

UNIVERSITY OF GHANA

COLLEGE OF ALLIED HEALTH

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

(HAEMATOLOGY UNIT)



**EFFECTS OF HEPARIN, ACID CITRATE DEXTROSE, AND EDTA ON PLASMODIUM
FALCIPARUM PARASITES MORPHOLOGY AND *IN VITRO* GROWTH RATE**

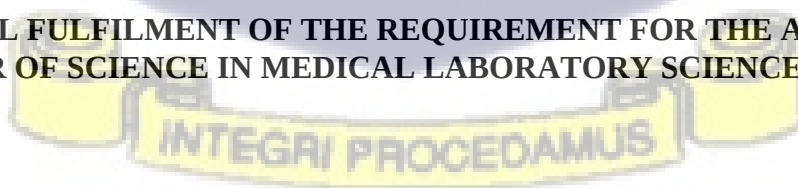
By

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**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON,
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DECLARATION

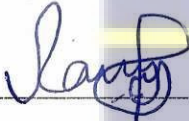
I, Sophia Eyia-Ampah, hereby certify that this thesis is my original research, except for cited references and that no section of this thesis has ever being presented for the granting of a degree or any qualification of that sort to this or any other institution. This research was done under the supervision of Prof. Micheal F. Ofori (Department of Immunology, Noguchi Memorial Institute for Medical Research, University of Ghana) and Dr. Ransord Kyeremeh, School of Biomedical Allied Health Sciences (SBAHS), University of Ghana – Korle Bu.

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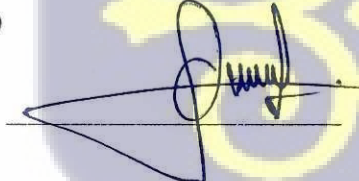
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DEDICATION

I dedicate this dissertation to my faithful God for his loving kindness, mercies, and grace throughout this MSc program. I further dedicate this research work to my parents, Mr. Samuel Eyia-Ampah and Madam Adwoa Woode, to my daughter, Benedicta Emefa Ekua Gati and to my brother, Kodwo Eyia-Ampah for all their prayers, encouragement, and support throughout this two-year period.



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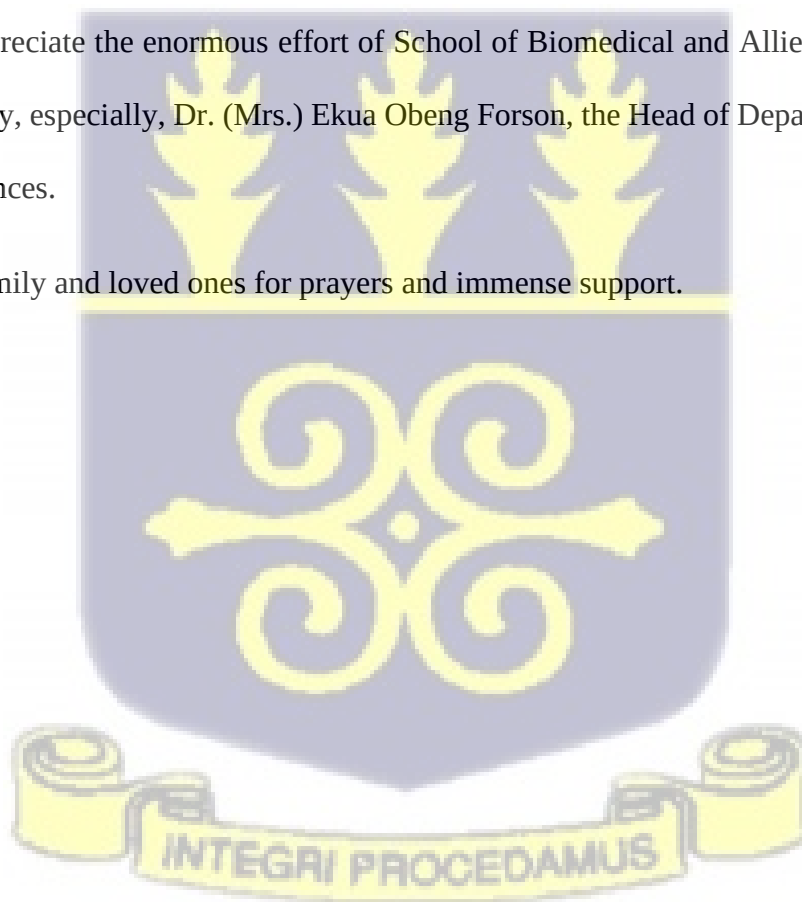
My utmost praise and thanks to the Almighty God for giving me the strength and grace to complete this work and for being with me throughout the most difficult moments.

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ABSTRACT

This study investigated the effects of three anticoagulants - Acid Citrate Dextrose (ACD), Heparin, and EDTA - on the *in vitro* growth and morphology of Plasmodium falciparum (3D7 strain). Using venous blood from five healthy donors, we established triplicate cultures for each anticoagulant and monitored parasitaemia daily for 14 days through microscopic examination. Our results demonstrated distinct patterns of parasite growth across the different anticoagulant conditions.

ACD-treated cultures showed the most favorable growth characteristics, reaching peak parasitaemia of 13.57% and maintaining well-preserved parasite morphology throughout the observation period. Heparin-supported cultures exhibited moderate growth (peak 7.4% parasitaemia) with some morphological variations, while EDTA-treated cultures displayed the lowest growth rates (peak 4.13% parasitaemia) and more frequent degenerative changes in parasite forms.

Statistical analysis using ANOVA ($F = 0.404$, $p > 0.05$) revealed no significant differences in mean parasitaemia levels among the three anticoagulant groups. However, the consistent trend of superior performance in ACD-treated cultures suggests its potential advantage for maintaining robust *P. falciparum* cultures. These findings have important implications for malaria research methodologies, particularly in studies requiring optimal parasite viability and standardized growth conditions.

The study provides evidence-based recommendations for anticoagulant selection in *P. falciparum* culture systems, with ACD emerging as the preferred choice for high-yield cultures, while Heparin and EDTA may serve as viable alternatives depending on specific experimental requirements.

These results contribute to the standardization of malaria research protocols and may enhance the reliability of *in vitro* studies investigating antimalarial drug efficacy and parasite biology.



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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The integrity and reliability of hematologic determinations, especially in malaria research, are significantly influenced by the methods used in blood sampling, the choice of anticoagulants, and the subsequent handling and storage of blood samples. This is particularly critical in studies assessing the effects of different anticoagulants, such as Acid Citrate Dextrose (ACD), Heparin, and EDTA, on the growth and morphology of *Plasmodium falciparum*. Malaria is a major tropical disease caused by parasites of the *Plasmodium* genus, it is transmitted to humans by female *Anopheles* mosquitoes when they ingest gametocytes, the form of the malaria parasite responsible for transmission, while deriving nutrition on the blood of an infected human.

Identifying malaria reservoirs and their role in subsequent transmission requires an understanding of the transmission capacity of gametocyte carriers; a prominent technique for this purpose is the use of mosquito feeding experiments. It has been shown that direct skin feeding assays, where mosquitoes feed directly on the skin of infected individuals, lead to higher rates of mosquito infection compared to direct membrane feeding assays (DMFAs) (Graumans et al., 2022, Collins et al., 2018). This has led to theories and hypotheses that gametocytes might hide in skin capillaries (Meibalan et al., 2021), or that the effectiveness of transmission might be impacted by technical aspects of DMFAs. Differential impacts on transmission efficiency may result from the anticoagulant selection made during DMFA blood collection (Lantero et al., 2020). Therefore, it becomes crucial to comprehend the effects of anticoagulants in order to optimize DMFAs and interpret results in light of natural malaria transmission.

The best thin blood films for diagnosing malaria are made from capillary or venous blood drawn directly from the patient, without the use of anticoagulants (Mathison & Pritt, 2017). In clinical laboratory settings, handling a multitude of samples from various sources often presents challenges in maintaining the integrity of each sample, especially in the context of malaria diagnosis (Daily et al., 2022). The stage of the parasite, how quickly the blood gets to the laboratory, and the kind of anticoagulant being used all have an impact on the results (Mathison & Pritt, 2017).

Plasmodium falciparum causes potentially fatal circumstances, particularly in pregnant women and children under five (WHO, 2020). In 2018, 99.7% of malaria cases in the WHO Africa Region were caused by *Plasmodium falciparum*, which led to the majority of the 405,000 deaths from the disease. According to estimates from the World Health Organization, 228 million cases of malaria occurred globally in 2018 (WHO, 2022).

