.63	0.003	0.15(0.001)
Don't know	20 (6.1 %)	2 (66.7 %)	1.82	0.14-23.25	0.646	1.11(0.94)
Bed net use						
Yes	132 (40.0 %)	6 (22.2 %)		Reference		
No	169 (51.2 %)	70 (25.1 %)	1.33	0.78-2.26	0.297	
Don't know	20 (6.1 %)	4 (20.0 %)	0.89	0.28-2.86	0.842	
IRS in previous 12 months						
Sprayed	27 (8.2 %)	6 (22.2 %)		Reference		
Notsprayed	279 (84.6 %)	70 (25.1 %)	1.17	0.45-3.02	0.742	
Don't know	20 (6.1 %)	4 (20.0 %)	0.88	0.21-3.63	0.854	
House construction Floor type						
Earth	243 (73.6 %)	59 (72.8 %)		Reference		

Local dung plaster Cement Wood Other	2 (0.6 %) 80 (24.2 %) 0 (0.0 %) 4 (1.2 %)	1 (1.2 %) 20 (25.0 %) 0 (0.0 %) 1 (1.2 %)	3.12 1.04 - 1.04	0.19-50.64 0.58-1.87 - 0.11-10.18	0.424 0.897 - 0.973	
Wall type Wooden plastered with Clay or mud Mud with cement plastering Iron sheets Brick or stone Other	 148 (44.9 %) 104 (31.5 %) 5 (1.5 %) 51 (15.5 %) 18 (5.5 %)	 41 (27.7 %) 17 (16.4 %) 0 (0.0 %) 15 (29.4 %) 7 (38.9 %)	 0.51 Empty 1.09 1.66	 Reference 0.27- 0.96 - 0.54- 2.19 0.60 - 4.58	 0.037 - 0.815 0.327	 0.47(0.046) - 1.31(0.49) 1.94(0.24)
Roof type Grass thatch Iron sheet Wood and mud Other	 223 (67.6) 3 (13.6 %) 80 (24.2 %) 4 (1.2 %)	 57 (70.4 %) 3 (3.7 %) 20 (24.7 %) 1 (1.2 %)	 0.46 0.97 0.972	 Reference 0.13- 1.61 0.54- 1.75 0.18- 9.52	 0.225 0.921 0.980	
Holes on the eave Yes No Don’t know	 148 (44.8 %) 168 (50.9 %) 10 (3.0 %)	 31 (20.9 %) 47 (27.9 %) 2 (20.0 %)	 1.47 0.94	 Reference 0.87- 2.47 0.19- 4.67	 0.149 0.943	 0.61(0.19) 0.24(0.15)
Electricity: Yes No Don’t know	140 (42.4 %) 189 (57.3 %) 0(0.0 %)	 36 (25.7 %) 45 (23.8 %) 0 (0.0 %)	 0.90	 Reference 0.54 - 1.50	 0.692	
Radio: Yes No Don’t know	 115 (34.9 %) 214 (64.9 %) 0 (0.0 %)	 26 (22.6 %) 55 (25.7 %) 0 (0.0 %)	 1.18	 Reference 0.69- 2.02	 0.535	
Television: Yes No Don’t know	 140 (42.4 %) 189 (57.3 %) 0 (0.0 %)	 36 (25.7 %) 45 (23.8 %) 0 (0.0 %)	 0.90 -	 Reference 0.54- 1.50	 0.692	
Water body in the neighbor hood No yes	 161 (48.8 %) 169 (51.2 %)	 40 (24.8 %) 41(24.3 %)	 0.97	 Reference 0.05-1.07	 0.061	 0.97(0.90)

6.3. Comparison of malaria parasite detection by RDT, microscopy and nested PCR

6.3.1. Diagnosis of symptomatic plasmodium infection

Of the 202 individuals presenting with malaria-related symptoms screened at the Adama malaria control center, 27.2 % were positive with the 18S nested PCR, while with the Carestart pf/pan RDT and Giemsa microscopy,12.9 % and 13.9 % suspected cases turned malaria positive respectively.

When the standard was Giemsa microscopy (Table-5), for the diagnosis of symptomatic *Plasmodium* infection, the Carestart pf/pan RDT presented a sensitivity of100.0%, a specificity of 100.0 %, PPV of 100.0 % and NPV of 100.0 %. The 18S nested PCR had equivalent sensitivity and NPV with RDT (100.0 %) but had a lower specificity and PPV than RDT (84.5 % versus 50.9%).

When 18S nested PCR was a reference, the Carestart pf/pan RDT presented a sensitivity of 50.0 %, a specificity of 100.0 %, PPV of 100.0 % and NPV of 84.6 %. Giemsa microscopy presented sensitivity of 50.9%, a specificity of 100.0 %, PPV of 100.0 % and NPV of 84.5 %. The agreement was 86.7 % (kappa value=0.60) between Carestart pf/pan RDT and 18S nested PCR and 86.6 % (kapa value=0.60) between Giemsa microscopy and PCR and 100.0 % agreement (kapa value=1) between Carestart pf/pan RDT and Giemsa microscopy.

Table-5:-Comparative performance of CareStart™ pf/pan RDT, Giemsa microscopy and 18S nested PCR for the diagnosis of Asymptomatic and Symptomatic malaria cases, Adama woreda East Shewa zone of Ethiopia, December 2016 to February 2017.

Standard	Population groups	Test methods	Sensitivity	Specificity	PPV	NPV	% of Agreement	Kapa Value
Giemsa microscopy	Asymptomatic	CareStart™ pf/pan RDT	62.5%(24%-92%)	100%(97%-100%)	100%(48%-100%)	97.6%(93%-100%)	97.7 %	0.76
		18S nested PCR	62.5%(24%-92%)	82.5%(75%-89%)	19.2%(6.6%-39%)	97.1%(92% -99%)	81.3 %	0.22
18S nested PCR	Asymptomatic	CareStart™ pf/pan RDT	15.4%(4.4% -35%)	98%(93%-100%)	66.7%(22%-96%)	82%(74%-88%)	80.5 %	0.14
		Giemsa	19.2%(6.6%-39%)	97.1%(92%-99%)	62.5%(25%-92%)	82.5%(75%-89%)	81.3 %	0.22
Giemsa microcopy	Symptomatic	CareStart™ pf/pan RDT	100%(87%-100%)	100%(98%-100%)	100% (87% -100%)	100%(98%-100%)	100%	1.00
		18S nested PCR	100%(88%-100%)	84.5%(78%-90%)	50.9% (37%-65%)	100%(98% -100%)	86.6 %	0.60
18S nested PCR	Symptomatic	CareStart™ pf/pan RDT	50%(36%-64%)	100%(98%-100%)	100%(87%-100%	84.6%(78%-90%)	86.7 %	0.595
		Giemsa	50.9%(37%-65%)	100%(98%-100%)	100%(88%-100%)	84.5%(78%-90%)	86.6 %	0.60
Giemsa microscopy	Total malaria cases	CareStart™ pf/pan RDT	91.2%(76%-98%)	100%(99%-100%	100% (89% -100%	99% (97% -100%)	99.07 %	0.95
		18S nested PCR	91.7%(78%-98%)	83.7%(79%-88%)	40.7%(30% 52%)	98.8%(96.5 %- 99.8%)	84.6 %	0.49
18S nested PCR	Total malaria cases	CareStart™ pf/pan RDT	37.2%(27%-49%)	99.2%(97%-100%)	93.5%(79%-99%)	83.2%(78%-87%)	84.2 %	0.46
		Giemsa microscopy	40.7%(30%-52%)	98.8%(97%-100%	91.7%(78%-98%	83.7%(79%-88%	84.6 %	0.49

6.3.2. Diagnosis of asymptomatic plasmodium infection

Out of 128 asymptomatic individuials screened with the three diagnostic methods,the 18S nested PCR, Giemsa microscopy and CareStart^{TM} pf/pan RDT detects plasmodium infection in 20.3 %), 6.3% and 4.1% individuials respectivelly.

