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Assessment of Platelet Count in Malaria Suspected Patients in Adama Referral Hospital, Adama, Ethiopia

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This is to certify that the thesis prepared by Feven Tilahun, entitled:

Assessment of Platelet Count in Malaria Suspected Patients in Adama Referral Hospital, Adama, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviation

AAPP	Anopheline antiplatelet protein
AT-III	Anti thrombin-III
APTT	Activated Partial Thromboplastin Time
CR1	Complement receptor 1
CSA	Chondroitin sulphate A
DIC	Disseminated intra vascular coagulation
HMIS	Health management information system
IEs	infected erythrocyte
IRBC	Infected red blood cell
PF	Plasmodium falciparum
PfEMP1	Plasmodium falciparum erythrocyte membrane protein 1
PT	Prothrombin time
TNF-α	Tumor necrosis factor alpha
TSP	Thrombospondin
WHO	world health organization

Abstract

Background: A higher risk of thrombocytopenia was identified in malaria patients with severe anemia, neurologic complications, pulmonary complications, liver dysfunction, renal impairment and severe hypoglycemia. However, there is limited information regarding platelet parameters abnormality associated with malaria infection in Ethiopia.
Objective: The objective of this study was to asses platelet count in malaria suspected patients in Adama Referral hospital, Adama.

Method: In this cross-sectional study which was conducted from February to September 2020, 442 malaria suspected patients were selected using convenient sampling technique. Data was collected using interviewer administered pre-tested questionnaire and clinical data was obtained from patient medical record. Complete blood count was performed using Beckman Coulter (DXH800) automated hematology analyzer and finger prick capillary blood was used for microscopic examination of malaria. Data was entered and analyzed by using (SPSS) version 24. Descriptive statistics was applied to determine the mean, frequencies, and percentages of the study parameters. Pearson correlation analysis and regression analysis was performed to see significance association between variables P value less than 0.05 was considered statistically significant.

Result: Of the 422 malaria suspects majority 206 were (48.8%) in the age group of 20-40 and 214 (50.7 %) were females. The overall magnitude of malaria was 23.9% (101/422) which is 56(26.9%) and 45(21.0%) in males and females, respectively. The overall magnitude of thrombocytopenia was 77(18.2%). Among thrombocytopenic patients, 55(71.4%) were positive for malaria. Magnitude was higher in *P. vivax* patients. There was negative correlation between parasite density and platelet count (r=-0.539 P=0.01) as well as with neutrophil % (r=-0.374 P=0.01).lymphocyte count % and parasite density had positive (r= 0.476 P=0.01) lymphocyte count # and parasite density had positive correlation (r=0.236 P=0.05).

Conclusion: The magnitude of thrombocytopenia was higher among malaria positive than negative patients and it was relatively more common among *P. vivax* than *P. falciparum* infected patients.

Key words: *Thrombocytopenia, Malaria, Complete Blood Count, Adama, Ethiopia*

1. Introduction

1.1 Background

Hematological abnormalities including thrombocytopenia are among the common complications of malaria infection. The most common complications encountered in malaria infection and they play a major role in malaria pathophysiology. Hematological changes can occur in the major cell lines, such as RBCs, leukocytes or white blood cells and platelets(1).

The five Plasmodium species well known to cause human malaria are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium Ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*(2).

Plasmodium species exhibit three life cycle stages in the human host the sprozoites, merozoite and the gametocytes. During blood meal, female anopheles mosquitos inoculates sprozoites into the blood stream of human host .The sprozoite travel to the liver and infect the liver cell where in a period of within 5-16 days, they grow divide and produce tens of thousands of haploid forms called merozoite per liver cell After 48 hr. parasite grow into ring and schizont stage A small percentage of merozoite leave the cycle of asexual multiplication and instead undergo differentiation in to the sexual forms of the parasite known as gametocyte ,The gametocytes then circulate in the blood stream and will eventually be ingested by mosquito during a blood meal .The gametocyte will further develop in to mature sex cell called gametes and undergo fertilization in the gut of mosquito toform zygote.The zygote elongates and become motile to form an ookinete which penetrate the mid gut wall of mosquito and develop into oocysts. theoocystgrows and rupture in to release sprozoite which make their way to the mosquito salivary gland and await inoculation into a human host during the mosquito next blood meal (3).

Anemia and thrombocytopenia are the most frequent malaria-associated hematological complications According to the World Health Organization Scientific Group1, the levels of hemoglobin below which anemia is likely to occur for a population living at sea level are: 11g/dl for children aged six months to six years, 12g/dl for children aged between 6

and 14 years, 13g/dl for adult males, 12g/dl for non-pregnant adult females and 11g/dl for adult pregnant females (4, 5).

An estimated 219 million cases of malaria occurred worldwide in 2017 compared with 239 million cases in 2010 and 217 million cases in 2016. The WHO African region still bears the largest burden of malaria morbidity, with 200 million cases (92%) in 2017, followed by the WHO South-East Asia Region (5%) and the WHO Eastern Mediterranean Region (2%) (6).

Malaria is one of the most prevalent human infections in the world. Data from household surveys conducted in 16 high-burden African countries between 2015 and 2017 showed that, among children aged under 5 years, the prevalence of any anemia was 61%, of which mild anemiawas 25%, moderate anemia 33% and severe anemia 3%. Among children who tested positive for malaria, the prevalence of any anemia was 79%, mild anemia 21%, moderate anemia 50% and severe anemia 8% (6).

Platelets play a critical role in the pathogenesis of malarial infections by encouraging the sequestration of infected red blood cells within the cerebral vasculature. Platelets also have well-established roles in innate protection against microbial infections (7).

Malaria-induced thrombocytopenia may also occur as a result of platelet death, which can be mediated through a process analogous to apoptosis in nucleated cells involving the activation of platelet caspases (24).

Study done Piguet PF et al in a mouse model of malaria, caspases have been shown to be activated causing platelet apoptosis. This was associated with an increase in the number of circulating platelet micro particles, a possible corollary of platelet apoptosis. CD40 is a cell receptor belonging to the TNF receptor superfamily that can modulate cell proliferation, differentiation and apoptosis. The thrombocytopenia in these mice could be decreased with pretreatment with anti CD40L antibodies or with caspases inhibitors, indicating that apoptosis was initiated by CD40L and that caspases were crucial (25). Malaria is an entirely preventable and treatable mosquito-borne illness.

Anopheline anti-platelet protein (AAPP) from a malaria vector mosquito may also have value as an alternative antiplatelet drug (17).

Acute and profound reduction in platelet count or thrombocytopenia is commonly observed. The phenomenon in humans is observed in infections caused by all Plasmodium species, as well as in most animal models of the disease. It has also been regarded a risk factor for mortality in African children with falciparum malaria (16).

Human platelets bind malarial-infected red cells and kill the parasite within these indicate a protective function of platelets in the early stages of erythrocytic infection distinct from their role in cerebral malaria. Thrombocytopenia frequently complicates malarial infection with Plasmodium falciparum. Plasmodium vivax also showed moderate thrombocytopenia (8).

1.2 Statement of the problem

Malaria associated with various hematological complications with anemia and thrombocytopenia as the most common.
The anemia is usually due to varied reasons ranging from hemolysis to co morbidities like
parasitic infections, folate, iron, and vitamin B12 deficiencies in endemic areas, antimalarial drug and further complicated by the coexistence of thalassemia and other hemoglobinopathies(9).

Hematological abnormalities are considered a hallmark of malaria and different studies have shown that abnormalities in many of these hematological values may lead to an increased clinical suspicion for malaria, thus initiating a prompt specific therapy even in the absence of a positive smear report for malaria (10).

A variety of hematological alterations like progressively increasing anemia, thrombocytopenia, and rarely disseminated intravascular coagulation (DIC) occur in *Plasmodium falciparum* infection.Thrombocytopenia is commonly observed in malaria. Splenic pooling and shortened platelet survival are responsible for the thrombocytopenia. Platelet parasitism may be another possible cause of shortened platelet life span (11).

There may be consumptive thrombocytopenia in cases with concomitant DIC. The derangement in the coagulation profile in malaria is highly sensitive measure to assess the severity of the disease. There is an accelerated coagulation cascade activity with accelerated fibrinogen turnover, consumption of antithrombin-III (AT-III) and increased concentration of fibrinogen degradation products (12).

Erythrocytes (RBCs) containing parasites and released cytokines are pro-coagulants. Prothrombin time (PT) and activated partial PT (APTT) are prolonged. Severe hemorrhage is reported in 5% of severe malaria. The patient may develop bleeding gums,

epistaxis, petechiae, subconjunctival hemorrhages, malena and hematemesis. This is more common in non-immune individuals in the temperate zone (13).

A variety of abnormalities of blood and bone marrow cells may be found in *P. falciparum* and *P. vivax* malaria. Severe anemia may occur in children with acute or chronic falciparum malaria with various degrees of parasitaemia. The possible pathogenesis of the hematological abnormalities may be parasite products, T-cell-derived cytokines, macrophage activation, macrophage-derived factors such as tumor necrosis factor-α, and macrophage dysfunction(7).

In the past it was thought that malaria is rarely associated with clinical features of thrombocytopenia like bleeding disorders and is usually an incidental finding on blood testing. Recent scientific evidences have invalidated this anecdote. It is pertinent that the finding of thrombocytopenia in patient may be an indication for a thorough lookout into the blood smear to rule out malaria as the cause (14).

This fact is especially important in the workup for thrombocytopenia in febrile patients. Thrombocytopenia may be associated with bleeding tendency; which is one of the important severe manifestations of Falciparum malaria (14).

Platelet phagocytosis could be mediated by the increase in p-selectin expression in the surface of activated platelet. Circulating p-selectin in plasma was elevated in *p.vivax* this might be reason thrombocytopenia is greater in *p vivax*(39).

Therefore, this study was conducted to assess platelet count among malaria suspected patients of Adama referral hospital at Adama, Ethiopia. Such data is limited in the study area.