When a female *Anopheles* mosquito feeds on blood from an infected person, consumed macrogametocytes (female gametocytes) and microgametocytes (male gametocytes) detect changes in the insect's midgut microenvironment and start sexual reproduction. After penetrating the macrogamete, the microgamete produces a zygote (Meibalan & Marti, 2017), which in 24 to 36 hours changes into an invasive and motile ookinete (Zhou et al., 2020).

It is possible to continuously cultivate *Plasmodium falciparum*'s intraerythrocytic developmental cycle (IDC) and, more recently, *Plasmodium knowlesi* *in vitro* (Moon et al., 2013). Since the conditions required for strong long-term *in vitro* replication of *Plasmodium falciparum* have not yet been established, samples of *Plasmodium vivax* and other human malaria species, such as *Plasmodium ovale* and *Plasmodium malariae*, must come directly from human infections. The IDC causes all of the symptoms of malaria, including cyclical fevers and hypoglycaemia, '*in vivo*,' or within the human body (Brown et al., 2020).

The *in vitro* culture of *Plasmodium falciparum* is a valuable method for studying the parasite's blood-stage development, which lasts about 48 hours, from invasion to the lysis of matured schizonts and the release of merozoites. The effectiveness of these culture systems relies on several factors, including the use of anticoagulants.

Both immature gametocytes and asexual phases that take place late in the intraerythrocytic developmental cycle (IDC) sequester in the bone marrow and microvasculature (Cao et al., 2019) (Neveu et al., 2018) to prevent removal by the host's spleen (Blasco et al., 2017). Consequently, a blood sample taken directly from an infected person may mostly contain mature gametocytes and early-stage asexual parasites, but an *in vitro* culture sample may contain an asynchronous combination of all stages.

Even though numerous biological advances in *Plasmodium* research have been made possible by contemporary *in vitro* culture technologies, no *in vitro* system is able to accurately mimic an *in vivo* infection. Many physical, dietary, and immunological variables that are different from those seen *in vitro* are presented to parasites *in vivo* (Brown & Guler, 2020). Heparin, one of the anticoagulants under investigation, has been shown to inhibit parasite growth and affect parasite morphology by disrupting merozoite invasion (Boyle et al., 2017). A third to half of *P. falciparum* clinical isolates' rosettes can also be partially disrupted *in vitro* by heparin, a highly-sulfated form of heparin sulphate that is mostly found in mast cells, according to certain studies (McQuaid & Rowe, 2020).

Antimalarial effectiveness against multiple stages of *Plasmodium* in humans has already been demonstrated by sulphated glycosaminoglycan (sGAG) heparin and similar compounds (Lantero, Fernandes, et al., 2020). It has been demonstrated that during host epithelial cell invasion,

Plasmodium ookinetes attach to chondroitin sulphate proteoglycans in the mosquito midgut as well as a synthetic polysulphonated polymer that mimics the structure of sGAGs found in the midgut epithelium. Additionally, ookinetes and ookinete-secreted proteins have shown a strong affinity for heparin (Marques et al., 2017).

Research may also be sparked by *in vitro* culture of *P. falciparum* mosquito stages, although the physiological and nutritional needs of each stage of the parasite have made it difficult to cultivate both human and mosquito stages. Research has been restricted to the sporogonic events that take place within the mosquito vector, despite notable advancements in the *in vitro* growth of *P. falciparum* strains that produce the exoerythrocytic stage (within liver cells) and the erythrocytic stage (within red blood cells) (Zhou et al., 2024).

In a recent study, it was seen that by competitively binding to particular AT-binding 3-O-sulfate (3-OS) containing GAGs (glycosaminoglycans) that convey d-helix-dependent protective signalling capabilities of the serpin, HRPII (histidine-rich protein II) was shown to impede the anti-inflammatory action of AT (antithrombin) (Biswas et al., 2022).

By interacting to the endothelium protein C receptor, the well-studied membrane protein *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) has been demonstrated to downregulate the protein C anticoagulant pathway. As a result, protein C's anticoagulant and anti-inflammatory effects are diminished (Aird et al., 2014). Additionally, by causing Src-dependent phosphorylation of VE-cadherin, HRPII prevents AT's barrier-protective role and causes endothelial cells' adherent junctions to break (Dinarvand et al., 2020). As hepatocyte receptors for sporozoite attachment, heparin and heparan sulphate function in the liver during the early stages of malaria infection. Heparin's antimalarial action in the blood stages, which has not been associated with any

resistances, works by preventing merozoite invasion of the erythrocyte (Lantero, Fernandes, et al., 2020).

According to a recent study, reference strains of *P. falciparum* grew similarly in fresh erythrocytes collected into the acid-citrate-dextrose (ACD) anticoagulant and those kept as packed erythrocytes at 4°C for two weeks. However, erythrocytes kept in the same settings for four weeks (growth of 35–58%) and six weeks (growth of less than 10%) showed a marked reduction in growth (Goheen et al., 2016). Different anticoagulants and storage buffers, such as ACD and CPDA (citratephosphate-dextrose modified with 0.2 g/L adenine), produced comparable outcomes (Basco, 2023).

In Standard Membrane Feeding Assays (SMFAs) using lithium heparin (LiHep), oocyst intensity was found to be significantly lower when sodium citrate (2.1-fold, $p=0.007$) and EDTA anticoagulants (261.6-fold, $p<0.001$) were used.

Sodium citrate and EDTA work as chelators of ionized calcium, a metal necessary for the coagulation cascade, while heparin, a sulphated polysaccharide, prevents thrombin from activating, slowing the synthesis of the clotting protein fibrin. These results show that heparin can affect *P. falciparum* gametocyte transmissibility, which could explain the discrepancies between DMFAs and cutaneous feeding tests (Graumans et al., 2022).

In summary, while significant research has explored the impacts of various anticoagulants on blood sample preservation and malaria transmission, there remains a gap in understanding their specific effects on *P. falciparum* growth and morphology during *in vitro* cultures. Prior studies have highlighted the inhibitory effects of heparin on merozoite invasion, but limited research has examined how anticoagulants like ACD and EDTA compare in terms of their influence on the

parasite's development, particularly in controlled laboratory settings. This study seeks to address these gaps by investigating the effects of Heparin, ACD, and EDTA on *P. falciparum* growth rates and morphological changes *in vitro*. The findings of this study will address the critical gap in knowledge regarding the interaction between anticoagulants and malaria parasites, contributing to improved diagnostic practices and potential therapeutic strategies.

1.2 Problem Statement

According to the Global Burden of Disease Study 2017, infectious diseases accounted for 550 million of the 2.5 billion disability-adjusted life years (DALYs) lost to all diseases worldwide. In 2017, lower respiratory tract infections (106 million DALYs), HIV/AIDS (54 million DALYs), diarrheal illnesses (81 million DALYs), and malaria (45 million DALYs) are the four infectious diseases that rank in the top ten worldwide causes of disease burden (Kyu et al., 2018).

Each year, more than 600,000 people die from vector-borne parasite infections, over 15% of all infectious diseases (WHO, 2022). Malaria, one of the most widespread vector-borne parasite diseases, causes over 400,000 deaths each year globally, with the majority of victims being children under the age of five (WHO, 2022).

The accurate study and diagnosis of malaria heavily rely on the observation of parasites in blood samples. However, the morphology of *Plasmodium falciparum* can be influenced by the anticoagulants used during the blood collection process, such as heparin, Acid citrate dextrose, and EDTA. These substances are critical for preventing blood coagulation and ensuring the viability of samples for laboratory analysis.

Despite their widespread use, there is a limited comprehensive understanding of how these anticoagulants affect the morphology and *in vitro* growth rate of *Plasmodium falciparum* parasites.

Alterations in parasite morphology could lead to misdiagnosis or misinterpretation of parasite load, potentially affecting patient treatment outcomes. Furthermore, understanding the impact of these anticoagulants on the growth rate of parasites could provide insights into their potential therapeutic effects or drawbacks.

1.3 Justification

Accurate diagnosis and understanding of malaria's pathophysiology are essential for effective treatment, control and ultimate elimination. In clinical and research settings, the integrity of blood samples is crucial for the precise detection of *Plasmodium falciparum*. The shape and developmental phases of the parasites may be changed by widely used anticoagulants that preserve these blood samples, such as heparin, acid citrate dextrose, and EDTA, which could result in inaccurate diagnoses. Current literature offers limited insights into how these anticoagulants affect the morphology and growth rate of the parasites. Misinterpretation of these changes could result in misdiagnosis or inappropriate treatment, exacerbating the disease's impact. Additionally, understanding these interactions might uncover pharmacological effects that could affect treatment efficacy or lead to new therapeutic strategies.