Using Giemsa microscopy as standard (Table-5),CareStart^{TM} pf/pan RDTpresented a sensitivity of 62.5 % ,a specificity of 100.0 % ,PPV of 100.0 % and NPV of 97.6 %. The 18S nested PCR presented equivalent sensitivity and NPV with RDT (62.5 % sensitivity and 97.1 % NPV) but a lower specificity and PPV (82.5 % versus 19.2 %).

When 18S nested PCR was gold standard,CareStart^{TM} pf/pan RDT presented a sensitivity of 15.4 % ,a specificity of 98.0 %, a PPV of 66.7 % and a NPV of 82.0 % .Giemsa microscopy presented a sensitivity of 19.2 % ,NPV of 82.5 % ,specificity 97.1 % and PPV of 62.5 % .

The level of agreement was 80.5 % (kappa value=0.14) between CareStart^{TM} pf/pan RDTand18S nested PCR and 81.3 % (kapa value=0.22) between Giemsa microscopy and 18S nested PCR and 97.7 % agreement (kapa value=0.76) between CareStart^{TM} pf/pan RDT and Giemsa microscopy.

6.3.3. Diagnosis of total malaria cases and malaria species

Out of the total 330 participants, the positivity rate was 24.6 % (81/330) by 18S nested PCR,10.9 % (36/330) by microscopy,9.4 % (31/330) by CareStart^{TM} pf/pan RDT. From 81 PCR, positive cases, 63% (51/81) and 59.3 % (48/81) were tested negative by CareStart^{TM} pf/pan RDT and Giemsa microscopy respectively. From 36 Giemsa microscopy positive individuals',18S nested PCR missed 8.3 % (3/36) and CareStart^{TM} pf/pan RDT missed 13.9 % (5/36) of them. CareStart^{TM} pf/pan RDT missed 9.1 % (3/33) of participants positive both by Giemsa microscopy and 18S nested PCR.

Using Giemsa microscopy as gold standard (Table-6), CareStart^{TM} pf/pan RDT performance was; sensitivity:91.2 %, specificity:100.0 %, PPV: 100.0 % and NPV: 99.0 % and the 18S nested PCR had sensitivity:91.7 %, specificity:83.7 %, PPV:40.7 % and NPV:98.8 %.

When 18S nested PCR was a gold standard, CareStart™ pf/pan RDT presented, sensitivity:37.2 %, specificity: 99.2 %, PPV:93.5 % and NPV:83.2 % and Giemsa microscopy presented, sensitivity:40.7 %, specificity:98.8 %, PPV:91.7 %and NPV: 83.7 %. The level of agrment was 84.2 % (kappa value= 0.46) between CareStart^{TM} pf/pan RDT and 18S nested PCR and 84.6 % (kapa value=0.49) between microscopy and18S nested PCR and 99.1% agreement (kapa value=0.95) between CareStart^{TM} pf/pan RDT and Giemsa microscopy.

Species wise analysis revealed that out of 81 nested 18S PCR posetive cases (PV=56,PF=19 and Mixed=6) ,microscope confirmes , only 40.0 % p.vivax cases , 30.0 % of p.falciparium cases and 33.3% of mixed infections. Similarlly CareStart^{TM} pf/pan RDT confirmes 41.8 % *p.vivax* and 15.0 % *p.falciparium* PCR posetive cases but miss all PCR detected mixed infections.

Using 18S nested PCR used as gold standard (Table-6) for the detection of *P. Falciparium*; CareStart™ pf/pan RDT performed sensitivity:15.8 %, specficity: 99.4 %, PPV: 60.0 % and NPV:95.1 % and for P. vivax indicators were: sensitivity:37.5 %, specficity: 98.9%, PPV: 87.5 % and NPV:88.6 %. Giemsa microscopy performed sensitivity:31.6 %, specficity: 99.4 % ,PPV:75 % and NPV:96.0 % for *P.Falciparium* and sensitivity: 39.3 % ,specficity:98.5 % ,PPV:84.6 % and NPV: 88.8 % for *P.vivax.*

When Giemsa Microscopy was gold standard, rather than 18S nested PCR,CareStart™ pf/pan showed a better *P. Falciparium* and *P. vivax* indicators.The indicators were:sensitivity:62.5 %, specficity:100.0 %, PPV:100.0 %, NPV: 99.1 % for *P.Falciparium* and sensitivity: 84.6 %, specficity: 99.3 %, PPV:91.7 % and NPV: 98.7 % for P. vivax.

Nested 18S PCR performed sensitivity:75.0 %,specficity:96.0 %, PPV:31.6 % and NPV:99.4 % for *P. Falciparium* and sensitivity: 84.6 %,specficity: 88.8 %, PPV:39.3 % and NPV:98.5 % for *P. vivax* .To detect *P.Falciparium* the level of agrment was 94.6 % (kappa value=0.23) between CareStart™ pf/pan RDT and 18S nested PCR and 95.5% (kapa value=0.43) between Giemsa microscopy and 18S nested PCRand 99.1 % agreement (kapa value=0.77) between CareStart™ pf/pan RDT and Giemsa microscopy. For *P. vivax,* the level of agrment was 88.5% (kappa value=0.47) between CareStart™ pf/pan RDT and18S nested PCR and 88.5% (kapa value=0.48) between Giemsa microscopy and 18S nested PCR and 98.2 % agreement (kapa value=0.87) between CareStart™ pf/pan RDT and Giemsa microscopy.

Table- 6:-Comparison of the performance of Carestart pf/pan RDT, Giemsa microscopy and 18S nested PCR in the discrimination of Plasmodium species, Adama woreda East Shewa zone of Ethiopia, December 2016 to February 2017.