1.3 Significance of the study

This study is useful for the physician to show them how the disease affect platelet count and consequence of low platelet count in order to avoid death rate due to malaria.

The physician will consider correlating malaria with platelet count when diagnosing patients and order blood film with complete blood count together for better management of patient.

Therefore, the patient will get good service and will be satisfied. This study also can be used as a reference for other study and for policy makers.

2. Literature review

Platelets and malaria

Platelets are the second most abundant cell in the bloodstream after erythrocytes. They are small (2–3 µm diameter) and discoid in appearance, a nucleate, and derived from the megakaryocytes of the bone marrow. Their primary physiological role is to regulate homeostasis, where they accumulate at sites of vascular injury and initiate coagulation to prevent bleeding; they also have well-known pathological roles in atherosclerosis and thrombosis. (15).

An estimated 219 million cases of malaria occurred worldwide in 2017. There were an estimated 435 000 deaths from malaria globally in the same year (13)

The significance of platelet counts in predicting malaria infection is getting attention as demonstrated by several studies (14, 16).

Southeast Asian adults and children with falciparum and vivax malaria may have increased risk of mortality due to severe malaria associated thrombocytopenia (18).

Across-sectional descriptive study done in Brazil in a total of 186 patients attending university of hospital between 2008-2013 were included in a study on hematological evaluation The study shows a mean hemoglobin level of 12.9g/dl mean hematocrit level of 38.5% and mean leucocyte count of 6278cells/mm^3. The mean platelet count was 114,823 cells/mm^3 16.7 %and 60.7% of the patient exhibited counts fewer than 50,000/mm_3 and between 50,000/mmm^3 and 150,000/mmm^3respectively (19).

In cross-sectional study done by LauraErhartet al (2004)hematologic profiles of persons with acute *Plasmodium falciparum or P. vivax* infection in Maesod in Thailand's western border with Myanmar were examined. The study compared with febrile, non-parasitemic persons also reporting to malaria clinics. Nine hundred seventy-nine subjects were malaria-negative, 414 were infected with *P.falciparum*,and 646 were infected with *P.vivax*. Persons with patent parasitaemia tended to have significantly lower white blood cell, red blood cell, platelet, and hemoglobin levels than those who were malaria-

negative. For the first time, a parallel trend in thrombocytopenia with parasitaemia was found to be associated with both *P. falciparum*, and *P. vivax* infection (12).

A prospective study done by Mumtaz Ali Shaikh et al in Pakistan investigated 200 diagnosed cases of malaria in Department of Medicine, LUMHS, Jamshoro/Hyderabad from February to December 2010. The diagnosis of malaria was carried out by thin and thick blood films. Platelet count was performed using an automated counter. Thrombocytopenia was classified as mild (50–150 x 10^3 cells/µl), moderate (20–50 x 10^3 cells/µl) and severe (<20 x 10^3 cell/µl). Results: The age of patients ranged from 16 to 80 (28±10.5) years, median age was 30 years. Among the study population, males were 124 (62%) and females were 76 (38%). Out of them 105 (52.5%) were cases of *Plasmodium falciparum*, 93 (46.5%) were of *P. vivax*, and 2 (1%) were of *P. malaria*. The data showed that 171 (85.5%) patients were having low platelet count; 141 (70.5%) had mild, 21 (10.5%) moderate, and 9 (4.5%) had severe thrombocytopenia. Twenty-nine (14.5%) patients had normal platelet count (20).

In a study done by (Arif M et al) Medical College Hospital, out of 100 cases, 32% were *P.falciparum*malaria, 65% were *P. vivax* malaria and 3% were mixed infection. Incidence of thrombocytopenia was 79%, out of which mild, moderate and severe degree of thrombocytopenia was seen 35.44%, 41.77% and 22.78% respectively Mild thrombocytopenia was commonly associated with *P. vivax* (52.08%) as compared with *P. falciparum* (10.71%) whereas severe thrombocytopenia was commonly associated with *P. falciparum* (46.43%) as compared with *P. vivax* (8.34%) (21).

In across sectional descriptive study done by MohammadShoaib Khan et al in India,a total of 500 malaria positive cases were included. Out of which, (100) each from Islamabad, Rawalpindi, and Deera Ismail Khan, while (200) from Peshawar. Out of 500 malarial positive cases, 444((88.8%) were infected with *Plasmodium vivax*, 45(9.0%) by *Plasmodium falciparum*, with an average platelet count of 151 ×10^9/L and 143×10^9/L respectively. Cases infected with both *Plasmodium vivax* and *Plasmodium falciparum* came out to be 11(2.2%) with an average of 145×10^9/L platelets. During the present study, no case of *Plasmodium malariae* and *Plasmodium ovale* were observed.

Gametocyte form in both *plasmodium vivax* and *falciparum*,caused most cases of thrombocytopenia while Ring form did not affect much(22).

A prospective study done by SaritaMohapatra et al in India institute of science New Delhi of 31 ,17 (55%) were classified as complicated and 14/31 (45%) were un complicated . Among 23 cases with thrombocytopenia, early stage of DIC was detected in 18 cases by the conventional methods.As platelet number begins to decrease early in infection prior to the onset of severe symptoms, it is unlikely that this is due to DIC. Immune-mediated clearance of platelets during malarial infection has been proposed as another important mechanism underpinning thrombocytopenia (23).

Study done by Martinez Salazar and TobonCastano in prospective study tastano in San Vicente foundation hospital and Pablo Tobon uribe hospital in Medellin 862 malaria patients were enrolled, including 533 (61.8%) patients infected with *Plasmodium falciparum*, 311 (36.1%) patients infected with *Plasmodium vivax* and 18 (2.1%) patients with mixed infections. The most frequently observed changes were low platelet count (PC) and high platelet distribution width (PDW), which were observed in 65% of patients; thrombocytopenia with <50,000 platelets/µL was identified in 11% of patients. Patients with complications had lower PC and plateletcrit (PT) and higher PDW values. A higher risk of thrombocytopenia was identified in patients with severe anemia, neurologic complications, pulmonary complications, liver dysfunction, renal impairment and severe hypoglycemia. The presence of thrombocytopenia (<150,000 platelets/µL) was associated with a higher probability of liver dysfunction (26).

A prospective cohort study was conducted among microscopically confirmed acute malaria patients aged, ≥18 years, who attended a tertiary care hospital in Manipal, Udupi, India from October 2014 till August 2016 Among 159 patients, 32 (20.1%) had severe malaria. 116 (73%) had infection with *P. vivax*, 37 (23%) *P. falciparum* and 6 mixed infection. Thrombocytopenia was seen in 32 (86.4%) of *P. falciparum* and 105 (90.5%) of *P. vivax* malaria cases. Patients with renal failure (p=0.02), shock (p=0.04) and liver dysfunction (p<0.001) had significantly lower platelet count compared to those who did not. Admission platelet count of 50,000 cell/mm3 had a sensitivity and specificity of 65.6% and 70.6% respectively, to discriminate severe malaria. A plateletcrit of 0.05%

had a sensitivity and specificity of 65.6 % and of 70.6% respectively. Thrombocytopenia was seen in 89.3% of malaria cases due to both *P. vivax* and *P. falciparum*. Platelet count and plateletcrit could be used as markers of disease severity. *P. vivax* malaria which has been traditionally regarded as 'benign' can be as sinister and menacing as *P. falciparum* malaria and hence warrants equal attention. Unnecessary transfusion of platelets should be avoided (27).

Based on the platelet's well-known abilities to bind both infected and non-infected red cells and the endothelium, they are thought to be a major mediator of cerebral malaria. Platelet binding to infected red cells involves the platelet receptors, CD36 and gC1qR, although on the erythrocyte only one molecule, *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) has been identified as a ligand for platelet CD36 (28).

Platelet-erythrocyte adhesion in human malaria, involving both infected and uninfected cells, has been variously observed as cell rosetting and clumping phenotypes (29).

Infected red cells also bind to the endothelium, principally to avoid the reticuloendothelial system and enable parasites to reproduce more effectively. Some of the endothelial receptors involved in parasite sequestration are also involved in platelet-erythrocyte binding. It is therefore possible that adhesion to platelets is a side-effect of the development of endothelial binding by infected red cells to avoid splenic clearance. Platelets are implicated in the development of cerebral malaria (CM), which is a complex collection of syndromes specific to *P. falciparum* infections and a major cause of death. The pathology of CM appears to involve the physical binding of infected red cells to the endothelial cells of small vessels in the brain Erythrocyte-endothelial binding occurs in many organs, but in the brain, this produces cerebral malaria. Binding is believed to result in the obstruction of blood flow, as well as stimulating leucocyte accumulation, which leads to localized intravascular inflammation, and activation and damage of the endothelium ultimately this could lead to disruption of the blood-brain barrier. Evidence for platelet involvement in CM has come from early observations reporting that platelets are seen in plugs in cerebral blood vessels(30).

Thrombocytopenia is a common pathological feature of malaria. It is reported that 22 of 26 (85%) patients with falciparum malaria and 30 of 39 (72%) patients with vivax malaria had depressed platelet counts below 150 × 109/lMalaria is often accompanied by splenomegaly. The increase in spleen size is due to an accumulation of macrophages, which phagocytize both infected and non-infected red cells (31).

Thrombocytopenia may be due to one of three reasons: a) Decreased production of platelets b) Increased destruction of platelets and c) Changing of distribution of platelets (32).

Scintigraphy studies, which transfused radiolabelled platelets into normal and malaria-infected patients, found no evidence for platelet pooling in the spleen, even in individuals with very large spleens; the distribution of platelets was indistinguishable from control individuals. However, a greatly reduced platelet half-life was noted in the malaria patients (33).

Consistent with these studies, there were no differences in platelet numbers between splenectomized and spleen intact rhesus monkeys infected with *P. cynomolgi* (34).