1.4 Hypotheses

1. Null Hypothesis (H_0): There is no significant difference in the mean parasitaemia levels and parasite morphology among the ACD, Heparin, and EDTA parasite blood cultures.
2. Alternative Hypothesis (H_1): At least one of the groups has a significantly different mean parasitaemia level.

1.5 Aims and Objectives

1.5.1 Aims

The main aim is to assess the effects of Heparin, EDTA and Acid citrate dextrose on *Plasmodium falciparum* parasite morphology and *in vitro* growth rates.

1.5.2 Objectives

- i. To assess the differences of *in vitro* growth rates of the *Plasmodium falciparum* parasites collected using three different anticoagulants and cultivated using microscopy.
- ii. To assess parasite and red cell morphology after cultures from all three anticoagulants using microscopy.
- iii. To establish the best anticoagulant that favours *in vitro* culturing of *Plasmodium falciparum* parasites.

1.5.3 Research Questions

1. How do the *in vitro* growth rates of *Plasmodium falciparum* parasites collected on heparin, acid citrate dextrose (ACD), and EDTA anticoagulants differ when cultivated?
2. What are the effects of heparin, ACD, and EDTA on parasite and red blood cell morphology?
3. Which anticoagulant is most suitable for the *in vitro* culturing of *P. falciparum* parasites?



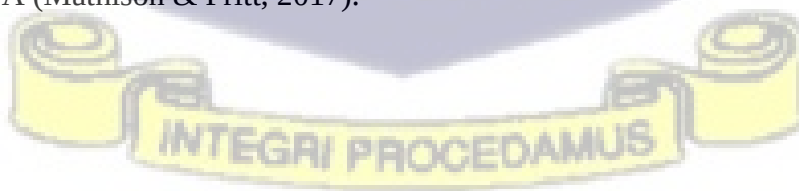
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Anti-coagulant

An anticoagulant is a blood thinner that prevents or delays coagulation of blood. There are different types of anticoagulants that act on blood in various ways to prevent blood clotting. Heparin, sodium citrate, and the sodium and potassium forms of ethylene diamine tetra-acetic acid (EDTA) are the anticoagulants most frequently used in human clinical haematology. Heparin is a naturally occurring endogenous anticoagulant that works in blood arteries both *in vivo* and *ex vivo* when blood samples are added to them. By preventing prothrombin from becoming active thrombin, heparin prevents fibrinogen from becoming fibrin. Thrombocyte aggregation and several haemostatic cascade events are suppressed by EDTA's anticoagulant action, which is based on the chelation of free Ca^{2+} ions (Witeska & Wargocka, 2011).

In human haematology, it is generally accepted that heparin should not be used for blood smears because it stains background blue or for WBC counts because it clots leukocytes, while EDTA should not be used to assess erythrocyte osmotic fragility since using too much of it could harm blood cells (Walencik & Witeska, 2007). Malaria detection using blood smears recommend the usage of EDTA tubes, when necessary, but for short periods to avoid the effects of prolonged exposure to EDTA (Mathison & Pritt, 2017).



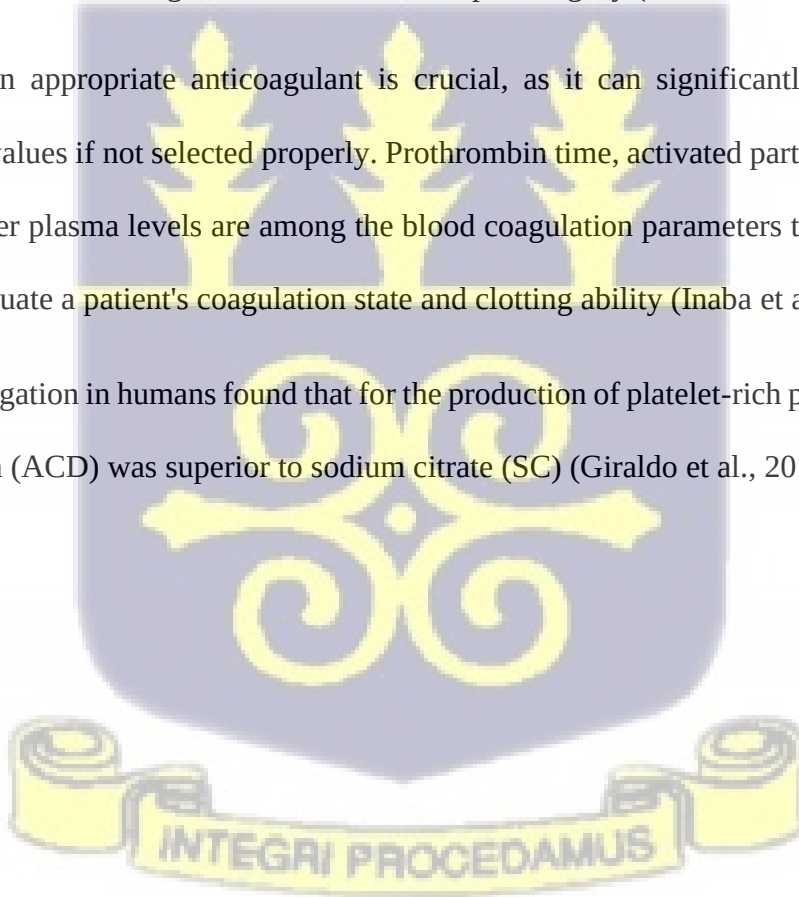
2.2 Anticoagulation

Anticoagulants are used in *in vitro* diagnostics to preserve whole blood for a range of haematological assays and to extract plasma for clinical chemistry and coagulation analysis. Clinical chemistry uses heparin, coagulation tests utilize sodium citrate, and hemocytometry uses ethylene diamine tetra acetic acid (EDTA) (Banfi et al., 2007).

While diverse anticoagulants have been employed for haematological analysis in different animal species, each anticoagulant has a unique reaction with the blood cells of various organisms (Sheikh & Ahmed, 2020). In fish blood analysis, for instance, the fast-clotting process of fish blood necessitates the use of anticoagulants to maintain sample integrity (Sheikh & Ahmed, 2020).

The choice of an appropriate anticoagulant is crucial, as it can significantly alter the actual haematological values if not selected properly. Prothrombin time, activated partial thromboplastin time, and D-dimer plasma levels are among the blood coagulation parameters that are commonly measured to evaluate a patient's coagulation state and clotting ability (Inaba et al., 2014).

However, investigation in humans found that for the production of platelet-rich plasma, acid citrate dextrose solution (ACD) was superior to sodium citrate (SC) (Giraldo et al., 2015).