Standard	Plasmodium species	Diagnostic Tests	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	% of agreement	Kappa Value
Giemsa microscopy	*P. Falciparium*	Carestart ™ pf/pan RDT	62.5%(25% - 92%)	100% (99%- 100%)	100% (48% - 100%)	99.1% (97%- 100%)	99.1%	0.77
		18S nested PCR	75% (35- 97%)	96% (93%- 98%)	31.6% (13%- 57%)	99.4% (98%- 100%)	95.5%	0.43
	P. vivax	Carestart ™ pf/pan RDT	84.6% (65%- 96%)	99.3% (98%- 100%)	91.7% (73%- 99%)	98.7% (97%- 100%)	98.2%	0.87
		18S nested PCR	84.6% (65% - 96%)	88.8% (85%- 92%)	39.3%(27%- 53%)	98.5% (96%- 100%)	88.5%	0.48
18S nested PCR	*P. Falciparium*	Carestart ™ pf/pan RDT	15.8% (3.4%- 40%)	99.4% (98%- 100%)	60% (15% - 95%)	95.1% (92%- 97%)	94.6%	0.23
		Giemsa Microscopy	31.6%(13%- 57%)	99.4% (98% - 100%)	75% (35%- 97%)	96% (93%- 98%)	95.5%	0.43
	P. vivax	Carestart ™ pf/pan RDT	37.5% (25%- 52%)	98.9% (97%- 100%)	87.5% (68%- 97%)	88.6% (85%- 92%)	88.5%	0.47
		Giemsa Microscopy	39.3% (27%- 53%)	98.5% (96%- 100%)	84.6% (65%- 96%)	88.8% (85%- 92%)	88.5%	0.48

7. DISCUSSION

The accuracy of Giemsa microscopy and CareStart TM pf/pan RDT routinely practiced diagnostic methods and nested 18S PCR were compared in asymptomatic and symptomatic malaria patients. The diagnostic accuracy of these methods was measured against Giemsa microscopy/18S nested PCR/ as gold standard. Most studies have however reported on the accuracy of these and other RDTs using Giemsa microscopy as gold standard (Ashton *et al*., 2010, Chanie *et al*., 2011, Eticha, 2016, Moges *et al*., 2012, Endeshaw *et al*., 2008). For a balanced comparison, a sub-analysis using 18S nested PCR as gold standard was also performed.

In a review of studies Microscopy detected on average only half of malaria infections detected by nested PCR (Golassa *et al*., 2015). Even some limited data show that RDT or Giemsa microscope missed nearly three quarters of infections detected by PCR (Umbers *et al*., 2015). Consistent with the aforementioned previous reports, in in my study RDT and Giemsa microscope failed to diagnose about 50/81 (61.7%) and 48/81 (59.3 %) *Plasmodium* infections detected by nested PCR respectively. In addition, RDT missed 14% of infections (any species) detected by Giemsa microscope. PCR detected 32 *P. vivax*, 14 *P. Falciparium* and 2 mixed infection of the 48 microscopy negative samples and 32 *P. vivax*, 15 *P. Falciparium* and 3 mixed infection in 50 of RDT negative samples. I also observed a marked underestimation of malaria infection when Giemsa microscopy and carestartTM pf/pan RDT were compared with nested 18S PCR. Thus, my finding implies the possibility that a significant number of malaria infected participants were left untreated for not being detected by Giemsa microscopy or carestartTM pf/pan RDT. Such diagnostic limitations of microscope and carestartTM pf/pan RDT might have serious implications for malaria control and elimination in the country. Misdiagnosis of plasmodium species increases the risk of complication or severity of the disease (Golassa *et al*., 2015).It can also lead to recrudescence and drug resistance (Kang *et al*., 2017).

Various factors are known to affect the performance of microscopy including; quality of equipment and reagents, the type and quality of the smear, skill of the technician, the parasite density, and the time spent on reading the smear. However, even under optimal conditions microscopy based diagnosis does not achieve the low detection limits that PCR-based methods yield (Alemu *et al*., 2014). The observed False negative results by RDT in my study may be caused by: (i) deletion or mutation of HRP-2 gene; (ii) the presence of blocking antibodies and (iii) presence of an inhibitor in the patient’s blood preventing development of the control line which also have been reported pervious in Ethiopian setting (Woyessa *et al*., 2013).While microscopy confirmed the presence of parasites only in 40.74% of PCR positive samples, PCR confirmed the presence of parasite DNA in 91.67 % of microscopy positive samples. Taking the detection limit of PCR as low as 0.002 parasites per µl, into consideration, it is not surprising to note the superiority of PCR over routine diagnostic methods (Golassa *et al*., 2015) Of course, nested 18S PCR missed 8.3% (3/36) of infections detected by Giemsa microscopy. The lack of detection of infections by PCR in these samples could be due to PCR might give false negative results due to modification (mutation) in the target sequence; deletion/insertion of sequence or degradation of DNA during sample preparation and storage. Alternatively, amplification may fail due to inhibition of PCR by sample components (Barker *et al*., 1994).

In line with previous findings (Kyabayinze *et al*., 2011) ,the CareStartTMpf/pan RDT in my study showed lower sensitivity than Giemsa microscope compared with nested PCR (37.2 % vs74.1 %), which is also lower than the sensitivity recommended by the WHO (Bastiaens *et al*., 2014). Also lower sensitivity of RDT (OptiMAL RDT) versus microscopy has been reported in immigrants' populations in Kuwait (66.0 % and 86.0 %.) (Iqbal *et al*., 1999) and in Venezuela (87.0 % versus 95.7%) (Rodulfo *et al*., 2007). In contrast to my finding, significantly higher sensitivity of RDT than Giemsa microscope has been reported with Paracheck- Pf® RDT in Angola (72.8% *versus* 60.0 %)(Fançony *et al*., 2013) and Binax NOW RDT in United States (97.0 % versus 85.0 %) (Stauffer *et al*., 2009).

Moreover, the estimates of the sensitivity of CareStart TM pf/pan tests performance depends on the reference standard used (Woyessa *et al*., 2013).The sensitivity of RDTs was lower (37.2 %)when compared to PCR but 91.2 % when compared to microscopy. A similar pattern was observed in western Kenya with sensitivity of RDTs 71.1–75.4 % when compared to PCR but >90 % when compared to microscope (Wanja *et al*., 2016) and in eastern Sudan with sensitivity of 69 % when compared to PCR but77.8 % when RDT was compared to microscope (Osman *et a*l., 2010) . This can be related with higher detection limit of nested PCR (as low as 0.002 P/µL) and lower and almost similar detection limit of RDT and microscopy (100 P/µL) (Kemleu *et al*., 2016).Compared to the less sensitive reference standard, Giemsa microscopy, RDT had a good performance 91.2 % and 100.0 % sensitivity and specificity respectively. The observed sensitivity of CareStartTMpf/pan RDT (91.2 %) in my study with Giemsa microscope as reference, is lower than previous reports of this test kit; 97.4% in Gam Gofa zone (Eticha, 2016);99.8 % in Pawe special woreda (Hailu *et al*., 2014); and 95.0 % in kola Diba (Moges *et al*., 2012) but higher than the study in Butajira(90.8 %), (89.7 %). This performance variation may relate with product lots, the impact of transportation and storage conditions of CareStartTMpf/pan RDT and also study seating(Eticha, 2016).Genetic variation of target antigens (genetic deletions, frame shift mutations or alterations in protein expression), can also lead to variation in performance of RDTs in different regions (Kozycki *et al*., 2017). However, the specificities of (100.0 % for CareStartTMpf/pan RDTand 98.8 % for Giemsa microscopy) reported in this study were higher than the specificity of CareStartTMpf/pan RDT reported from Butajira 82.7 % (Woyessa *et al*., 2013), Pawe Special Woreda (97.7 %) (Hailu *et al*., 2014) and Kola Diba (94.2%) (Moges *et al*., 2012).