In a prospective study which was conducted for a period of six months. patients of all ages who were either hospitalized or attending OPD were included. Out of 394 requests for peripheral blood smear examination, 120 patients were positive for malaria parasite. Of these 92 were positive for *Pv*, 18 for Pf and ten had mixed infection A total of 120 patients were included in the study and identified positive for malaria parasites on peripheral smear examination with conventional microscopy. Platelet count was done on a fully automated, quantitative, hematology analyzer. Thrombocytopenia was noted in 63.33% cases. The mean platelet count in *Plasmodium vivax(Pv*) malaria was 1,27,652/µl (SD 78,269) with a range of 8000-3,50,000/µl, as against *Plasmodium falciparum* (*Pf)* malaria where the mean platelet count was 78,500/µl (SD 51,485) with a range of 9000-1,90,000/µl. Platelet count < 50,000/µl was noted in only 17.4% cases of *Pv* malaria as against 33.3% cases of *Pf* malaria (35).

A retrospective study wasdone by Anita B et al over a period of one year from December 2011 to November 2012. A total number of 75 cases were included in this study which

was conducted at a tertiary care teaching Centre in North Karnataka Sixty six cases of *P. vivax* and nine cases of *P. falciparum* were included Thrombocytopenia was the most common abnormality noted in 71 (94.66%) of 75 cases. Among those who were infected by *P. falciparum,* one case had severe thrombocytopenia while eight (88.88%) had moderate thrombocytopenia. In case of *P. vivax* infection four (6.06%) patients had severe thrombocytopenia while 51(77.27%) had moderate degree of thrombocytopenia. An increase in mean platelet volume was seen in 19 *vivax* infected cases (28.78%). Anemia was present in 5 cases of *P. falciparum* and 25 cases of *P. vivax* infection. Leucopenia was observed in four and 23 cases of *P. falciparum* and *P. vivax* respectively (36).

In a study conducted in S.P. Medical College and from associated Group of Hospitals, Bikaner, India by Kochar KD from January 2007- July 2008 Shows that out of 1064 cases of malaria 49.34% had *Plasmodium falciparum* mono infection, 43.23% had *Plasmodium vivax*infection and 7.22% had mixed malaria infection. Platelet count <150,000 per microliter of blood was observed in 16.9%, 31.09%, and 3.04% of *Plasmodium falciparum, Plasmodium vivax,* and mixed infection, respectively. From the observation the study was evident that thrombocytopenia is a common hematological finding in patients with *plasmodium* infection and it notifies the association of severe thrombocytopenia was strongest with *Plasmodium vivax* infection as compared to *Plasmodium falciparum* and mixed infection (37).

In Liberia, Mahmoud and Yasir studied a total of 145 patients who had *P. falciparum* malaria. Out of these 109 (75.18%) had thrombocytopenia. They concluded an extended search for malarial parasite in patients having thrombocytopenia on smear. Mild-to-severe thrombocytopenia observed in hospitalized patients was considered enough to alert the possibility of malarial infection, as *P. falciparum* was found to be common species in these patients. The study also connotes that *falciparum* malaria is more common at lower platelet counts as compared to *vivax* infection and overall the chances of finding *falciparum* malaria are almost twice than that of finding *vivax* malaria in thrombocytopenic patients(38).

In study done in Ethiopia by Amsalu Arota a total of 340 study participants were included in the study, out of which 170 were malaria cases, and the remaining 170 were malaria negatives. An institution-based cross-sectional study was conducted. Malaria diagnosis was based on thick and thin blood films microscopy. Hematological parameters were determined by using an automated, CELL-DYN 1800 hematology analyzer. Malaria parasite density was determined by counting the asexual parasites against 200 WBCs, and then calculated by using the standard formula. The prevalence of thrombocytopenia and anemia in malaria patients was 84% and 67%, respectively. There was an inverse correlation between *P. falciparum* and *P. vivax* parasite density and lymphocyte count, as well as platelet count. (42)

There is limited information in the relation between thrombocytopenia and malaria infection particularly in the study area hence this study tried to address this gap.

3. Objective

3.1 General objective

- To asses Platelet counts in malaria suspected patients in Adama Referral hospital, from February to September 2020 Adama, Ethiopia.

3.2 Specific objective

- To determine the magnitude of thrombocytopenia in malaria suspect patients.
- To determine the association betweenparasite loadand platelet count.
- To determine malarial species specific effect on platelet count in malaria positive Patients.

4. Materials and Method

4.1 Study Area

The study was conducted in Adama Hospital Medical College. The hospital is located in Central Ethiopia, Oromia regional state, in Adama town 99 km from Addis Ababa on Ethio-Djibuti main road. It was established in 1942 by Italian Missionaries. The hospital was initially named as Haile Mariam Mammo Memorial Hospital But later it was renamed as Adama Hospital Medical College by Oromia regional state health bureau after it enrolled students in different programs like accelerated medicine, emergency surgery and some specialty in 2012. Currently the college hospital has catchment population of about 5 million serving as referral hospital for all nearby hospitals and the adjacent regions. It has capacity of 200 beds for inpatient with five disciplines (Surgery, Internal medicine, pediatrics, Gynecology/Obstetrics and ophthalmology) with four pharmacies (OPD, ward, emergency and ART pharmacy) and serves about 850 patients per day at OPD during working hours and on average 52 patients per day after working time in private wing clinic. The hospital has about 465 workers of which 257 were health professionals and the remaining are administrative workers and teachers. The hospital is now working in collaboration with Adama General Hospital (40).

4.2 Study design and period

A cross sectional study design was conducted on malaria suspected patient attending at Adama Referral Hospital from February – Sep, 2020

4.3 Population

4.3.1 SOURCE POPULATION

All malaria suspected patient attending in ADAMA referral hospital

4.3.2 STUDY POPULATION

Malaria suspected patients who sent to the laboratory.

4.4 Inclusion criteria and Exclusion criteria

4.4.1 Inclusioncriteria

- Malaria suspected patients of all ages that present to Adama referral hospital and Willing to participatein the study.

4.4.2 Exclusion Criteria

- Patient who has taken anti-malaria drug within 2 weeks

4.5 Study variable

4.5.1 Dependent variable

- Platelet count

4.5.2 Independent variable

- Socio demographic characteristics (age, sex,)
- Malaria status, Alcohol intake, pregnancy, bacterial Infection infectivity status.

4.6 Sample Size Calculation and Sampling Method

4.6.1 Sample size calculation

- Sample size is calculated from the total study population that fulfill in inclusion criteria by the following formula

- $n = \frac{(z\alpha/2)2\ x/\ pq}{D2}$
- n= minimum sample size required
- P= proportion of prevalence 0.5
- q =1-p
- d= the margin of sampling error tolerated = 0.05
- Z= the standard normal value

$$n= \frac{1.96^2 X\ (0.5)\ (0.5)}{0.05^2}$$

n=384

Add10% non-respondent thus final sample size(nf) will be

nf =384+ (384*10%)

=384+38.4

=422

Therefore, the final sample size(nf)was 422

4.6.2 Sampling Method

Convenient non probability technique was used to select all malaria suspect patients attending in the hospital

4.7 Measurement and Data Collection

4.7.1. Data Collection Procedure

Socio-demographic and clinical data was collected using interviewer administered structured questionnaire. The questionnaire was designed in English and translated into local Language (Afan Oromo and Amharic). After brief explanation about the aim of the study, the participants were requested to take part in the study voluntarily. After obtaining consent/assent, about five milliliters of venous blood was collected for CBC evaluation more specifically for platelet count determination and capillary blood was also collected for microscopic examination of malaria.

4.7.2. Laboratory Analysis

Sample collection and processing

The diagnosis of malaria was based on blood film microscopy. Both thick and thin blood film was prepared with capillary blood collected from a finger prick. Thin film was fixed with absolute methanol. Then, blood film was stained with 10% Giemsa stain for 10 minutes and examined with an 100× oil immersion objective by experienced microscopists. Peripheral blood smear examination is a gold standard for the diagnosis of malaria infection. To determine the density of malaria parasite; two experienced microscopists independently counted the asexual stage Plasmodium parasite on a slide against 200 WBCs in thick blood film from each of the malaria cases.

Venous blood of 5 mL was collected by Venipuncture into an EDTA tube from each study participant for CBC. Beckman Coulter (DXH800) automated hematology analyzer was used to perform, complete blood count and gives results in printouts. Hematological analysis was achieved in accordance with the standard protocol and manufacturer instructions of the hematology analyzer machine by an experienced Senior Laboratory Technologist.

4.7.3 Beckman Coulter (DXH800) Hematology Analyzer Principle

The DxH 800 CBC analysis based on Coulter principle, performs 100 samples per hour of 27 hematological parameters. CBC is essential analytical test that evaluates the three main cellular components; WBC, RBC and platelets. Sample preparation and data

collection occurs in SAM and CBC modules on the DxH 800. The data analysis is handled by the system manager. The method of counting and sizing in combination with an automatic diluting and mixing device for sample processing, and a single beam photometer for hemoglobinometry. The WBC differential uses VCS technology. Analysis and classification of WBCs use three simultaneous measurements of individual cell volume (V), high frequency conductivity (C), and laser light scatter (S). The scatter gram plots the cells based upon the measurements of these three parameters.

The Beckman Coulter method accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle such as a cell, in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of electrical path between the submerged electrodes on either side of the aperture. This causes a measureable electronic pulse. For counting, the vacuum used to pull the diluted suspension of the cells through the aperture must be at a regulated (reproducible) volume. While the number of the pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume. The hemoglobin is photo metrically measured at 525 nm using lysed WBC dilution drains to the cuvette from the WBC analysis (counted). The lytic reagent rapidly and simultaneously destroys the RBC and converts Hgb into stable pigment, which is proportional to the concentration of Hgb.

4.7.4Giemsa stain principle

Principle

A small drop of blood is placed near the frosted end of a clean glass slide. A second slide is used as a spreader. The blood is streaked in a thin film over the slide. The slide is allowed to air-dry and is then stained.