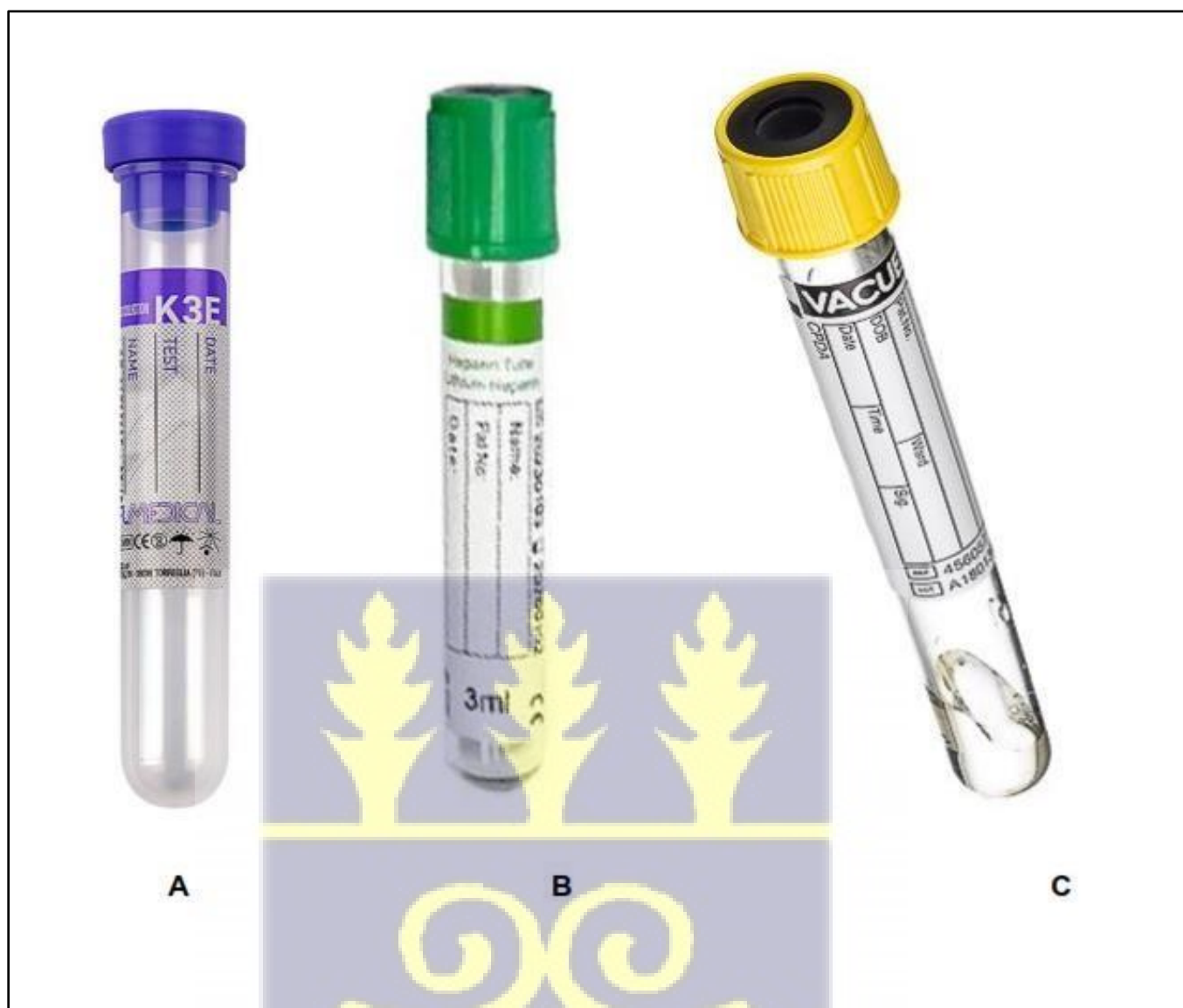


Figure 2.1: Anticoagulant sample collection tubes. This figure contains images of EDTA tube with a purple cap (A), heparin- green cap (B) and ACD- yellow cap (C).

2.3 Heparin

Heparin is a sulphated glycosaminoglycan that was first isolated from liver tissue a century ago, hence its name (Zhang et al., 2019). It can be found in the intestinal mucosa, vascular wall, lung, and other places. Heparin's unique Penta saccharide sequence gives it anticoagulant properties once it binds to antithrombin and prevents factors Xa and IIa from activating in the coagulation

cascade. The most common anticoagulant medication is heparin, which is found naturally in animals (Oduah et al., 2016). Heparin is commonly used to prevent or treat thrombosis-related illnesses such as embolic disorders and myocardial infarction, as well as to anticoagulate blood during treatments like cardiovascular surgery, cardiac catheterization, cardiopulmonary bypass, and haemodialysis. Many activity investigations have shown that synthetic low-molecular-weight heparin (LMWH) and heparin derivatives, which have been developed recently, have a variety of pharmacological effects, such as anticoagulant, anticancer, antiangiogenic, and anti-inflammatory properties (Lazrak et al., 2017; Lima et al., 2017).

Heparin is a type of glycosaminoglycan (GAG), a group of linear polysaccharides that carry a negative charge. GAGs vary based on their specific polysaccharide structures and include types such as heparan sulphate, chondroitin sulphate, hyaluronic acid, dermatan sulphate, and keratan sulphate (Lima et al., 2017). Low-molecular-weight heparin (LMWH), ultralow-molecular-weight heparin (ULMWH), and unfractionated heparin (UFH) are the three forms of heparin anticoagulants that are most frequently available. UFH, a naturally occurring heparin derivative obtained from animal tissues, has a molecular weight between 3,000 and 30,000 Da (Fang & Tang, 2020).

Research has demonstrated the antimalarial properties of sulfated polysaccharides (SPS), specifically heparin (acidic sulfur-containing GAG) and heparin-like polysaccharides. (Lantero, Aláez-Versón, et al., 2020) Heparin works by blocking cytoadhesion, thereby lowering parasitaemia. The apical surface of merozoites contains various molecules that bind to receptors on erythrocytes. Heparin targets many of these molecules on the parasite's surface that are crucial for invading red blood cells. Thus, the authors consider the chance of heparin resistance developing

to be low, as it is unlikely that numerous molecules could rapidly adapt to resist the polysaccharide (Kobayashi et al., 2013).

When heparin is present, the contact between the apical end of merozoites and the surface of erythrocytes is disrupted, preventing invasion. Heparin binds to proteins of the parasite's DBL and RBL families. In this case, the polysaccharide little affects erythrocytes, and the only factor limiting localization is the apical surface of the parasite. Leitgeb and colleagues (Leitgeb et al., 2011) found that heparin similarly inhibits the invasion of merozoites in sialic acid- *Plasmodium falciparum* dependent and sialic acid-independent ways. Moreover, heparin inhibits the distortion of erythrocyte membranes. However, because heparin produced severe bleeding in patients, it was discontinued (Dans et al., 2020).

Marques and colleagues (Marques et al., 2016) demonstrated how sulphated galactan from the red algae *Botryocladia occidentalis* has antimalarial and anticoagulant qualities. This substance has antimalarial properties comparable to heparin. It considerably slowed *Plasmodium falciparum*'s development in *in vitro* cultures. In contrast to heparin, which had an IC₅₀ of 4.1 µg/mL and an IC₉₀ of 8.0 µg/mL, galactan had an IC₅₀ of 3.5 µg/mL and an IC₉₀ of 6.8 µg/mL.

It should be mentioned that during *Plasmodium* infections, the blood maintains free merozoites for a short time. As a result, the authors speculated that the binding mechanism might also take place inside the erythrocyte. Heparin was introduced to the culture to support this claim. Confocal microscopy was used to demonstrate that heparin bound intra-erythrocyte-developing merozoites and entered live erythrocytes (Marques et al., 2016).

2.3.1 Heparin's Applications in Malaria Research

2.3.1.1 Antimalarial Drug Synergy

Heparin has been investigated for its ability to enhance the effectiveness of existing antimalarial drugs. Research has indicated that when combined with antimalarial treatments, heparin may improve drug efficacy by modulating the immune response and affecting the parasite's life cycle. (White, 2022) This synergy could lead to more effective treatment regimens, particularly for severe malaria cases that often exhibit high mortality rates.

2.3.1.2 Modulation of Inflammatory Responses

In severe malaria, the host's inflammatory response plays a critical role in disease progression. Heparin's anticoagulant properties can potentially mitigate the severe inflammatory responses associated with malaria infection, such as those leading to cerebral malaria and acute respiratory distress syndrome (Xiao et al., 2024). Studies have suggested that heparin may help in reducing the risk of complications by targeting the pro-inflammatory pathways activated during malaria infection (Tripathi et al., 2023).

2.3.1.3 Potential for Drug Delivery

Heparin's unique properties also position it as a potential carrier for targeted drug delivery in malaria treatment. By conjugating heparin with antimalarial agents, researchers aim to enhance the bioavailability and targeted action of these drugs, thereby maximizing therapeutic outcomes while minimizing side effects. This novel approach is being explored in preclinical models, indicating promising results that warrant further investigation (Rathmes et al., 2020).

2.4 Ethylenediaminetetraacetic

The ability of Ethylenediaminetetraacetic (EDTA) to best maintain the cellular components and shape of blood cells has historically made it the preferred anticoagulant for haematological tests.

(Banfi et al., 2007; Lippi & Plebani, 2012) highlighted that EDTA, through its unique properties, ensures that blood cell structures remain intact, providing accurate results in haematological analyses. The polyprotic acid EDTA can chelate or form complexes with metal ions in a 1:1 metal: EDTA ratio due to its chemical structure, which consists of two amine groups with lone pair electrons and four carboxylic acid groups. This chelation capability is central to its anticoagulant function, as EDTA binds metal ions, such as calcium (Ca^{2+}), which are essential cofactors in various enzymatic processes and the blood clotting cascade (Witeska & Wargocka, 2011). By removing free calcium ions, EDTA effectively suppresses thrombocyte aggregation and disrupts several processes within the haemostatic cascade, preventing clot formation and enabling blood analysis.