In most seating's microscopy and RDTs miss between 30.0 % to 50.0 % of asymptomatic infections compared to nucleic acid amplification tests (Okell *et al*., 2009). Early study from, Myanmar, reported that 38 asymptomatic cases which were not detected by microscopic examination, were identified by molecular detection method. In my study, the CareStartTMpf/pan RDT and Giemsa microscope also showed a substantially low performance compared to 18S nested PCR,15.4 % and 19.2 %, for Giemsa microscope and CareStartTMpf/pan RDT respectively. These findings are consistent with previous studies reporting, low sensitivity (26–32 %) of four RDTs (Carestart™, First-Response®, Parascreen® and SD-Bioline®). T) and field microscopy) relative to PCR, among asymptomatic pregnant women in eastern Indonesia (Ahmed *et al*., 2015) Low sensitivity of RDTs (37.5 % for ICT/29.1 % for OptiMAL) than Giemsa microscope (50.0 %) was also reported in Canada (Ndao et al., 2004). But in contrast with studies reporting higher sensitivity of RDT than Giemsa microscope.:-(93.9 % for SD Bioline® Pf/Pan test and 8.5 % for Giemsa microscope,) in Yemen (Alareqi et al., 2016) and (70.0 % for Optimal-IT RDT versus 22.5% for field microscopy) in Brazil amazon (Andrade *et al*., 2010) The Specificity of RDT and Giemsa microscope were comparable ,with 100.0 % for both methods in asymptomatic cases and (98.0 % for RDT vs 97.1 % for Giemsa microscope) among symptomatic cases. Similarly, higher specificity (100.0 %) have been reported for CareStartTM pf/pan, SD Bioline pf/pan,NanoSign and Asan Easy RDTtest and Giemsa microscope in south Korea (Kim *et al*., 2013).Such high specificity of CareStart TM pf/pan RDT in the present study is also in agreement to the high specificity (96.1 %) recorded for the CareStart™ HRP-2 RDT tested in an earlier study (Atroosh *et al*., 2015) but in contrast with a previous study comparing four brands of *Pf*HRP2-based RDTs ([Bioline SD , First response malaria , Paracheck , ICT diagnostics) for f*alciparum* malaria diagnosis among febrile patients in Malawi, where specificity of 39.0 –68 .0 % was reported (Chinkhumba *et al*., 2010).

Compared to 18S nPCR, I observed equivalent sensitivity of CareStart TM pf/pan RDT (50.0 %) and light microscope (50.9 %) and was even poorer for asymptomatic (15.4% for CareStart TM pf/pan RDT and 19.2 % for Giemsa microscope) cases which was in line with early studies. A study in Papua New Guinean reported better sensitivity of RDT in symptomatic individuals than in Asymptomatic individuals (Umbers *et al*., 2015).

Moreover, in terms of detecting different species of malaria the performance of the CareStartTM RDT and Giemsa microscopy was low. Similarly, a study from Papua New Guinean reported that RDT and Giemsa microscope missed 88.0 % (22/25) and 76.0 % (19/25) of *P. vivax* infections, respectively (Umbers *et al*., 2015). Another study by Huetmaker found species miss identification by RDT compared to microscopy and PCR in 4/10 (2.5 %) samples (Heutmekers *et al*., 2012).

Compared to PCR, Giemsa microscopy and RDT performed better in detecting *P. vivax* than detecting *P. falciparum* and mixed infected subjects with comparable sensitivity. A similar pattern was reported from northwest Ethiopia (Alam *et al*., 2011);Solomon island (Harris *et al*., 2010) and Colombia (Zurovac *et al*., 2006). A possible explanation for this might be that in *P. falciparum* which usually are only ring-stage parasites in blood smears and they are smaller than the ring-stage and mature trophozoites of *P. vivax* and may be easier to miss by microscopy particularly when staining is less than perfect (Harris *et al*., 2010).

Early studies, reported the low diagnosis reliability of microscopy for species specific and mixed-infections in endemic areas (Reyburn *et al*., 2007). A study from China-Myanmar documented misdiagnosis of mixed infection by microscope among 31 PCR confirmed cases (Kang *et al*., 2017) and a study from Venezuela reported that mixed infection was only detected by PCR, while the RDT and Giemsa microscope diagnosed it as *P. falciparum* or *P. vivax infections (*Rodulf2007*).* In line with this, I observed great misdiagnose of plasmodium species (17 *P. falciparum* and all mixed infections) with RDT and (14 *P. falciparum* and 4 mixed infections) with Giemsa microscope compared to nested PCR.

8.CONCLUSION

With slight difference to detect asymptomatic cases and mixed infections, overall the performance of CareStart TM pf/pan RDTs to detect malaria was equivalent to Giemsa microscopy. 18S nested PCR assay showed better performance in all circumstances; in asymptomatic, symptomatic and species-specific diagnosis. Better performance by 18S nested PCR was observed particularly in monitoring the asymptomatic carriage of malaria and in making diagnosis of symptomatic malaria to a species-specific level.

9.STRENGTH and LIMITATION

My study has the following strength:

- I include Asymptomatic and Symptomatic cases.
- Standard procedures were used in all the procedures.

Weaknesses of my study are.

- Quantitative assessments of parasitaemia was not performed.

10. RECOMMENDATIONS

According to these findings, I would like to recommend the following points:

- ✓ Given the ease of performing and interpreting results; the observed equivalent performance of CareStart TM pf/pan RDTs to Giemsa microscopy done by WHO certified malaria microscopists underlines its value as alternative or parallel use in resource limited setups, like ours.
- ✓ Incorporation of PCR techniques in the Ethiopian guideline especially on the way to elimination looks more important.

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ANNEXES

Annex I: Participant information and assent form for children between (6 months and 18 years)

1.Participaint information

Hello, how are you? My name is ______________ I came from ___________ This is an interview to be done with you for a study that is being conducted at Addis Ababa University, college of health Science, School of medicine.

Title of the study: -"Comparative Assessment of Microscopy, rapid diagnostic tests RDT and Polymerase chain reaction as Malaria Diagnostic Tools "

1) Purpose of the study

Early and adequate diagnosis and prompt treatment is one of the main strategies in malaria prevention, control and effective disease management. Reduction of malaria morbidity and drug resistance intensity plus the associated economic loss of these two factors require urgent scaling up of the quality of parasite-based diagnostic methods. Moreover, an investment in anti-malarial drug development or malaria vaccine development should be accompanied by a parallel commitment to improve diagnostic tools and their availability to people living in malarias areas. Data on the sensitivity and specificity of each test methods is important information to help guide test selection by national malaria control programmers.