- Giemsa stain is a mixture of eosin and methylene Blue. When applied to blood cells, the dyes produce multiple colors based on the ionic charge of the stain and the various components of the cell. The eosin ions are negatively charged and stain basic cell components an orange to pink color. The methylene blue ions are positively charged and stain the acid cell components in varying shades of blue. The neutral components of the cell are stained by both components of the dye producing variable colors.

- **Eosin** is anionic and acts as an acidic dye. It is negatively charged and can react with positively charged, acidophilic components in the tissue, such as amino groups in proteins in the **cytoplasm.**
- **Methylene blue** stains the nucleic acids. It is a basic dye and bind well to DNA (negative charge). It stains the dead cells and thus differentiates it from the living cells because the dead cells will take up the stain easily than the live cells.( )

4.8 Data Quality Assurance

Data quality assurance

The standard operating procedures- (SOPs) were followed in all the laboratory procedures, and training was then given to laboratory assistants by the principal investigator before starting laboratory work.

Pre-analytical:- in this phase the laboratory professional assigned at reception checked the sample quality and its accuracy by checking the patient request with full information and proper labeling, handling and transportation of the sample.

Based on sample acceptance and rejection criteria, the sample was evaluated for clot, hemolysis and lipemia and any sample with those problem was rejected and also patient request that is not properly filled with necessary patient information and miss match of sample identification were rejected.

To ensure the accuracy of malaria diagnosis, 10% of the positive and negative samples were re-examined by an independent laboratory technologist, who was blinded to the results of the first Slide-reader. For an automated hematology analyzer, a daily quality assurance check was performed in accordance with manufacturer's recommendations.

Analytical phase: - in this phase the patient specimen was prepared for testing and ends when the test result is interpreted and verified. Doing this the specimen was rechecked for correct labeling and identifications again by the personnel which analyze the sample. After checking the sample accuracy the personnel checked the machine which is

Beckman Coulter (DXH800) and by doing daily, weekly or as needed maintenance. After doing the maintenance the personnel processed the daily QC.

Post-analytical phase: Post-analytical quality assurance is insured by using appropriate result formats and registrations.

4.9 Data Analysis and Interpretation

First data was entered in epidata and checked for completeness and coded. It wasthen importedinto and analyzed by using SPSS version 24.0. The appropriate descriptive statistics was applied to determine the mean, frequencies, and percentages of the study parameters.

Regressionanalysis to see association of platelet count with different associated factor and Pearson correlation analysis was used to test the association between two continuous variables.. For all statistical tests, $P<0.05$ was considered as statistically significant. Finally, all results were described and presented using tables and figures.

4.10Ethical Considerations

The study was ethically approved by Department Research ethics Review committee of Department of Medical Laboratory Sciences, AAU. Approval letter was also obtained from Oromia Health Bureau and finally, a letter of permission to conduct the study was obtained from Adama Referral Hospital. Written informed consent/assent was obtained from each participant after thoroughly explaining the nature of study and their right to withdraw from the study at any time during the study. Confidentiality of data was maintained throughout the study and patient with confirmed malaria case and critical CBC result was sent to physician for possible intervention

4.11 Dissemination of Result

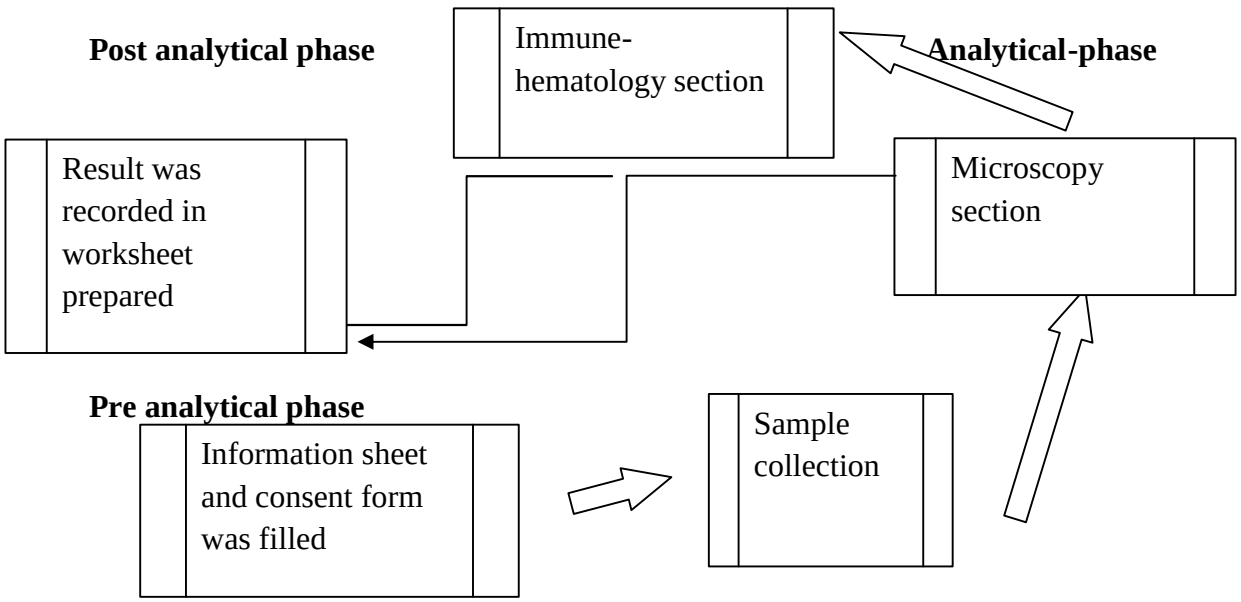
The finding of this study will be disseminated to Ministry of Health, Bureau, and Addis Ababa Health office Department. The finding will also be disseminated to different health organizations that will have a contribution to improve the utilization of hematological parameters for malaria control services. The finding willalso be presented in Department of medical Laboratory Sciences, various seminars and workshops. It will alsobe published in peer reviewed a scientific journal

4.12 OPERATIONAL DEFINITION

- Thrombocytopenia: A condition in which there is an abnormally low number of platelet in the circulating blood below138,000cells/microliter. Ethiopian Normal reference interval of platelet count is 138,000-462,000cells/microliter.(41)
- Anemia: A physiologic and/or clinical condition where an individual exhibits inability to maintain normal tissue oxygenation because of decreased hemoglobin content Ethiopian Normal reference (Hg RI,12-17.1g/dl), RBC (RI 4.3-5.9 trillion/liter) number or packed red blood cells (38-55%).(41)

4.13. Work Flow

Figure 1 work flow



5. Result

5.1Socio demographic characteristics

In this study,422 malariasuspected patients andmajority (48.8%) of the patients werein the age group of 20-40. Of the total suspect, 214(50.7 %) of the respondent were females Majority do not take alcohol and had no history of bacterial infection (Table 1).

Table1. Socio demographic and clinical characteristics of malaria suspected patients admitted to Adama Referral Hospital, Adama, Ethiopia (n=422)

Variable		**Frequency**	**Percent**
Age			
< 20		149	35.3%
20-40		206	48.8%
40-60		49	11.6%
>60		18	4.3%
Total		422	100.0%
Sex			
Male		208	49.3%
Female		214	50.7%
Alcohol Intake	Yes	44	10.42%
	No	378	89.57%
Pregnancy Status	Positive	4	1.8%
	Negative	210	98.2%
History of Bacterial infection	Had history	22	5.2%
	No history	400	94.8%

From total of 422 participants 101(23.9%) of them had malaria positive confirmed by microscopy. From this, 56(26.9%) males and 45(21.0%) females were malaria positive as summarized in Table 2. Malaria positivity increased with increasing age. Among malaria infected patient, 71(70.3%) had *Plasmodium falciparum* whereas 28 (27.7%) had *Plasmodium vivax* infection and only 2(2% n=101) had mixed infection (Table 2).

Table 2: Age, sex and malaria positive species distributionamong malaria suspected patients admitted to Adama Referral Hospital, Adama, Ethiopia (n=422)

Variable		**Malaria status**		**Total**
		Positive	**Negative**	
Sex	**Male**	56(27%)	152(73%)	208
	Female	45(21%)	169(79%)	214
Age group	**≤ 20**	26(17%)	123(83%)	149
	20-40	54(26%)	152(74%)	206
	40-60	14(29%)	35(71%)	49
	≥60	7(39%)	11(61%)	18

Species	***P.vivax***	***P.falciparum***	**Mixed**	**Total**
Frequency	28	71	2	101
Percent	27.7%	70.3%	2%	100%

5.2 Status of thrombocytopenia among malaria suspect study group

Only 49(48.51%)of malaria positive patients had platelet count greater than 138,000/microliter. Majority(51.48%) of malaria positive patients had low platelet count less than 138,000/ microliter. (Table3)

Table 3: The magnitude of thrombocytopenia among malaria suspected patient in Adama Referral Hospital, Adama, Ethiopia. (n=422)

Malaria status	**Thrombocytopenia (%)**		**Total**
	Yes (%)	**No (%)**	
Positive	52(51.48%)	49(48.51%)	101(23.93%)
Negative	12(3.73%)	309(96.26%)	321(76.06%)
	64(15.16%)	358(84.83%)	422(100%)

5.3. Thrombocytopenia status in the presence of different species

Mean platelet count of malaria positive patients was 149,790cells /microliter (SD=103,899). while those of malaria negative patientswas 295,900/microliter (SD=118,737). (Table 4)

Table 4: Comparison Mean and standard deviation of platelet count between malaria positive and malaria negative patient attending at Adama Referral Hospital, Adama, Ethiopia**.**

		Platelet count		
Malaria status		**Mean**	**Std. Deviation**	**p-value(95%CI)from t test**
Positive	101	149,790	103.899	< 0.05
Negative	321	295,900	118.737	

The risk of thrombocytopenia was more in *Plasmodium vivax* 16(57% n=28) than *Plasmodiumfalciparum* 34(48% n=71). Magnitude of severe thrombocytopenia in*plasmodium vivax* 5(17.8% n=28)infection was higher than *Plasmodium falciparum* 11(15.49% n=71). (Table 5)

Table 5: The severity of thrombocytopenia in different malaria species among patients admitted at Adama Referral Hospital, Adama, Ethiopia.