When blood is collected in tubes without additives, clotting occurs rapidly due to the presence of free calcium ions. However, in the presence of EDTA, the chelation of Ca^{2+} ions prevent clotting, making it a valuable tool in blood collection for laboratory testing. This property has made EDTA a reliable anticoagulant not only for haematology but also for genetic and enzymatic studies. For instance, EDTA-stabilized blood samples have been used for genotyping, as demonstrated in the study by (Bonetti et al., 2016; Ogretmen et al., 2014), where EDTA was used to genotype the eNOS gene in a study investigating the correlation between the eNOS Glu298Asp polymorphism and the risk of hypertension among patients with psoriasis. Such applications underscore the versatility of

EDTA in clinical research beyond its anticoagulant role. The International Council for Standardization in Haematology (ICSH) officially recommends K_2EDTA as the preferred formulation for blood analysis (Mannuß, 2020). However, other formulations such as K_3EDTA and Na_2EDTA remain in use, particularly for specific tests. For example, Na_2EDTA is used in

combination with sodium fluoride for blood glucose measurements (Bonetti et al., 2016). These different formulations reflect EDTA's adaptability to various analytical contexts while maintaining its core functionality of anticoagulation.

Despite its widespread use, EDTA has several well-documented effects on platelets, which should be considered during analysis. One such effect is the time-dependent morphological change that platelets undergo in EDTA-anticoagulated blood. Platelets lose their normal discoid shape and swell over time, a process that results in an increase in the mean platelet volume (MPV).

This phenomenon, which is most pronounced in K_3EDTA formulations, has been a topic of concern, as the swelling of platelets can artificially elevate MPV values, potentially leading to misinterpretations in clinical assessments (Banfi et al., 2007). Studies have found K_3EDTA to cause more significant platelet swelling over time compared to Na_2EDTA , suggesting that different EDTA formulations may have varying effects on platelet morphology and, consequently, on the reliability of MPV measurements (Mannuß, 2020). The implications of this swelling are critical, particularly when using MPV as a diagnostic marker, as it is a key parameter in evaluating conditions such as thrombocytopenia and other platelet-related disorders (Lancé et al., 2012)

2.4.1 Effects of EDTA on Platelets

Ethylenediaminetetraacetic acid is widely used as an anticoagulant in blood sample analysis due to its ability to chelate calcium, which is essential for platelet aggregation and blood clotting. Platelets (PLTs) are not entirely stabilized by EDTA, and with time, some morphological changes may transpire. When platelets come into contact with EDTA, they change from their typical discoidal shape to a spherical one in a time-dependent manner. This morphological alteration can affect platelet function and has implications for the accuracy of platelet counts and other platelet related parameters (Karpatkin & Freedman, 1978).

Modern haemocytometers are capable of measuring the mean platelet volume (MPV), a parameter that reflects the average size of platelets in the blood. However, the clinical use of MPV has been somewhat limited due to various preanalytical issues, including those caused by the interaction between EDTA and platelets. Despite these limitations, MPV remains a valuable clinical marker in certain conditions. For instance, MPV tends to be normal in cases of autoimmune thrombocytopenia but is elevated in conditions such as disseminated intravascular coagulation (DIC), microangiopathies, and other pathologies that impair platelet maturation and release (Karparkin & Freedman, 1978).

One of the notable effects of EDTA on platelets is its inhibition of both platelet aggregation and the release of platelet extracellular vesicles (EVs) (Buntsma et al., 2022). Clinical research is becoming more interested in extracellular vesicles, which are tiny membrane-bound particles that are essential for intercellular communication. (Ramirez et al., 2018) demonstrated that EDTA stabilizes the concentration of EVs in blood samples for up to 6 hours when stored at room temperature, making it feasible to study EVs within this time frame. However, beyond this time limit, EDTA's stabilizing effects may diminish.

Studies have shown that while platelet and erythrocyte EV concentrations remain stable in EDTA anticoagulated blood for up to 48 hours, the detected EVs are predominantly larger in size. This suggests that the concentration of smaller EVs decreases over time, with only the larger EVs being detected after prolonged storage. (Buntsma et al., 2022) observed that the EV concentrations in their study were an order of magnitude lower than those previously reported, indicating that smaller vesicles might degrade or become undetectable with extended storage times. This highlights the importance of processing EDTA-anticoagulated samples in a timely manner for accurate EV analysis (Buntsma et al., 2022; Wisgrill et al., 2016).

Excess EDTA can affect other blood cells, including red blood cells and leukocytes, in addition to platelets. These cells may atrophy and develop degenerative alterations if the EDTA concentration in the blood above 2 mg/ml. When measured by centrifugation, this may cause a substantial drop in the packed cell volume (PCV) of red blood cells and a rise in the mean cell hemoglobin concentration (MCHC). Because the pieces are big enough to be counted as platelets by automatic cell counters, the platelets may enlarge and finally disintegrate when exposed to too much EDTA, creating an erroneously high platelet count. (McNamara, 2017).

EDTA has also been associated with the activity of naturally occurring antiplatelet autoantibodies, which can cause platelet aggregation *in vitro*. This aggregation can sometimes be observed as platelet adherence to neutrophils in blood films. Such phenomena may complicate the interpretation of blood smears and lead to diagnostic inaccuracies, particularly when assessing platelet function or counts in EDTA-anticoagulated samples (McNamara, 2017).

2.4.2 Calcium Ion Dependency

The serum proteins uptake ability of *P. falciparum* is notably affected by the availability of calcium ions. The addition of calcium chloride (CaCl_2) to naturally coagulated serum (NCS) containing EDTA can enhance parasite growth, indicating that a sufficient concentration of calcium is necessary for optimal uptake of serum proteins by the parasite (Van Schalkwyk et al., 2013). Conversely, removing calcium from plasma-derived serum (PDS) treated with EDTA decreases the serum proteins uptake ability, emphasizing the importance of calcium in the lifecycle of the malaria parasite (Van Schalkwyk et al., 2013).

2.4.3 EDTA and Malaria Parasite Viability

The use of EDTA as an anticoagulant has been shown to influence the viability of malaria parasites during sample processing. Studies indicate that EDTA can inhibit the growth of *Plasmodium* spp.

in vitro, affecting the interpretation of findings related to parasite load and drug efficacy (WhartonSmith et al., 2021). Consequently, researchers have had to carefully calibrate EDTA concentrations to ensure that while blood samples remain fluid, the viability of the parasites is preserved for accurate analyses.

2.4.4 Improvement of Sample Collection Techniques Using EDTA

The introduction of EDTA in malaria research has also led to improvements in sample collection techniques. EDTA anticoagulated blood can be stored for extended periods without significant degradation, allowing for longitudinal studies and retrospective analyses of stored samples (Zahraini et al., 2021).

2.5 Acid Citrate Dextrose

Acid citrate dextrose (ACD) is a widely utilized anticoagulant solution composed of citric acid, sodium citrate, and dextrose, primarily employed in blood collection and preservation (Schneider et al., 2017). It functions by chelating calcium ions, which are essential for the blood coagulation process, thereby preventing clot formation and maintaining the integrity of blood samples (Dao et al., 2025).

ACD's significance extends beyond routine blood collection, as it plays a critical role in malaria research by facilitating the analysis of blood samples from infected individuals without the complications of coagulation (Bowen & Remaley, 2014).

In malaria studies, ACD enables researchers to collect, process, and analyse samples effectively, allowing for the investigation of parasite biology and host responses, which are vital for understanding malaria infections and evaluating new therapeutic approaches (Bowen & Remaley, 2014) Its formulation has paved the way for more sophisticated blood preservation solutions,

solidifying its status in both clinical and research contexts. However, the use of ACD is not without challenges; potential limitations, such as the risk of mild coagulation if not administered correctly and its varying effects on different assays, necessitate careful consideration in research design and clinical applications (Aninagyei et al., 2018).

Overall, ACD stands out as a vital tool in both haematology and tropical medicine, facilitating advancements in understanding malaria pathology while ensuring high-quality blood samples for a variety of diagnostic and therapeutic purposes (Mohanty et al., 2021).