We therefore ask your permission to participate in this study and also for long term storage of samples from this study. In case of further research on stored samples, all identifying information such as your name or address will be removed from the data and ethics approval will be sought.

2) Study procedures

You or your child will be asked to donate a small finger prick blood sample at the beginning of the study. We use the sample to determine the presence of malaria parasites in the blood. We are aiming to enroll all malaria suspected individuals coming to the health center. We will also ask you some questions related to malaria (risk factors for the disease). All individuals who have a fever and have a positive test RDT s will be treated.

3) Voluntary participation

Your decision not to participate or to withdraw from participation will not affect the care you or your child 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. If you chose not to participate, you have access to the same level of clinical care.

4) Discomforts and Risks

You or your child might feel a small amount of discomfort during blood sampling and you or your child may have a small amount of bruising or bleeding where the blood sample was taken. This is considered not to be harmful. We will use sterile equipment to collect the blood sample and the small wound that may arise from the procedure will be treated adequately. The volume of blood is less than half of a teaspoon and too small to influence you or your child's health and the blood will quickly be replaced by your/your child's body. There is no discomfort associated with the collection of sample from your or your child's finger.

5) Benefits

You or your child will receive free clinical care for the duration of the study. You or your child will not be paid for participation in this study but will be given treatment of the disease if you or your child have the parasite in your or your child's blood.

6) Confidentiality statement

The records concerning your/your child's participation are to be used only for the purpose of this research project. Your/your child's name will not be used on labels on laboratory specimens or in any report resulting from this study. At the beginning of the study, we will give you/your child 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 and under lock and key. Only senior members of the study team will have access to information linking your/your child's name with your/your child's study number.

7) Long term storage of samples

We will ask your consent to store your/your child's blood samples for long term storage. New techniques may become available to study the research questions we want to answer in this study. We will anonymize your samples by removing the name and any identifying information. If further studies are conducted on stored study material, ethics approval will be sought.

8) Questions and freedom to withdraw from the study) Freedom to ask questions

If you have questions regarding this study or would like to be informed of the results after its completion, please do not hesitate to contact: Getaneh Tegegne, School of Medicine, Department of Medical Biochemistry, Addis Ababa University, Ethiopia.

Cell phone: -0918140704

email: getanehtegegne21@gmail.com

Annex II. Informed assent agreement form for children between (6 months and 1 8years)

"Comparative Assessment of Microscopy, rapid diagnostic tests RDT and Polymerase chain reaction as Malaria Diagnostic Tools "

I, ____ (PARTICIPANT'S NAME), having full capacity to, do hereby consent to participation of my child in the research study entitled "Comparative Assessment of Microscopy, RDTs and PCR techniques as Malaria Diagnostic Tools", under the principal investigator Mr. Getaneh Tegegne. The implications of my voluntary participation, the nature, duration and purpose; methods and means by which it is to be conducted; and the inconveniences and hazards which may reasonably be expected have been explained to me by _________________________, and are set forth in the Informed Consent Explanation, which I have signed. I have been given an opportunity to ask questions concerning this investigational study, and any such questions have been answered to my full and complete satisfaction. If there are any further questions that may arise, I may contact Mr. Getaneh Tegegne (0918140704) School of Medicine, Department of Medical Biochemistry, Addis Ababa University, Ethiopia. I understand that I may at any time during the course of this study revoke my consent and withdraw my child from the study without prejudice; however, my child may be requested to undergo further examinations if, in the opinion of the physician, such examinations are necessary for his or her wellbeing.

I **understand** / **do not understand** the practical consequences of this study, asking me to provide small finger prick blood samples(circle)

I **approve** / **disapprove** of part of the blood sample to be analyzed outside Ethiopia (circle)

I **approve** / **disapprove** of part of the sample to be stored for future analyses (circle). If studies are conducted using stored study material, approval from ethics committees will be sought.

I **agree** / **disagree** to take part in this study (circle)

Thumbprint if subject is unable to sign

Participant's name: _______________________________________

Participant's signature: ______________________________________

Impartial witness's name: ______________________________________

Impartial witness's signature: _________________________________ Date:

Local investigator's name and signature: _____________________________

Annexes III: Parental/guardian information and consent form for adults (18 years and above)

1.Parental/guardian information for adults (18 years and above)

Hello, how are you? My name is ______________ I came from ___________ This is an interview to be done with you for a study that is being conducted at Addis Ababa University, college of health Science, School of medicine.

Title of the study: -"Comparative Assessment of Microscopy, rapid diagnostic tests RDT and Polymerase chain reaction as Malaria Diagnostic Tools "

1) Purpose of the study

Early and adequate diagnosis and prompt treatment is one of the main strategies in malaria

prevention, control and effective disease management. Reduction of malaria morbidity and drug resistance intensity plus the associated economic loss of these two factors require urgent scaling up of the quality of parasite-based diagnostic methods. Moreover, an investment in anti-malarial drug development or malaria vaccine development should be accompanied by a parallel commitment to improve diagnostic tools and their availability to people living in malarias areas. Data on the sensitivity and specificity of each test methods is important information to help guide test selection by national malaria control programmers.

We therefore ask your permission to participate in this study and also for long term storage of samples from this study. In case of further research on stored samples, all identifying information such as your name or address will be removed from the data and ethics approval will be sought.

2) Study procedures

You will be asked to donate a small finger prick blood sample at the beginning of the study. We use the sample to determine the presence of malaria parasites in the blood. We are aiming to enroll all malaria suspected individuals coming to the health center. We will also ask you some questions related to malaria (risk factors for the disease). All individuals who have a fever and have a positive test for RDTs will be treated.

3) 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. If you chose not to participate, you have access to the same level of clinical care.

4) Discomforts and Risks

You might feel a small amount of discomfort during blood sampling and you may have a small amount of bruising or bleeding where the blood sample was taken. This is considered not to be harmful. We will use sterile equipment to collect the blood sample and the small wound that may arise from the procedure will be treated adequately. The volume of blood is less than half of a teaspoon and too small to influence you or your health and the blood will quickly be replaced by your body. There is no discomfort associated with the collection of sample from your finger.

5) Benefits

You will receive free clinical care for the duration of the study. You will not be paid for participation in this study but will be given treatment of the disease if you have the parasite in your blood.

6) 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 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 and under lock and key. Only senior members of the study team will have access to information linking your name with your study number.

7) Long term storage of samples

We will ask your consent to store your blood samples for long term storage. New techniques may become available to study the research questions we want to answer in this study. We will anonymize your samples by removing the name and any identifying information. If further studies are conducted on stored study material, ethics approval will be sought.

8) Questions and freedom to withdraw from the study) Freedom to ask questions

If you have questions regarding this study or would like to be informed of the results after its completion, please do not hesitate to contact: Getaneh Tegegne, School of Medicine, Department of Medical Biochemistry, Addis Ababa University, Ethiopia.