			Grade of thrombocytopenia			
			Mild **100,000-138,000**	**Moderate** **50,000-100,000**	**Severe** **<50,000**	**Total**
Species	***P.vivax***	Frequency	5	6	5	28
		Percentage %	17.8%	21.4%	17.8%	27.7%
	P.falciparum	Frequency	11	12	11	71
		Percentage %	15.49%	16.9%	15.49%	70.29%
	Mixed	Frequency	0	1	1	2
		Percentage %	0.0%	50%	50%	2.0%
Total		Frequency	16	19	17	52
		Percentage %	30.76%	36.53%	32.69	100.0%

5.4 Severe thrombocytopenia status in the presence and absence of different associated factors in malaria positive and malaria negative patients

Patients those have history of bacterial infection were more likely to develop severe thrombocytopenia than has no history of bacterial infection (AOR=1.583: CI 0.198-12.621) and those who were *plasmodium vivax* are 2 times more likely to develop severe thrombocytopenia (AOR=2.493: CI 0.110-56.560) subjects however, there was no statistically significant association between overall platelet count of the study participant and the variables .Malaria positive patients have 9 times risk of severe thrombocytopenia AOR=9.180(95%CI= (1.011-83.336) there was no significant difference in overall platelet count of the study participant by difference in alcohol intake shown in Table 6.

Table 6: Risk of severe thrombocytopenia in malaria species and other factors in patientsattending at Adama Referral Hospital, Adama, Ethiopia**.**

Factors	**Crude OR**	**95%CI**	**P value**	**Adjusted OR**	**95% CI**	**P value**
Alcohol intake						
Yes	0.128	0.0014-1.148	0.066	0.067	0.007-0.662	0.021
No						
History of bacterial infection						
Yes	1.235	0.220-6.922	0.810	1.583	0.198-12.621	0.665
No						
Malaria species						
P.vivax	1.200	0.59-24.472		2.493	0.110-56.566	0.566
P.falciparum	1.091	0.61-19.630	0.953	1.558	0.083-29.201	0.767
Malaria status						
Positive	7.824	0.871-70.261	0.066	9.180	1.011-83.336	0.049
Negative						

5.5The correlations between platelet count and parasite densities by species type

Among *P.vivax* infected patient, 31.0% of patient had heavy parasite load while 35.7% of P. falciparum infected had heavy parasite load. All of patient with severe thrombocytopenia had heavy parasite load with *P. falciparum* account for higher percentage (64.7%) than *P.vivax* (29.4%). (Table7)

Table7:grade of thrombocytopenia and parasite density with in malaria species in patients attended at Adama Referral Hospital, Adama, Ethiopia**.**

Grade of thrombocytopenia			Species			Total
			P.vivax	P.falciparum	mixed	
100-138	**parasite load**	**1-100**	4	7		11
		101-5000	7	27		34
		>5000	0	1		1
	Total		11(23.91%)	35(76.08%)		46
50-100	**parasite load**	**1-100**	2	1	1	4
		101-5000	4	10		14
		>5000	0	2		2
	Total		6(30%)	13(65%)	1(5%)	20
<50	**parasite load**	**101-5000**	2	1	1	4
		>5000	4	11	0	15
	Total		6(32%)	12(63%)	1(5%)	19
Total	**parasite load**	**1-100**	6	8	0	14
		101-5000	13	38	1	52
		>5000	9	25	1	35
	Total		28(27.72%)	71(70.29%)	2(2%)	101

There was significant negative correlation between parasite density and platelet count (r=-0.539 P=0.01). Parasite density and neutrophil count % had negative correlation (r=-0.374 P=0.05) while lymphocyte count % and parasite density had positive (r= 0.476 P=0.01) lymphocyte count # and parasite density had positive correlation(r=0.236 P=0.05) (Table8)

Table 8: correlation of parasite density with CBC parameters

Uwbc	-0.110
Wbc	-0.112
Rbc	-0.154
Hgb	-0.169
Hct	-0.159
Mcv	-0.006
Mch	-0.063
Mchc	-0.145
Rdw	0.038
Rdwsd	0.058
Plt	-0.539**
Mpv	0.129
ne%	-0.374*
ly%	0.476**
mo%	-0.006
eo%	0.086
ba%	0.149
ne#	-0.174
ly#	0.236*
mo#	-0.042
eo#	0.037
ba#	0.031
Nrbc	0.156
nrbc#	0.079

6. Discussion

Certain hematological change is associated with malaria which is the major public health problem especially in developing countries. Anemia and thrombocytopenia are among the major complications(11). The current study aimed to assess platelet count in malaria suspected patients in Adama Referral hospital, Adama, Ethiopia.

In this study from a total of 422 study participants 101(23.9%) of them had malaria positive result confirmed by microscopy. The overall magnitude of thrombocytopenia was 64(15.16%). In other study which is done by Gill MK et al higher magnitude of thrombocytopenia (63.3%) in malaria positive patients compared to this study.(35)

In this study Among thrombocytopenic patients 52 (51.48%) were positive for malaria and 12(3.73%) of the thrombocytopenic patients were negative.among malaria positive patient, 28 (27.7%) had *Plasmodium vivax* whereas 71(70.3%) had *Plasmodium falciparum* infection and only 2(2%) had mixed infection. as study by Erkurt MA et al Thrombocytopenia occur in malaria may be due to increased destruction of platelet (32)

In this study Mean platelet count of malaria positive patientsis 149,790 /microliter While that of malaria negative is 295,900/microliter where as in study by Gill MK et alThe mean platelet count in Plasmodium vivax was 127,652/µl, as compared to*Plasmodium falciparum* (Pf) malaria where the mean platelet count was 78,500/µl.

In the currentstudy thrombocytopenia was detected in 57% of the *P.vivax* cases while 47.88% of the *P. falciparum* cases as determined platelet count of less than 138,000 cells per microliter Whereas a study conducted in S.P. Medical College by Kochar KD et al showed that, out of 1064 cases of malaria, Platelet count <150,000 per microliter of blood was observed in 16.9%, 31.09%, and 3.04% of Plasmodium falciparum, Plasmodium vivax, and mixed infection, respectively.(37) thrombocytopenia greater in *plasmodium vivax* due to circulating p-selectin in plasma was elevated in *p vivax* this might be reason thrombocytopenia is greater in *p vivax* .(39)

In this study The magnitude of severe thrombocytopenia was comparable in of *Plasmodium vivax* (17.8%)and *plasmodium falciparum* (15.49%) Patients those who had *plasmodium vivax* are 2 times more likely to develop severe thrombocytopenia.. From the observation was evident that thrombocytopenia is a common hematological finding in patients with plasmodium infection and it notifies the association of severe thrombocytopenia was strongest with *Plasmodium vivax* infection as compared to *Plasmodium falciparum* and mixed infection.

On the other hand a very high rate of thrombocytopenia was reported by a study done by Anita B ET AL in North Karnataka which recruited sixty six cases of *P. vivax* and nine cases of *P. falciparum*. Thrombocytopenia was the most common abnormality noted in 71 (94.66%) of 75 cases (36).

A prospective study done by Mumtaz Ali Shaikh et al in Pakistan was conducted on 200 diagnosed cases of malaria. Of them 105 (52.5%) were cases of Plasmodium falciparum, 93 (46.5%) were of *P. vivax*, and 2 (1%) were of *P. malaria*. The data showed that 171 (85.5%) patients were thrombocytopenic (20).

In this study 9(31%) of *P.vivax* infected patients had heavyparasite load while 25(35.7%) of patient with *P. falciparum* had heavy parasite load There was an inverse correlation which is negative correlation between parasite density and platelet count (r=-0.539 P=0.01) and whilelymphocyte count % and parasite density had positive (r= 0.476 P=0.01) lymphocyte count # and parasite density had positive correlation(r=0.236 P=0.05)

In Ethiopia in a study done by Awoke and Aorta a total of 340 study participants were included ,out of which 170 were malaria cases, and the remaining 170 were malaria negatives The prevalence of thrombocytopenia and anemia in malaria patients was 84% and 67%, respectively. There was an inverse correlation between *P. falciparum* and *P. vivax* parasite density and lymphocyte count, as well as platelet count(42). Negative correlation parasite load and platelet count may be due to platelet shortened life span.(33)

7. Conclusionand recommendation

7.1Conclusion

The magnitude of thrombocytopenia was higher among malaria positive than malaria negative patients. Thrombocytopenia was relatively more common among *P.vivax*than *P.falciparum* infected patients. Therefore, thrombocytopenia can be taken as a predictor of complicated malaria is better predicted by severity of thrombocytopenia.

7.2Recommendation

- A clinician that suspects a patient for malaria diagnosis using clinical manifestations can use platelet parameter as another supporting predictorif laboratory technologists' register severe thrombocytopenia in parallel patients that live around malaria endemic area, malaria diagnosis should be considered.
- Since finding of low platelet count in patients significantly indicates the presence of malaria, laboratory technologists are highly recommended to consciously scan, detect and identify malaria parasite before reporting it as negative for hemo parasite.

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8. Strength and limitation of the study

8.1Strength of the study

- This study includes all age group in order to show more affected age group .
- This study quantifies parasite density to associate relative risk of severe thrombocytopenia in patient with *P.vivax* and to show effect of parasite load in severe thrombocytopenia.

8.2Limitation of the study

- The study did not include the death rate of malaria due to effect of severe thrombocytopenia in malaria positive patient further investigation must be done to show death rate due to effectof thrombocytopenia.