2.5.1 Role in Malaria Research

In as much as more information on the use of ACD is required in malaria research, Scientists have demonstrated the importance of the use of ACD as an anticoagulant in collecting, processing, and analysing samples effectively, allowing for the investigation of parasite biology and host responses, which are vital for understanding malaria infections and evaluating new therapeutic approaches (Bowen & Remaley, 2014).

2.6 Anticoagulant in Molecular Biology, Virology, and Infectious Diseases Research

The preferred anticoagulants in molecular biology are EDTA and ACD. Since heparin binds to DNA denaturation products and prevents DNA amplification using polymerase chain reaction (PCR) technology, its usage is not advised (Narayanan, 1997). Restriction enzymes may also be inhibited by EDTA and ACD, albeit these substances are usually eliminated during the precipitation stages of this process. For HIV-1 RNA quantitation by PCR, EDTA is advised. Although plasma must be separated within six hours of blood collection, ACD may also be utilized (Coad, 1992; Narayanan, 1997).

Acid–citrate–dextrose (ACD) or citrate–phosphate–dextrose (CPD), which is made by combining citrate and dextrose, enhances the long-term preservation of red blood cells for particular testing and transfusion requirements (McNamara, 2017)

2.6.1 Anticoagulants in Medicine

EDTA is given as calcium disodium edetate in pharmacology, which stops it from attaching to calcium in the body. Despite having a substantially higher affinity for calcium, edetate disodium is no longer utilized in chelation therapy because of the increased risk of hypocalcaemia. Outside of medicine, EDTA is widely employed across various industries to eliminate toxic metal ions. It is commonly found in cosmetics, food production, and has numerous applications in scientific laboratories (George & Brady, 2024; Wax, 2013).

For the prevention and treatment of thrombotic disorders such atrial fibrillation (AF), pulmonary embolism (PE), and deep vein thrombosis (DVT), doctors use unfractionated heparin, an anticoagulant (Warnock & Huang, 2024).

Additionally, heparin is used to stop excessive bleeding during dialysis, including continuous renal replacement therapy (CRRT), heart surgery, and extracorporeal circulation (Mulloy et al., 2016)

Heparin is commonly used in hospitals for various off-label purposes, such as in patients with acute coronary syndromes (ACS) and those undergoing percutaneous coronary intervention (PCI).(Warnock & Huang, 2024) Warfarin for mechanical and bioprosthetic valves and heparin to transition to oral anticoagulation will be administered while the patient is in the hospital. Heparin is advised by the American College of Chest Physicians (ACCP) for numerous more thrombotic conditions: Cardioversion, endocarditis, systemic emboli, or venous thrombosis in atrial fibrillation (Hemker, 2016)

2.7 Malaria Burden

Malaria continues to rank among the most important global public health concerns. Malaria is spread in 85 countries in five WHO regions. According to the World Malaria Report 2020, there were 241 million cases of malaria worldwide in 2020 (uncertainty range: 218-269 million) and 627,000 fatalities from malaria worldwide (uncertainty range: 583-765 thousand). The WHO African Region has the highest malaria burden, with an estimated 95% of cases and 96% of fatalities; 80% of all deaths in this region occur in children under the age of five. (WHO, 2022). Malaria particularly causes 10% of all deaths in Ghana and more than 25% of deaths in children under five (Heuschen et al., 2022).

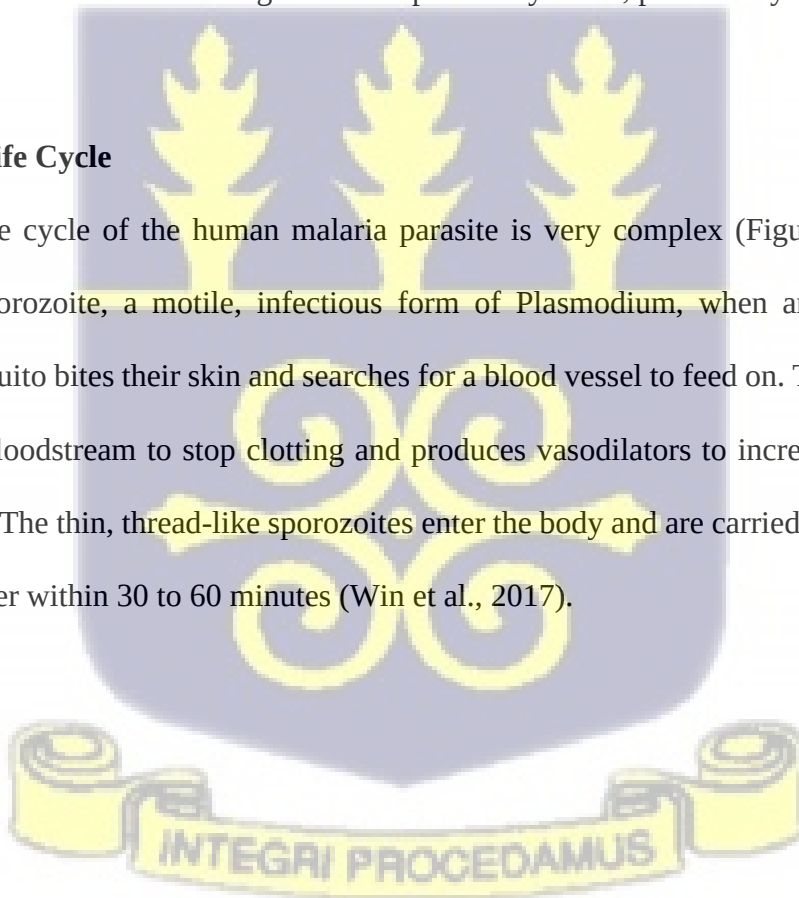
Malaria remains one of the leading causes of morbidity and mortality in sub-Saharan Africa. In 2020, there were 12% more malaria deaths in sub-Saharan Africa than in 2019. Disruptions in the delivery of malaria prevention, diagnosis, and treatment during the pandemic were responsible for around two-thirds of these extra deaths (47,000). Malaria accounted for about one-third of all OPD visits in 2020 (WHO, 2022). With a nationwide frequency of 14% in 2019, malaria is regarded as endemic in all regions of Ghana (Aheto et al., 2024) and accounts for 10% of all deaths (Heuschen et al., 2023). Community-based Health Planning and Services (CHPS), the Ghana Strategic Plan for Malaria Control, the Child Health Policy 2007–2015, and National Health Insurance were all adopted in Ghana to lower the prevalence of malaria from 2014–2020. These measures were necessary due to the high disease burden of malaria in Ghana (Aheto, 2019; Awine et al., 2017). Malaria cases decreased over time as a result of this approach until COVID-19 struck in 2020. The global coronavirus disease 2019 (COVID-19) epidemic was declared a Public Health Emergency of International Concern at the end of January 2020 (WHO, 2022). Even before the first cases were identified in their countries, many African governments immediately responded to

this threat by implementing control measures, such as border closures, movement restrictions, social distance, and school closures (WHO, 2022). It was predicted that the negative effects of the COVID-19 pandemic will reverse the progress made in the last 20 years in managing malaria in the highly endemic African countries (Sherrard-Smith et al., 2020). The COVID-19 pandemic has, in fact, had a detrimental effect on the accessibility and uptake of malaria prevention and treatment services, especially the community-based Mass Test Treat and Track (MTTT) interventions.

In certain regions, this has led to a rise in the number of patients reporting complicated malaria in medical facilities (Cheng et al., 2022). One of the most significant global public health issues is malaria, which continues to have a significant impact everywhere, particularly in Ghana and Sub-Saharan Africa.

2.7.1 Malaria Life Cycle

The intricate life cycle of the human malaria parasite is very complex (Figure 2.2). A person contracts the sporozoite, a motile, infectious form of Plasmodium, when an infected female Anopheles mosquito bites their skin and searches for a blood vessel to feed on. The insect secretes saliva into the bloodstream to stop clotting and produces vasodilators to increase its chances of finding a vessel. The thin, thread-like sporozoites enter the body and are carried by the circulatory system to the liver within 30 to 60 minutes (Win et al., 2017).