Cell phone: -0918140704

email: getanehtegegne21@gmail.com

Annex IV. Informed consent agreement for adults (18 years and above)

"Comparative Assessment of Microscopy, rapid diagnostic tests RDT and Polymerase chain reaction as Malaria Diagnostic Tools "

I, ____ (PARTICIPANT'S NAME), having full capacity to, do hereby consent to my participation in the research study entitled" Comparative Assessment of Microscopy, rapid diagnostic tests RDT and Polymerase chain reaction as Malaria Diagnostic Tools " under the principal investigator Mr. Getaneh Tegegne. The implications of my voluntary participation, the nature, duration and purpose; methods and means by which it is to be conducted; and the inconveniences and hazards which may reasonably be expected have been explained to me by _________________________, and are set forth in the Informed Consent Explanation, which I have signed. I have been given an opportunity to ask questions concerning this investigational study, and any such questions have been answered to my full and complete satisfaction. If there are any further questions that may arise, I may contact Mr. Getaneh Tegegne (0918140704) School of Medicine, Department of Medical Biochemistry, Addis Ababa University, Ethiopia. I understand that I may at any time during the course of this study revoke my consent and withdraw my participation from the study without prejudice; however, I may be requested to undergo further examinations if, in the opinion of the physician, such examinations are necessary my wellbeing.

I **understand** / **do not understand** the practical consequences of this study, asking me to provide small finger prick blood samples (circle)

I **approve** / **disapprove** of part of the blood sample to be analyzed outside Ethiopia (circle)

I **approve** / **disapprove** of part of the sample to be stored for future analyses (circle). If studies are conducted using stored study material, approval from ethics committees will be sought

I **agree** / **disagree** to take part in this study (circle)

Participant's name: ______________________________________

Participant's signature: ___________________________________

Impartial witness's name: ____

Impartial witness's signature: _______________________________

Local investigator's name and signature: ____________________________

Thumbprint if subject is unable to sign

Annex-V: -Questionnaire

1. English version of Questionnaire based interview

Title of the study: - "Comparative Assessment of Microscopy, rapid diagnostic tests RDT and Polymerase chain reaction as Malaria Diagnostic Tools "

The interviewer begins by introducing himself and the purpose of the study to the participants, requesting the respondents for participation in the survey, thanking the participant for giving consent to take part in the study.

Date: __ __/__ __/__ __ __ __(dd/mm/yyyy)

1. Participant's name: ____
2. Participant's code: __ __/__ __ __ __/ __ __ __
3. Participant's telephone number: __ __ __ __ __ __ __ __ __ __
4. Date of Birth: __ __/__ __/__ __ __ __(dd/mm/yyyy)
5. Age (in years) ____years
6. Gender: a. Male b. Female
7. Woreda (District) name: ____
8. Name of town or village: ____
9. Household number: ____
10. What is the nearest health facility to your house?

a. Hospital
b. Health center
c. Clinic
d. Health post

11. How far is the nearest health institution located (time it takes, in minutes, to arrive there with the available transportation)? ______________________________minutes
12. Have you (has the child) had malaria in the last three weeks?

a. Yes b. No c. Don't know

13. If yes, when exactly? ____
14. Was treatment given?

a. Yes, name of the drug if known_______________________________________
b. No
c. Don't know

15. Parent/guardian name (for children <18 years old): ____________________________
16. What kind of water body exists in your neighborhood? (you can give more than one answer)

a. None
b. River
c. Lake
d. Pond
e. Swamp
f. Stagnant water
g. Other (Specify) ____

17. How far (in minutes) is the nearest water body located from your house on walking? __________(minutes)
18. Does your household have any of the following?

a. Electricity:
i. Yes ii. No iii. Don't know
b. Radio:
i. Yes ii. No iii. Don't know
c. Television:

i. Yes ii. No iii. Don't know

19. What type of roof was used for the construction of the house?
a. Grass that chc. Wood and mud
b. Iron sheeted.
c. Other

20. What type of wall was used for the construction of the house?
a. Wooden plastered with Clay or mud
b. Mud with cement plastering
c. Iron sheets
d. Brick or stone
e. Other___________

21. What is the main material of the house's Floor?
a. Earth
b. Local dung plaster
c. Cement
d. Wood
e. Other_____________

22. Are the eaves open or closed?
A. Open
b. Closed
c. Partially open
d. No eaves/not applicable

23. Do you own a bednet for yourself (the child in the study)?
a. Yes b. No c. Don't know

24. Did you use the bednet (for the child in the study) during the last night?
a. Yes b. No c. Don't know

25. How many people permanently live in the house? _____________________________

26. How many bednets are available in the house? ________________________________

27. At any time in the past 12 months, have the interior walls of the house been sprayed against mosquitoes?
a. Yes b. No c. Don't know

28. How many months ago was the house sprayed? (If less than one month, record '00' months ago)? ____

29. Have you (your child) made any overnight trips outside of (the DISTRICT) in the last 3 months?
a. Yes b. No c. Don't know

30. If yes, where did you (your child) spend the most time during this overnight trip and when exactly? ____

31. What time do you (does the child) enter the house usually in the night? ___________

32. What time did you (the child) enter the sleeping space last night? _______________

33. What time do you (the child) leave the sleeping space in the morning? ___________

34. What time do you (the child) leave the house in the morning? ___________________

35. Temperature (^{o}C): ____

if ≥37.5^{o}C carry out RDT

if <37.5 ^{o}C skip to Hb results

36. RDT taken?
a. Yes b. No

37. If not, why not? ____

38. RDT results
a. Positive b. Negative c. Invalid

If positive, refer to the nearest health institution for treatment to be given

39. Hb taken? A. yes b. No
40. If not, why not? ____
41. Hb result: ____

If < 8 g/dL, refer to the nearest health institution for treatment to be given

42. Microtainer sample taken?

a. Yes
b. No

43. If not, why not? ____
44. Microscope slide film taken? a. Yes b. No
45. If not, why not? ____
46. Filter paper taken?

a. Yes
b. No

If not, why not? ____

47. 2.Amharic version of questionnaire-based interview

የመረጃ ማሰባሰቢያ ቅጽ

የጥናቱ ርዕስ፡- በኢትዮጵያ ለወባ ምርመራ የምንጠቀምባቸውን ቴክኒኮች (RAPID DIAGNOSTIC TEST/RDT, MICROSCOPY AND POLMERASE CHAIN REACTION /PCR) በማወዳደረ የመመረመረ ዐቅማቸውን መገምገምና መረዳት