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10. Annex

Annex I. Information Sheet and consent form

Addis Ababa University College of Health Sciences Department of Medical Laboratory Science You are invited to participate in a study to be conducted by MSc student at Addis Ababa, College of Health Sciences, and Department of Medical Laboratory Science.

Please read the following statements and ask any unclear points before you agree to participate.IntroductionThe topic of this study “Assessment of platelet count in malaria suspected patients)

The aim of the study is by using hematology test we will assessment of malaria effect on platelet count. If you are not interested to participate or if you once decide to participate and withdraw at any time, there will be no consequences and you will get all the services provided in the hospital with no problems.

If you decide to participate, you have to sign on the consent form and you may obtain a copy of this information sheet. What is expected from me as a participant of the study? As a participant of this study, you are expected to participate for giving blood sample.

In addition, you are expected to give answers for some questions about your health and socio-demographic conditions. You need to know that your results might be discussed with other appropriate individual out of this hospital. But your name, address and phone number will not be disclosed and rather than Identification code will be used in such conditions.

How much time will I spent to participate in this study? You will spend 20-25 minutes until the specimen is collected, the consent form is signed .

How my information is to be kept in secret? All information that you give and the results from your sample will be used for this study only, only limited numbers of professionals will have access to the information.

All the information will be encoded in a computer and saved with password protection. What are the benefits from participation? Since this study is MSc student research, there

will not be payments for participants. But your Participation is important for the establishment good assessment of platelet count.

Please direct any questions or problem you may encounter during this study to:

Feven TilahunMob: +251920196276Email: feven7197@gmail.com

Advisors: .AsterTsegaye 0911696085tsegayeaster@yahoo.com,; Mr.Mogeswordofa email heranmakmow@gmail.com for additional information, please contact Department of Medical Laboratory Sciences, Addis

Ababa University, Institutional Review Board (IRB) office;

Tel: +2511911107099

P.O Box: 9086, Addis Ababa, Ethiopia

Agree to participate? Yes No

የተሳታፊዎችመረጃቅጽጥናቱን:

የሚያጠናዉፌቨንጥላሁንበአዲስአበባዩንቨርሲቲየጤናሳይንስኮሌጅየህክምናላቦራቶሪሳይንስትምህርትክፍልየወባበሽ
ታምርመራበታዘዘላቸዉህመምተኞችላይስለፕሌትሌትየደምህዋስአጠቃላይሁኔታእናየፕሌትሌትንየደምህዋስቁጥርስ
ለሚቀንሱተያያዥነትስላላቸዉሁኔታዎችለማወቅሲባልለሚደረገውጥናትየተዘጋጀየእጩተሳታፊዎችየፍቃደኝነትመጠ
የቂያውልየጤናድርጅትስም---------- መለያ(ኮድ)----------የመረጃሰብሳቢውስም----
----------የተከበሩተሳታፊእንደምንአሉ፣ስሜ----------ይባላል::

በአሁኑሰአትበአዲስአበባዩንቨርሲቲየጤናሳይንስኮሌጅህክምናላቦራቶሪሳይንስትምህርትክፍልየማስትሬትዲግሪትምህ
ርቴንበመከታተልላይየምገኝስሆንጥናቴንለማካሄድአስቤአለሁ::

የጥናቱመነሻርዕስየሚያተኩረውየወባበሽታምርመራበታዘዘላቸዉህመምተኞችላይስለፕሌትሌትየደምህዋስአጠቃላይ
ሁኔታእናየፕሌትሌትንየደምህዋስቁጥርስለሚቀንሱተያያዥነትስላላቸዉሁኔታዎችስለማወቅይሆናል::

ስለሆነምበጥናቱላይለመሳተፍፍቃድኛከሆኑለጥናቱአስፈላጊየሚሆንከ3-
5ሚ.ሊየሚሆንየደምናሙናከእርስዎእንወስዳለን::

ይህየደምናሙናበሚወሰድበትወቅትመጠነኛህመምሊሰማዎትይችላል::ይሁንእንጂህመሙከፍተኛችግርአያመጣም::

የሚሰጡትየደምናሙናከታቀደለትአላማውጪአይውልም፤

በሚሰጡትየደምናሙናየሚሰራውየላቦራቶሪስራበሚስጥርኮድ (መለያ) ሲሆንስምእንደመለያአንጠቀምም::

ውጤቱምከመረጃሰብሳቢዎቹእናከጥናትባለሙያውውጪለማንምግልጽአይሆንም::

በዚህጥናትበመሣተፍዎየሚያገኙትግላዊጥቅምወይምክፍያአይኖርም፤

በደምናሙናዎላይበሚሰራውየላቦራቶሪስራምክፍያአይጠየቁም::

በተጉአዳኝነትየነዚህንየፕሌትሌትህዋሳትንየሚቀንሱበሽታዎችአሉ::ስለነሱምለማወቅየተዘጋጀመጠይቅአለ::

ለዚህጥናትአላማስኬትእነዚህንጥያቄዎችመጠየቅእናመሙላትአስፈላጊይሆናል::

በዚህጥናትላይየመሳተፍወይምያለመሳተፍሙሉመብትአለዎት፤

በዚህጥናትባለመሳተፍዎአሁንእያገኙባለዉየህክምናአገልግሎትወይምወደፊትሊያገኙበሚፈልጉትአገልግሎትላይምን
ምአይነትተጽዕኖአይኖረዉም::የተሳታፊምላሽ፡

በዚህጥናትለመሳተፍወይምላለመሳተፍወይምባልፈለኩሰአትለማቋረጥነጻነኝ፤

በዚህጥናትላይበመሳተፌወይምባለመሳተፌበዚህተቐምበወቅቱወይምለወደፊቱበማገኘዉአገልግሎትላይምንምአይነት
ተጽእኖአያሳድርም::ከዚህበታችያለዉፊርማዬበዚህጥናትለመሳተፍፍቃደኛመሆኔንያረጋግጣል፡

ስላደረጉልንትብብር

እናመሰግናለን!ፌቨንጥላሁን፤የሕክምና፡ላቦራቶሪ፡ቴክኖሎጂ፡ዲፓርትመንት፣የጤናሳይንስ፡ኮሌጅ፣አዲስ፡አበባ፡
ዩኒቨርሲቲ- አዲስአበባ፣ኢትዮጵያ

 ኢ-ሜይል፣feven7197@gmail.com

ስልክ+251-920-19-62-76

ይህመረጃ: በጥንቃቄ:የሚያዝ: ይሆናል፡፡በመጨረሻም: የጥናቱ: ውጤት :ለሚመለከተዉ :አካል: ለጥናቱ: አላማና:ለህክምና:ባለሙያዎች: ብቻ: የሚገለፅ:ይሆናል፡፡

ያስታውሱ፤ስለዚህ:ጥናት:ማንኛውም: ጥያቄ: ካለዎት: በማንኛውም: ጊዜ: ከዚህ: በታች: በተጠቀሱት; አድራሻዎች; መጠየቅ: ይችላሉ፡፡

እኔምየጥናቱ: ተሳታፊ: ይህንን: በመገንዘብ: ጥናቱ: ላይ: ለመሳተፍ: ተስማምቼያለሁ፡፡

ፊርማ ----------

መረጃውን፡የሰበሰበው፡ግለሰብ፡ስም----------

ፊርማ ----------

የዋና: ተመራማሪው; አድራሻ፤

ፌቨንጥላሁን፤የሕክምና፡ላቦራቶሪ፡ቴክኖሎጂ፡ዲፓርትመንት፣የጤና፡ሳይንስ፡ኮሌጅ፣አዲስ፡አበባ፡ዩኒቨርሲቲ-አዲስአበባ፣ኢትዮጵያ

 ኢ-ሜይል፡feven7197@gmail.comስልክ 251-920-19-62-76

1. Consent form for parents/guardians

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that my child participates in this study provided he/she gives assent.

To collect her/his blood and be a participant in this study and understand that I have the right to withdraw my child from the study at any time .

Print name of participant, date and signature or thumb impression of participant

_____ ____ /____ /____ (dd/mm/yy)

_____

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ ____/____/_____ (dd/mm/yy)

_____

Print name of researcher, date and signature of researcher

_____ _____/____/_____ (dd/mm/yy)

_____

2. Assent form for children aged 12-17 years

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily assent that I would participate in this study provided my parents/guardians give their consent.

To collect my blood and be a participant in this study and understand that I have the right to withdraw from the study at any time .

Print name of participant, date and signature or thumb impression of participant

_____ ____ /____ /____ (dd/mm/yy)

_____

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ ____/____/_____ (dd/mm/yy)

_____

Phone number (parents/guardians)

Print name of researcher, date and signature of researcher

_____ _____/____/_____ (dd/mm/yy)

_____

3 .Consent form for adults (≥18 years)

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that I would participate in this study.

To collect my blood and be a participant in this study and understand that I have the right to withdraw from the study at any time .

Print name of participant, date and signature or thumb impression of participant

_____ ____ /____ /____ (dd/mm/yy)

_____

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ ____/____/_____ (dd/mm/yy)

_____Phone number

Print name of researcher, date and signature of researcher

_____ _____(dd/mm/yy) _____

AnnexII. Questionnaire onmalaria effect on platelet count

Addis Ababa University College of health Science School of allied health sciences Departmentof medical laboratory science.

Socio demographic characteristics. Code _____

1. Sex of the patient 1.Male 2.Female.

2. Age of the patient _____

3.Are you enduring consumer of alcoholic beverage?

☐ ☐ Yes No

4.Are you pregnant if female?

☐ ☐ Yes No

5.Are you taking anti-malarial drug currently /quinine/chlorquine?

☐ ☐ Yes No

6.Do you have a history of infection ?