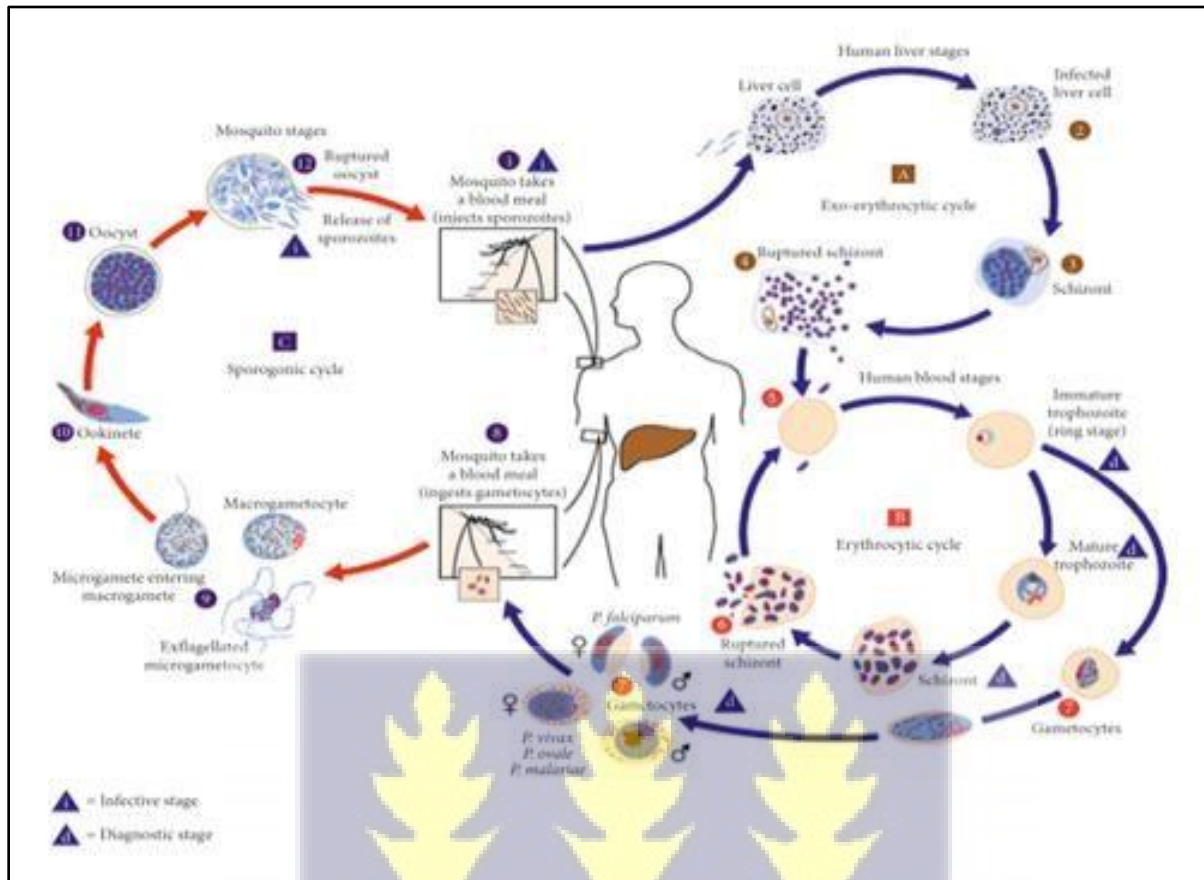


Figure 2.2: Life cycle of malaria parasite. This figure illustrates the lifecycle of the malaria parasite including the stages in the human liver, blood and in the mosquito (Nureye & Assefa, 2020a)



	vivax	ovale	malariae	falciparum
Ring Stage				
Trophozoite				
Schizont				
Segmenter				
Gametocytes				

Figure 2.3: Shapes of *Plasmodium* Species Infecting Humans under normal conditions,
(Extracted from Sutherland et. al. 2010)

The sporozoites evolve into schizonts over a 7–12-day period, and they can produce up to 30,000 merozoites that rupture the hepatocytes (Gazzinelli et al., 2014; WHO, 2013). However, some vivax and ovale sporozoites develop into hypnozoites, which can cause relapses in infected

individuals and remain dormant in the liver for months or years. (Walker et al., 2010). It's interesting to note that some years after leaving an endemic location, patients were reported to have recurred falciparum malaria. The *falciparum* exhibits a latent period at least infrequently. (Nureye & Assefa, 2020b). The merozoites then invade RBC to develop by ingesting hemoglobin, starting the asexual cycle (Figure 2.2). The parasite progresses from the early ring stage to the late trophozoite and then, after mitotic divisions, to the schizont stage, which consists of 6 to 32 merozoites, depending on the species. (Nureye & Assefa, 2020b).

The liberated merozoites invade other RBCs to continue the life cycle after the erythrocytic schizont ruptures. Cyclic fevers typically happen right before or during RBC lysis, when schizonts rupture to produce new infectious merozoites. This occurs every 48 hours in situations of tertian malaria and every 72 hours in cases of quartan malaria. Certain merozoites go through this recurrent cycle by developing into erythrocytic gametocytes, which are male and female sexual forms with a common nucleus, and then waiting for a female *Anopheles* mosquito to come along that is looking for blood. (CDC, 2010).

Gametogenesis is then triggered when the mosquito consumes gametocytes. The macrogametes are penetrated or fertilized by the flagellated microgametes produced by exflagellation, producing zygotes. The zygotes develop into spherical oocysts after changing into ookinetes. The oocyst grows and produces a large number of sporozoites as a result of the frequent divisions of its nucleus (WHO, 2013). When the sporozoites are fully matured, the oocyst bursts, releasing them into the hemocoel, the mosquito's internal cavity. When the sporozoites reach the salivary glands, the life cycle is finished (Figure 2.1). When sporozoites from the mosquito's salivary glands infect a new human host, the malaria life cycle is maintained (CDC, 2010; WHO, 2013).

2.8 Theoretical Framework

This study on the effects of Heparin, Acid Citrate Dextrose (ACD), and Ethylenediaminetetraacetic Acid (EDTA) on *P. falciparum* parasite morphology and *in vitro* growth rate is grounded in two primary theoretical perspectives: the Host Cell Modification Theory and the Calcium Dependency Theory. These theories provide essential insights into how different anticoagulants may influence the ability of *P. falciparum* to invade, develop, and proliferate within red blood cells (RBCs). By understanding these mechanisms, researchers can better interpret variations in parasite growth and morphologies across different anticoagulant treatments.

2.8.1 Host Cell Modification Theory

The Host Cell Modification Theory suggests that anticoagulants alter the physical and biochemical properties of RBCs, which may in turn affect *P. falciparum* infection and growth. When a parasite invades an RBC, it relies on specific surface receptors, membrane fluidity, and intracellular conditions to establish a favourable environment for its development. Anticoagulants such as Heparin, ACD, and EDTA can interfere with these factors by modifying the RBC membrane, altering receptor availability, or affecting intracellular signalling pathways.

For example, Heparin is known to interact with cell surface proteins and can prevent the formation of essential receptor-ligand interactions needed for *P. falciparum* invasion. ACD, which contains citric acid, lowers blood pH and alters RBC metabolism, potentially creating unfavourable conditions for parasite survival. EDTA, as a chelating agent, binds to divalent cations, which may disrupt critical cellular interactions. If an anticoagulant makes the RBC environment more conducive to parasite survival, parasitaemia levels in that group will be higher. Conversely, if it disrupts key RBC properties, parasite growth will be hindered.

Understanding how anticoagulants modify RBCs is crucial for explaining variations in *P. falciparum* growth across different blood samples. If the study reveals a significant difference in parasite growth among anticoagulant groups, it could indicate that certain anticoagulants enhance or suppress parasite development by modifying host cell properties. This aligns with the alternative hypothesis, which suggests that at least one anticoagulant group will show significantly different parasitaemia levels compared to the others. If no significant differences are observed, it would support the null hypothesis, indicating that the anticoagulants do not meaningfully alter the parasite's ability to thrive within RBCs.

2.8.2 Calcium Dependency Theory

The Calcium Dependency Theory explains how calcium chelation by anticoagulants disrupts intracellular processes that are essential for *P. falciparum* development. Calcium is a critical element in the malaria parasite's life cycle, playing a central role in signalling pathways, merozoite invasion, egress, and metabolic regulation. When calcium availability is altered, parasite survival and replication may be compromised.

ACD and EDTA are strong calcium chelators, meaning they bind to free calcium ions in the blood, reducing their availability. This depletion can have profound effects on the parasite's ability to regulate intracellular signalling, leading to impaired invasion, delayed development, or even cell death. Studies have shown that calcium-dependent proteases and kinases are necessary for *P. falciparum* schizont maturation and merozoite egress. If these processes are disrupted, parasites in EDTA- and ACD-treated samples may exhibit slower growth rates compared to Heparin-treated samples, where calcium levels are relatively preserved.