መረጃአሰባሳቢውእራሱንእናየጥናቱንዓላምበማስተዋወቅእንዲሁምየጥናቱንተሳታፊዎችለመሳተፍፍቃደኛመሆናቸውንበማመስ ገንይጀምራል።

ቀን፡-__ __/__ __/__ __ __ __

<u>ስክሪን አንድ</u>

1. የተሳታፊው ስም፡-________________________________
2. የተሳታፊው ኮድ፡-________________________________
3. የተሳታፊው የስልክ ቁጥር፡-____________________________
4. የተማሪዉ የትውልድ ቀን፡- __ __/ __ __/ __ __ __ __
5. የተማሪዉ ዕድሜ (በዓመት) ______________________ዓመት
6. ፆታ፡-
   a. ወንድ
   b. ሴት
7. የወረዳዉ ስም፡-________________________________
8. መነደሩ (ከተማው) ያለበት ከተማ ስም፡-________________________
9. የመኖሪያ ቤት ቁጥር፡-______________________________
10. በቅርበት ያለው የጤና ተቋአም ምንድን ነው?
    a. ሆስፒታል
    b. ጤና ጣቢያ
    c. ክሊኒክ
    d. የጤና ኬላ
11. በቅርብ ያለው የጤና ማዕከል ለመድረስ ምን ያህል ይፈጃል (የሚፈጀው ሰኣት በደቂቃ) ፡-__________ደቂቃ
12. ልጅዎ (እርስዎ)ባለፉት ሶስት ሳምንታት ወባ በሽታ ይዞት ያውቃል?
    a. አዎ
    b. አያውቅም
    c. አላውቅም
13. መልስዎ አዎን ከሆነ በትክክሉ መቼ ነበረ? ________________________
14. ህክምና ተሰቶት ነበረ?
    a. አዎ, የመድሃኒቱን ስም ይጥቀሱ ________________________
    b. አልተሰጠውም (ኝም)
    c. አላውቅም
15. የቤተሰብ/የአሳዳጊ ስም (ዕድሜያቸው ከ18 ዓመት በታች ለሆኑ) ፡-__________________
16. በቤትዎ ቅርብ ምን ዓይነት የውሃ አካል ይገኛል? (አንድና ከዛም በላይ መምረጥ ይችላሉ)
    a. ምንም የለም
    b. ፈሳሽ ወንዝ
    c. ሃይቅ
    d. ኩሬ
    e. ረግረግ
    f. የተኛ ውሃ
    g. ሌላ ካለ ይጥቀሱ ____________________
17. በቅርብ ያለው የውሃ አካል ከመኖሪያ ቤቱ በእግር ጉዞ ምን ያህል ደቂቃ ይርቃል? ____________ደቂቃ
18. መኖሪያ ቤታችሁ የሚከተሉት አሉት?
    a. ኤሌክትሪሲቲ

i. አለው ii. የለውም iii. አላውቅም

b. ሬዲዮ

i. አለው ii. የለውም iii. አላውቅም

c. ቴሌቪዥን

i. አለው ii. የለውም iii. አላውቅም

19. መኖሪያ ቤታችሁ ከምን አይነት ጣራ ነው የተሰራው?

a. ከሳር ክዳን
b. ከቆርቆሮ
c. እንጨት እና ጭቃ
d. ሌላ (ይገለጽ)_____________

20. ቤቱ ከምን አይነት ጣራ ነው የተሰራው?

a. ከእንጨት እና ጭቃ ምርጊት
b. ጭቃ በሲሚንቶ ምርጊት
c. ከቆርቆሮ
d. ከድንጋይ ወይም ብሎኬት
e. ሌላ (ይገለጽ)_______________

21. የቤቱ ወለል ከምንድነው የተሰራው?

a. ዓፈር
b. እበት የተለቀለቀ
c. ሲሚንቶ
d. እንጨት (ጣውላ)
e. ሌላ (ይገለጽ)_______________

22. ጣራው ወደዉጭ ክፍት ነው ወይስ ዝግ?

a. ክፍት
b. ዝግ
c. መለስተኛ ክፍት

23. ለራስዎ (ለልጅዎ) አጎበር ይጠቀማሉ?

a. አዎ b. አልጠቀምም c. አላውቅም

24. ለራስዎ (ለልጅዎ)ባለፈው ምሽት አጎበር ተጠቅመዋል?

a. አዎ b. አልተጠቀምኩም c. አላውቅም

25. በቤቱ ውስጥ ስንት ሰዎች ይኖራሉ? ____

26. ስንት አጎበሮች አለዎት? ____

27. ባለፉት 12 ወራት የመኖሪያ ቤታችሁ ውስጡ የወባ መከላከያ ተረጭቶ ያዉቃል?

a. አዎ b. አያውቅም c. አላውቅም

28. ከ ስንት ወር በፊት ነው የወባ መከላከያ የተረጨው? ____

29. ባለፉት 3 ወራት እርስዎ (ልጅዎ) የሌሊት ጉዞ አድርጎ(ገው) ያውቃል(ሉ)?

a. አዎ b. አልጠቀምም c. አላውቅም

30. መልስዎ አዎን ከሆነ አብዛኛውን ምሽት የት ነበር የሚያሳልፈው(ፉት)? ______________________________

31. እርስዎ (ልጅዎ) ምሽት ላይ ብዙ ጊዜ በስንት ሰዓት ወደ ቤቱ ይገባል? ______________________________

32. እርስዎ (ልጅዎ) ባለፈው ምሽት ወደ መኝታ ቦታው ስንት ሰዓት ላይ ነው የገባው(ቡት)? ___________________

33. ልጅዎ ጠዋት ላይ ብዙ ጊዜ በስንት ሰዓት ከ ቤት ይወጣል? ____________________________________

34. እርስዎ (ልጅዎ) ባለፈው ጠዋት በስንት ሰዓት ከቤት ወጣ(ጡ)? ________________________________

35. የሰውነት ሙቀት (^oC)

ከ37.5 ^oC በላይ ከሆነ RDT ይሰራ

ከ37.5 ^oC በታች ከሆነ ወደ Hb ምርመራ ቀጥል/ይ

36. RDT ተወስዶአል?

a. አዎ
b. አልተወሰደም

37. ካልተወሰደ ለምን? ____________________

38. RDT ዉጤት

a. ፖዚቲቭ b. ኔጌቲቭ c. ኢንቫሊድ

ፖዚቲቭ ከሆነ በቅርብ ወዳለው የጤና ማዕከል ይመራ ህክምና እንዲሰጠው

39. Hb ተሰርቶአል?

a. አዎ b. አልተወሰደም

40. ካልተወሰደ ለምን? ____________________

41. Hb ዉጤት፡-

ከ8g/dL በታች ከሆነ አይረን ይሰጥ

42. በቲዩብ ደም ተወስዶአል?

a. አዎ b. አልተወሰደም

43. ካልተወሰደ ለምን? ____________________

44. የማይክሮስኮፕ መመልከቻ መስታወት ላይ ደም ተወስዶአል?

a. አዎ b. አልተወሰደም

45. ካልተወሰደ ለምን? ____________________

46. የደም ጠብታ ወረቀት ላይ ተወስዶአል?

a. አዎ b. አልተወሰደም

47. ካልተወሰደ ለምን? ____________________

3.Afan Oromo version of questionnaire-based interview

Gaafannoo Yaada Guuruf Qopaha'e

Mataduree Qoranichaa: Itiyoopiyaa keessatti dhibee busaa qorachuudhaaf kan fayyadamnu "Maayikirooskoppii, Rapid DiagnosticTest fi Polymerase Chain reaction' kan jedhaman dandeetti maxxantuu dhibee busaa addaan baasuu isaanii wal madaalchisuun qorachuu.