☐ ☐ Yes No

Annex III. Standard Operating Procedure

Lab sop for Giemsa

SOP FOR BLOOD FILM PREPARATION

- Principle: Giemsa stain is a mixture of eosin and methylene Blue. When applied to blood cells, the dyes produce multiple colors based on the ionic charge of the stain and the various components of the cell. The eosin ions are negatively charged and stain basic cell components an orange to pink color. The methylene blue ions are positively charged and stain the acid cell components in varying shades of blue. The neutral components of the cell are stained by both components of the dye producing variable colors.
- **Eosin** is anionic and acts as an acidic dye. It is negatively charged and can react with positively charged, acidophilic components in the tissue, such as amino groups in proteins in the **cytoplasm.**

Methylene blue stains the nucleic acids. It's a basic dye and bind well to DNA (negative charge). It stains the dead cells and thus differentiates it from the living cells because the dead cells will take up the stain easily than the live cells

The thick film is used as a screening test to establish the presence of malaria,

And the thin film is used to identify the species of the organism. Examination of malaria Blood films by microscopy is a basic technique, which remains the gold standard for the Diagnosisof malaria. Good quality blood films are essential to establish accurate diagnosis.

Using venous blood:

1. Using a micropipette, place a 2µl drop of blood in the smaller circle and 6 µl in the bigger circle of the slide (pre-labeled) placed over the template. Do not delay Between applying and spreading the drop.

Preparation of the thin film

2. Working quickly, obtain a second clean and polished slide (spreader) and place it front of the small drop blood at a 30º - 45º angle. Pull back the slide and hold until the blood is Evenly spread along the edge of the slide. Do not delay between applying and spreading the drop.

3. Rapidly push the slide forward in a single, smooth, continuous motion. Avoid Hesitation or jerky motions when spreading the blood. (A feathered end of the film should

have red blood cells that are lying individually without overlapping and relatively evenly Distributed).

Preparation of thick blood film

1. With one corner of the spreader slide, in a circular motion, spread the blood out to make a circle with approximately 1cm (1/3 inch) in diameter, finishing off at the center.
2. The ideal thickness of the smear should allow for printed text to be readable when it is Placed on it. 3. Discard the spreader into an appropriate slide container and DON'T re-use it for another patient's blood sample.
4. Allow both the blood films to air dry in a horizontal position on a slide tray or folder. If EDTA blood is used, drying should be between 24–72 hours. Slow drying prevents cracking. Avoid using a fan or blow dryer to dry these slides.

SOP for Staining: Good quality staining of blood films is essential to establish accurate diagnosis.

Procedure: Fixing the thin film

1. When the films are completely dry, fix ONLY the thin film by dipping if in absolute methanol
for approximately 30seconds. Care must be taken not to fix any portion of the thick film.
2. Allow the film to dry.

Staining the thick and thin films

1. Gently pour 3% or 10% Giemsa working solution in to the staining jar.
2. Put the slides in a rack inside the staining jar; the slides should be fully submerged/covered
with the stain.
3. Stain for 30-45minutes and 10-15minutes for 3% and 10% Giemsa working solutions, Respectively.
4. Pour clean water gently in to the jar to float off the iridescent scum on the surface of the stain.

Alternatively, gently immerse the whole jar in a vessel filled with clean water.

5. Gently pour of the remaining stain, and rinse slides again in clean water for a few seconds.

Pour the water off.

6. Wipe the back of each slide with paper towels.

7. Dry the slides in a vertical position with the thin film down wards.

4. Allow both the blood films to air dry in a horizontal position on a slide tray or folder. If EDTA blood is used, drying should be between 24–72 hours. Slow drying prevents cracking. Avoid using a fan or blow dryer to dry these slides.

SOP for Staining: Good quality staining of blood films is essential to establish accurate diagnosis.

Procedure: Fixing the thin film

1. When the films are completely dry, fix ONLY the thin film by dipping if in absolute methanol

for approximately 30seconds. Care must be taken not to fix any portion of the thick film.

2. Allow the film to dry.

Staining the thick and thin films 1. Gently pour 3% or 10% Giemsa working solution in to the staining jar.

2. Put the slides in a rack inside the staining jar; the slides should be fully submerged/covered

with the stain.

3. Stain for 30-45minutes and 10-15minutes for 3% and 10% Giemsa working solutions, respectively.

4. Pour clean water gently in to the jar to float off the iridescent scum on the surface of the stain.

Alternatively, gently immerse the whole jar in a vessel filled with clean water.

5. Gently pour of the remaining stain, and rinse slides again in clean water for a few seconds.

Pour the water off.

6. Wipe the back of each slide with paper towels.

7. Dry the slides in a vertical position with the thin film down wards.

Evaluation of a well-stained thin film

1. The background should be clean and free from debris; the color of erythrocytes is a pale
green pink.
2. Neutrophil leukocytes have deep purple nuclei and well-defined granules.
3. The chromatin of malaria parasites is a deep purplish red and cytoplasm a clear purplish blue. 4. Stippling should show up as Schuffner's dots in erythrocytes containing P. vivax or P. ovale, and Maurer's spots in erythrocytes containing thelarger ring forms of P. falciparum.

Evaluation of a well-stained thick film

1. The background should be clean and free from debris, with a pale mottled-grey color derived
from the lysed erythrocytes.
2. Leukocytes nuclei are a deep, rich purple.
3. Malaria parasites are well defined with deep-red chromatin and pale purplish blue cytoplasm. In P. vivax and P. ovale infections the presence of Schuffner's stippling in the "ghost" of the
host erythrocyte can be seen especially at the edge of the film.

Evaluation of staining quality

1. A MBF that is too pinkish suggests low pH or over-staining.
   2. A MBF that is too bluish or purplish suggests high pH or under-staining.
   SOP FOR EXAMINATION OF MALARIA BLOOD FILMS
   Focusing and scanning the blood film Procedure:
   1. Place the MBF (Malaria Blood Film) on the microscope stage, switch on the light and adjust
   the light source optimally by looking through the ocular and the 40X objective.
   2. Place a drop of immersion oil on the dry stained slide. To avoid cross contamination, ensure
   that the immersion applicator never touches the slide.
   3. Slowly change to the oil immersion objective, and a thin film of oil will form between the
   slide and the lens.

Evaluation of a well-stained thin film

1. The background should be clean and free from debris; the color of erythrocytes is a pale

green pink.

2. Neutrophil leukocytes have deep purple nuclei and well-defined granules.

3. The chromatin of malaria parasites is a deep purplish red and cytoplasm a clear purplish blue.

4. Stippling should show up as Schuffner's dots in erythrocytes containing P. vivax or P. ovale, and Maurer's spots in erythrocytes containing thelarger ring forms of P. falciparum.

Evaluation of a well-stained thick film

1. The background should be clean and free from debris, with a pale mottled-grey color derived

from the lysed erythrocytes.

2. Leukocytes nuclei are a deep, rich purple.

3. Malaria parasites are well defined with deep-red chromatin and pale purplish blue cytoplasm. In P. vivax and P. ovale infections the presence of Schuffner's stippling in the "ghost" of the

host erythrocyte can be seen especially at the edge of the film.

Evaluation of staining quality

1. A MBF that is too pinkish suggests low pH or over-staining.

4. Adjust the light source optimally by looking through the 10x ocular (eyepiece) and the100X objective and use the fine adjustment knob to focus the field; the lens should not be allowed totouch the slide.

5. Examine the slide in a systematic fashion. Start at the left end of the thick film and begin reading at the periphery of the field and finish at the other end. When the field is read, move theslide right to examine adjacent fields.

Examining the thick blood film

1.Scan the thick film under oil immersion objective (x100) and ascertain whether a smear is positive or negative. 2.Use the "WHO BenchAids in the Diagnosis of Plasmodium Infections"

3.If positive, determine all species and stages present.

4.Read a minimum of 200 oil immersion fields before declaring a slide negative. If time permits,

scan the whole thick film**.**

Examining the thin blood film

1. When species is doubtful on the thick film, or mixed infections are suspected, a careful

examination of the parasite morphology should continue on the thin smear for verification.

2. If different species are observed, this should also be recorded.

Result Interpretation - Report" Parasite species, stage If it is negative, report "No haemoparasite seen'.

- One asexual parasite form that is 100% typical must be observed for a slide to be called

POSITIVE. A slide is positive if both chromatin and perfect cytoplasm OR chromatin and pigment are observed.

-Parasites are considered atypical (<100%) if classical features are distorted i.e. cytoplasm is not well defined, ring is broken, ring is not the correct color or shape etc…

-If atypical parasites are observed, both thick and thin films should be examined carefully.

Alternately a follow-up slide can be prepared.

SOP Beckman Coulter (DXH800) Hematology Analyzer

Purpose

This SOP provides general information about Beckman Coulter (DxH800) automated Hematology Analyzer.

Principle

The DxH 800 CBC analysis based on Coulter principle performs 100 samples per hour of 27 hematological parameters. CBC is essential analytical test that evaluates the three main cellular components; WBC, RBC and platelets. Sample preparation and data collection occurs in SAM and CBC modules on the DxH 800. The data analysis is handled by the system manager. The method of counting and sizing in combination with an automatic diluting and mixing device for sample processing, and a single beam photometer for hemoglobinometry. The WBC differential uses VCS technology. Analysis and classification of WBCs use three simultaneous measurements of individual cell volume (V), high frequency conductivity (C), and laser light scatter (S). The scatter gram plots the cells based upon the measurements of these three parameters.

The Beckman Coulter method accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle such as a cell, in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of electrical path between the submerged electrodes on either side of the aperture. This causes a measureable electronic pulse. For counting, the vacuum used to pull the diluted suspension of the cells through the aperture must be at a regulated (reproducible) volume. While the number of the pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume. The hemoglobin is photo metrically measured at 525 nm using lysed WBC dilution drains to the cuvette from the WBC analysis (counted). The lytic reagent rapidly and simultaneously destroys the RBC and converts Hgb into stable pigment, which is proportional to the concentration of Hgb.

Specimen collection

The phlebotomist collects a 3- or 5-mL K3 EDTA tube on all SPs aged 1 year and older following established venipuncture protocol and procedures (a 1-2% dilution effect occurs in this liquid EDTA tube).