Odeeffanoon kuni meeshaalee eleektiroonikaa gargaaramuun sassaabama. Namni odeeffanoo kana sassaabus mataa isaa fi kaayyoo qoranichaa ibsuun hirmaattoni qoranichas yaada isaanii fedhiiniin waan kennaniif dursee galateeffachuun eegala.

Guyyaa:-__ __/__ __/__ __ __ __

1.Maqaa Hirmaataa: ____

2.Koodii Hirmaataa: __ __/__ __ __ __/ __ __ __

3.Lakk. Bilbila Hirmaataa: __ __ __ __ __ __ __ __ __ __

4.Guyyaa dhalootaa: __ __/__ __/__ __ __ __

5.Umurii (waggaan)____

6.Saala:

a. Dhiira
b. Dhalaa

7.Aanaa: ____

8.Maqaa Magaalaa ykn gandaa: ____

9.Lakk. Manaa: ____

10.Manni yaalaa mana keessanitti dhiyeenyan argamu kami?

c. Hospitaala
e. Kiliinika fayyaa
d. Buufata fayyaa
f. Kellaa Fayyaa

11.Mana yaalaa dhiyootti argamu ga'uuf yeroo hangam (daqiiqaan) fudhata? Daqiiqaa______________________________

12.Torban sadan darban kana keessa isin ykn daa'imman keessan dhibee busaa dhukkubsattaii/dhubsatanii beektu?

g. Eeyyee
h. Lakki
i. Hin beeku

13.Yoo deebi'iin keessan eeyee ta'e, yoomi laata? ________________________________

14.Yaalii argattaniittuu?

j. Eeyyee yoo jettan, maqaa daawwaa yoo beektan______________________________
k. Lakki
l. Hin beeku

15.Maqaa maatii/Guddistuu (ijollee umurii waggaa 18 gad ta'aniif): ____________________________

16.Mana keessaniitti dhiyeenyaan qaamoleen bishaanii argamu kami? (deebi'ii tokkoo ol kenuun ni danda'ama)

m. Hinjiru
n. Laga
o. Garba bishanii
p. Burqaa/yaatuu
q. Chaffee
r. Bishaan chiisaa
s. Kan biro (Haa ibsamu) ____

17. Bishaan mana keessaniitti dhiyeenyaan argamu kana ga'uuf deemsa miillaa hangamii (daqiiqan) ta'a? Daqiiqaa _________________________

18.Manni keessan kanneen armaan gadii ni qabaataa?

t. Ibsaa eleektiriikaa:
i. Eeyyee ii. Lakki iii. Hin beeku

u. Radiyooo:
i. Eeyyee ii. Lakki iii. Hin beeku

v. Televizninii:
i. Eeyyee ii. Lakki iii. Hinbeeku

19.Baaxii manni keessanii maal irraa ijaareame?

w. Chitaa
x. Qorqoorroo
y. Mukaa fi dhoqqee
z. Kanbiroo (haa ibsamu) ______________

20.Gidgiddaan mana maalirraa hojjetame?

aa. Muka dhoqeen maragame
bb. Simmintoon garafame
cc. Qorqoorroo
dd. Xuubii ykn dhagaa
ee. Kanbiroo (haa ibsamu) ______

21.Lafti mana keesanii mal irraa tolfame?

ff. Biyee
gg. Dhoqee looniin lolla'amaa
hh. Simintoo
ii. Muka
jj. Kanbiroo (haa ibsamu) ______

22.Baaxiin ykn xaaraan manaa gara bakkeetti banaa moo cufaadha?

KK. Banaadha
ll. Cufaadha

Walakkan banaadha

23.Matta keessaniif ykn daa'imman keessaniif agoberii ni fayyadamtuu?

a. eyyeeb. akki c.in beeku

24.Kalessa galgala mataa keessaniif (daa'ima keessaniif) agobera siree fayyadamtaniittuu?

a. eyyee b. akki c.in beeku

25.Mana kessan keessa dhaabbataan nama meeqatu jirata? ________________________

26.Agoobera siree meeqa qabdu? ________________________________

27.Ji'oota 12n darban kana keessatti manni kessan qoricha farra bookee busaatiin biifamee beekaa?

a. eyyee b. akki c.in beeku

28.Ji'oota meeqan dura manni keessan qoricha farra bookee busaan kan biifame? (Yoo ji'a tokkoo gadi ta'e, jia '00' jechuun galmmessi) _____________________________

29.Ji'oota 3n darban kana keessa isinis ykn ijoolleen keessan adeemsa halkanii taasiftanii/taasisanii beektuu?

a. Eeyyee b. Lakki c. Hin beeku

30.Deebi'iin keessan eeyyee yoo ta'e, galgala yeeroo hedduu eessatti dabarsitu/dabarsu? ____

31.Isin ykn ijolleen keessan galgala yeroo baay'ee sa'a meeqatti manatti galtu/galu? ___________

32.Isin ykn ijolleen keessan Kaleesa galgala sa'a meeqatti gara iddoo chiisaa deemtan /deeman? _______________

33.Isin ykn ijolleen keessan yeroo baay'ee ganama sa'a meeqatti mana baatu/ ba'u? ________________

34.Isin ykn ijolleen keessan kaleessa ganama sa'a meeqatti manaa batan ykn ba'e/bate?

35.Ho'a qaamaa (°C): ____

yoo ≥37.5°C ol ta'e RDTn haa hojjetamu

yoo <37.5 °C gadi ta'e gara qorannaa Hb tti haadarbu/tu

36.RDTn fudhatameeraa?

a. Eeyyee b. Lakki

37.Yoo lakki ta'e, maaliif? ____

38.Bu'aan qoranaa RDT?

a. Poozetiivii b. Nagatiivii b. Gatii hinqabu

Yoo poozetiivii ta'e, gara buufata fayyaa dhihootti yaliif haa ergamu/ttu

39.Hbn hojjetameeraa?

a. Eeyyee b. Lakki

40.Yoo lakki ta'e, maaliif? ____

41Bu'aan qorannoo Hb: ____

yoo 8 g/dL gadi ta'e Ireenii kenniif

42.Hujumoo maayikirootiin dhiigni fudhatameeraa?

a. Eeyee b. Lakki

43.Yoo lakki ta'e, maliif? ____

44.Isliidii maayikirooskoppii irratti dhiigni fudhatameeraa?

a. Eeyyee b. Lakki

45.Yoo lakki ta'e, maaliif? ____

46.Waraqaa cophsa dhiigaa irratti fudhatameeraa?

a. Eeyyee b. Lakki

47.Yoo hinfudhatamne ta'e, maaliif? ____