Sample preparation

The aspiration pump activates and aspirates 165 uL of sample. After the probe is removed from the specimen tube a second pull of the aspiration pump draws the blood through the BSV pathway, verifying a proper aspiration at the blood detectors. With each

cycle, the BSV directs the delivery of the sample and DxH diluent to the WBC (approximately 6 mL diluent and 28 uL of sample are combined with 1.08 mL of DxH Cell Lyse for a final dilution of 1:251) and RBC (approximately 10 mL of DxH diluent and 1.6 uL of sample are mixed for a final dilution of 1:6250) triple aperture baths.

Reagents

- ✓ Coulter DxH Diluent (10L)
- ✓ Coulter DxH Cleaner (5L)
- ✓ Coulter DxH Cell lyse (5L)
- ✓ Coulter DxH Diff Pack (1x1900mL Erythrolyse-II & 1x850 mL Stabilize)
- ✓ Coulter DxHRetic Pack
- ✓ Coulter Retic-X Cell control
- ✓ Coulter S-CAL calibrator
- ✓ Coulter Body fluid control
- ✓ Coulter Latron CP-X control
- ✓ Coulter LIN-X Linearity control
- ✓ Coulter 6C Cell Controls Tri Pack

Supplies

- ✓ 3-mL K2 or K3 EDTA Vacutainer tube for whole blood, peritoneal, pleural, and hyaluronidase pretreated-synovial fluid
- ✓ Barcode labeled Tube Rack
- ✓ Clorox Bleach, 5.25% sodium hypochlorite
- ✓ Cotton gauze pads
- ✓ Stapler Punch
- ✓ Staplers
- ✓ Printer ribbon
- ✓ A4 size paper

Equipment

- ✓ Beckman Coulter (DxH 800) machine
- ✓ Printer

- ✓ Barcode Reader
- ✓ Screen touch Monitor
- ✓ UPS
- ✓ Environmental and Safety control
- ✓ Universal precautions must be used when handling, processing and disposing of patient samples.
- ✓ Do not expose to large temperature variations and direct sunlight.
- ✓ Avoid shocks and vibrations.

Calibration

For best performance and tracking normal process, verifying and calibrating all the CBC parameters using Coulter S-CAL is necessary except WBC-diff, NRBC and Retic which done by authorized Beckman Coulter representative and no need of calibrating VCSn parameters. When to calibrate:

- ➢ At installation
- ➢ After replacement of any component that involves dilution characteristics (such as BSV) or the primary measurements (such as aperture)
- ➢ When advised to do so by Beckman Coulter Representative

Verification failure

Quality Control

Coulter S-CAL calibrator, retic-X cell, body fluid, Latron CP-X, LIN-X Linearity and 6C Cell Controls Tri Pack control are used as control material. A quality control should be performed:

Before any start of operation - prior to analyzing samples

at least every 8 hours during operation

after replenishment of components

after maintenance

If there is any doubt about the accuracy of the analysis values.

Procedure

Check to see that the reagents needed for the number of the samples to be processed for the day are available.

Turn on the IPU switch and log on screen will appear on the computer. Enter the user name and password.

Wake up/ Go online the main unit on the machine. Daily-check, auto rinse, temperature stabilization and background check will be automatically performed, and the "READY LED turns on (ready for analysis) will appear

Permissible background counts

Parameter	Limits for whole blood	Parameter	Limit for Body fluid
WBC	< = 0.05[x 103cells/µL]	TNC	< = 20 cells/mm3
Diff-WBC	< = 100 events	RBC	< = 1000 cells/mm3
RBC	< = 0.005 [x106cells/µL]		
HGB	< = 0.10 [g/dL]		
PLT	< = 3.0 [x103cells/µL]		
NRBC Region	< = 10 events		
NRBC Total	< = 60 events		
Ret	< = 600 events		

Perform quality control analysis on 3 levels of control blood material (low, normal and high), Latron and Latron Primer to verify that the instrument is performing within the specified ranges of the quality control material

If the result of quality control is inacceptable range, run the blood samples. Samples can be run in Single/manual mode, Sampler mode.

Single Tube/Manual mode

Click the single tube icon at the top of the computer.

Input the tube position number or use bar code reader.

Mix gently invert (10x) put the tube appropriately in the position labeled purple for whole blood or light green for the body fluid

Ask “Add diluent” for whole blood if it is inadequate

Fill the necessary information like MRN, test items (CDR/CBC/WBC-NE), and click “Submit”.

Remove the tube when the analysis is overReview and print the result.

Sampler mode

Click the sampler icon at the top of the computer.

Prepare and put the samples on a barcode labeled tube rack.

Fill the necessary information like tube position number similarly on the tube rack, MRN.

Once again check the rack number and tube position number in the Rack Number/Tube Position Confirmation dialog box.

Position the tube rack on the sample station so that the tube rack taken for analysis momentarily by the magnetic interaction and start analysis automatically

Review and print the results.

Quality control procedure

Bring all the control materials to room temperature except Latron CP-X (already at room temperature) putting them on sample mixer.

Wake up/ Go Online the screen and log on screen will appear on the computer. Enter the user name and password.

Turn on the main unit on the machine. Self-check, auto rinse, temperature stabilization and background check will be automatically performed, and the "READY LED turns on (ready for analysis) will appear.

Click the Controller button on the Menu screen.

Double-click the QC Analysis icon on the Controller Menu and select QC File dialog box.

Select a QC file and click OK.

Gently invert eight times the control tubes

Hold the opened control tube under the sample probe and press the start switch button.

Accept the control result if are within the range of the target limit or repeat the analysis if control results are out of the target limit.

All control data are managed using software that provides graphical reports (Levey-Jennings graphs, and monthly cumulative histograms).

Calculations

Not applicable

Performance Characteristics

Method was verified for intended use.

Uncertainty measurement

Interferences/Limitations

The following is a list of possible substances/factors that may affect listed parameters.

WBC and TNC

Platelet aggregation, giant platelets, nucleated RBCs, cryoglobulins, lyse resistant RBCs in patients with hemoglobinopathies and severe liver disease.

RBC

Very high WBC count, cold agglutinins, severe microcytosis, fragmented RBCs, large number of giant platelets, in vitro hemolysis.

HGB

Severe lipemia, heparin, certain unusual abnormalities that resist lysing, abnormal proteins in blood plasma, leukocytosis (above 100,000/µl).

MCV

Very high WBC count, cold agglutinins, large number of giant platelets

HCT, MCH and MCHC

Similar to RBC and MCV affecting factors.

PLT

Pseudo thrombocytopenia, giant PLTs, PLT aggregation, microcytosis.

NRBC

Lyse resistant red cells, malarial parasites, very small or multi-population lymphocytes and precipitated elevated proteins

Differential

Hypo granular, agranular, lyse resistant red cells, very small or multi-population lymphocytes, precipitated elevated proteins, elevated triglycerides, transient basophilia due to high temperature exposure, blasts are detected but not enumerated by internal algorithm using acquired events or histogram

Reticulocytes

Numerous erythrocyte inclusion stained by new methylene blue, hemoglobinopathies like SS or SC

Body fluids

Cellular debris, improper mixing

CSF

Decreased manual count and correlation due to low albumin and lipid levels, in turn accelerated cell lysis, delay in analysis.

Critical values

WBC <2,000 or >40,000 x 103/ µL

HGB <7g/dl

Platelet<50,000/mm3

Result reporting

Results are reported from automated printing and through computer

Result Interpretation

Certain disease states are defined by an absolute increase or decrease in the number of a particular type of cell in the bloodstream and many types of anemia.

Biological Reference Interval

Parameter	Female	Male	Unit	Parameter	Female	Male	Unit
WBC	3.8 – 11.8	3.2 - 10.6	x103/µL	MCH	24.7-32.8	23.8-33.4	pg
Ne	42.7-76.8	43.5-73.5	%	MCHC	32.3-35.6	32.5-36.3	g/dl
Ly	16.0-45.9	15.2-43.3	%	RDW	12.3-17.7	12.1-16.2	%
Mo	4.3-10.9	5.5-13.7	%	RDW-SD	37.6-50.3	36.5-45.9	fL
Eo	0.5-7.0	0.8 – 8.1	%	PLT	179-408	152-348	x103/µL
Ba	0.2-1.3	0.2-1.5	%	MPV	7.9-10.8	7.4-11.4	fL
Ne#	1.9-8.2	1.7-7.6	x103/µL	NRBC	0.0-0.3	0.0-0.6	/100WBC
Ly#	1.1-3.1	1.0-3.2	x103/µL	NRBC#	0.00-0.02	0.00-0.02	x103/µL
Mo#	0.2-0.9	0.3-1.1	x103/µL	RET	0.51-2.17	0.42-2.23	%
Eo#	0.0-0.5	0.0-1.5	x103/µL	RET#	0.0230-0.0935	0.188-0.1086	x106/µL
Ba#	0.0-0.1	0.0-0.1	x103/µL	RBC	3.63-4.92	4.63-6.08	x106/µL
HGB	10.9-14.3	13.7-17.5	g/dl	HCT	31.2-41.9	36.7-47.1	%
MCV	75.5-95.3						

Annex IV Work Sheet Preparation Data Entry

Table 9: Worksheet preparation For Malaria report

T= Tropzoite G=Gametocyte S=Shizonts PF=plasmodium falciparum

PV=plasmodium VivaxMx= Mixed Dt= Date

Slide no	Negative	Specious			Stage			Parasite load reported	Dt
		PF	PV	Mx	T	G	S		

code	stage	WBC	NE	LY	MO	EO	BA	# NE	# LY	# MO	# EO	# BA	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	RDW-SD	PLT	MPV	NRBC	# NRBC	RET	# RET

Table10: worksheet for CBC result report

11. Declaration

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

M.Sc. candidate: Feven Tilahun (B.Sc.)

Signature:_____

Date of submission:_____

This thesis has been submitted with our approval as advisors.

Advisor: Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: MogesWordofa (MSc)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.