This theory directly aligns with the study's hypotheses. If anticoagulants that deplete calcium lead to lower parasitaemia levels, it would support the alternative hypothesis, indicating that

anticoagulants influence parasite growth. If no significant difference is found, the null hypothesis would be validated, suggesting that calcium depletion does not substantially impact *P. falciparum* proliferation under *in vitro* conditions. By investigating these effects, the study contributes to a deeper understanding of how anticoagulant selection may impact experimental outcomes in malaria research.

2.8.3 Implication of the Study

These theoretical perspectives provide a strong foundation for interpreting variations in *P. falciparum* growth rates under different anticoagulant conditions. If anticoagulants significantly alter RBC properties or calcium availability, they could influence parasite viability and replication, leading to differences in parasitaemia levels. The observation of these variations will allow help to determine whether anticoagulant-induced changes in host cell properties impact parasite survival, thereby addressing the study's central hypotheses.

This study has practical implications for malaria research and diagnostics. Anticoagulants are commonly used in blood sample collection and malaria culture studies, yet their effects on parasite viability are often overlooked. If certain anticoagulants enhance or suppress parasite growth, researchers may need to adjust their protocols to minimize bias in experimental results. For instance, if an anticoagulant leads to artificially high parasite counts, it could impact drug sensitivity assays or treatment efficacy studies. Similarly, if an anticoagulant suppresses parasite growth, it may lead to underestimations of parasitaemia levels in clinical or laboratory settings.

By applying the Host Cell Modification and Calcium Dependency theories, this study will contribute to our understanding of how anticoagulants influence *P. falciparum* biology. The findings could guide the best practices for malaria research, ensuring that anticoagulant selection is optimized to produce accurate and reproducible results.

2.9 Conceptual Framework

The conceptual framework visually represents the relationship between the selected anticoagulants—Heparin, Acid Citrate Dextrose (ACD), and Ethylenediaminetetraacetic Acid (EDTA)—and their effects on *Plasmodium falciparum* morphology and *in vitro* growth rate. This framework is designed to provide a structured approach to understanding how anticoagulants influence parasite development by altering the red blood cells (RBCs) that serve as host cells for the parasite.

At the core of the framework are the independent variables: Heparin, ACD, and EDTA (Figure 2.11). These anticoagulants each have distinct mechanisms of action that affect RBCs differently, thereby influencing the parasite's survival and growth within the host cells. Heparin, for instance, prevents blood clotting by inhibiting thrombin and other clotting factors, but it may also interfere with parasite invasion and development. ACD, which chelates calcium, prevents clotting primarily by disrupting calcium-dependent coagulation pathways, but in doing so, it may also affect intracellular processes crucial for parasite survival. EDTA, a strong metal ion chelator, acts by binding to calcium and magnesium, thereby destabilizing various enzymatic activities within RBCs, which could, in turn, impact parasite growth.

These interactions between anticoagulants and RBCs result in observable changes in two key dependent variables: parasite morphology and *in vitro* growth rate. Changes in parasite morphology include variations in size, shape, and developmental stages (ring, trophozoite, and schizont stages). The *in vitro* growth rate is measured through parasitaemia levels over time, providing insight into how well the parasite can proliferate under different anticoagulant conditions.

This conceptual framework serves as a guide for systematically analysing how these anticoagulants influence *P. falciparum* in a controlled experimental setting. By establishing a structured model, it becomes possible to interpret how anticoagulant-induced changes in RBC properties translate to differences in parasite morphology and growth dynamics.

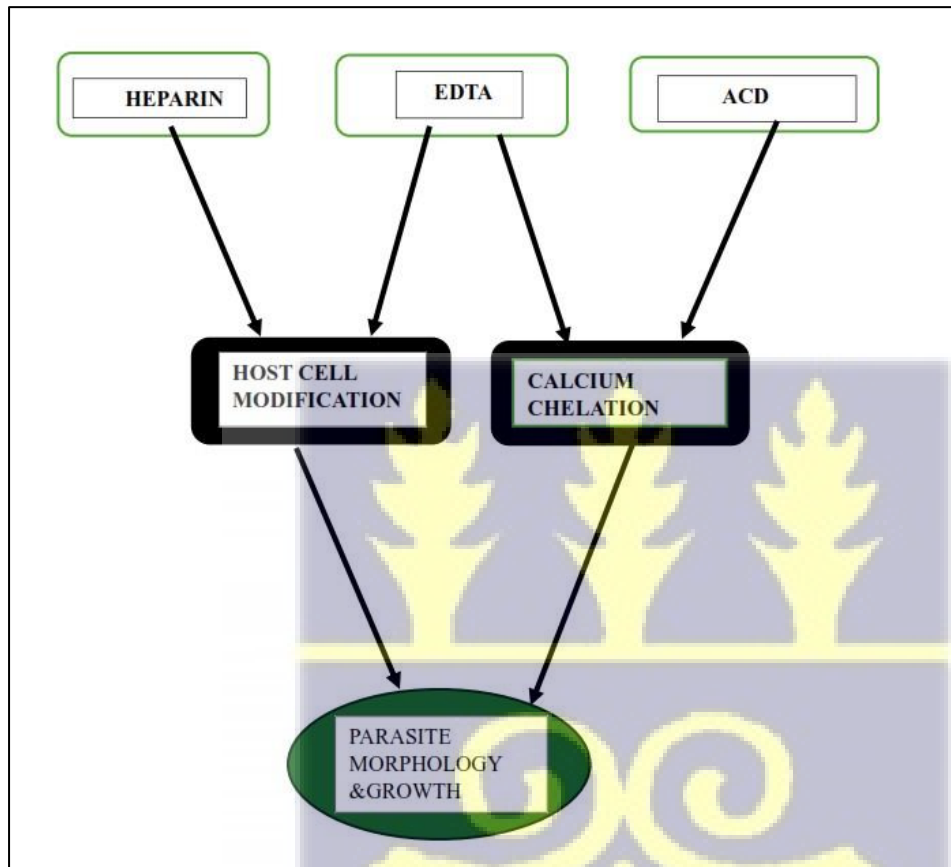


Figure 2.4: Conceptual framework of this study

CHAPTER THREE

3.0 METHODOLOGY

Introduction

In this chapter, the research methodology used for the study is presented in detail. It outlines the research design, data collection methods, sampling techniques and analysis procedures. This chapter provides a rationale for the chosen methods, ensuring they align with the research objectives and questions. This chapter also discusses the ethical considerations and limitations of the methodology, ensuring transparency in the research process. By the end of this chapter there will be a clear understanding of how the research was carried out and the steps taken to ensure accuracy and reliability in the findings.

3.1 Study Design

An experimental research design was employed to investigate how different anticoagulants (heparin, Acid Citrate Dextrose, and EDTA) affect the morphology and growth rates of *Plasmodium falciparum*. In this approach, the effects of the different anticoagulated blood on the parasites are observed and reported. Venous blood samples were taken from five participants (three males and two females all of the same ages, that is 28 year).

3.2 Study Site

The study was carried out at Noguchi Memorial Institute for Medical Research in the Immunology department's parasite culture laboratory.



3.3 Inclusion/ Exclusion Criteria

3.3.1 Inclusion Criteria

Adults with only Blood Group O Rh D positive, sickling negative individuals, individuals with no chronic health conditions, and individuals who are not on any antibiotics and antimalaria two weeks before and during period of sampling were included.

3.3.2 Exclusion Criteria

Adults with blood groups other than Blood Group O Rh D positive (O⁺), sickling positive individuals, individuals with chronic health conditions, and individuals who are on antibiotics and antimalarial two weeks before and during period of sampling.

3.4 Ethical Considerations

The University of Ghana's School of Biomedical and Allied Health Sciences Ethics and Protocol Review Committee approved the conduct of this study. Ethical approval was also obtained from the Noguchi Memorial Institute for Medical Research. Participants were briefed on the research aims during consent-seeking. They signed informed consent after reviewing project details sheet, receiving a copy afterward. Their rights, including withdrawal, were respected throughout.

3.5 Blood Sampling Method

For blood sample collection, informed consent was obtained from volunteers, and after signing the consent forms questionnaires were administered and completed. The questionnaire was used to collect information on age, sex, and whether the individual was on any antimalarial or antibiotic drug. After the participant was deemed to qualify, blood sample was tested to confirm whether their blood groups were O⁺ (O positive) blood type and were also sickling-negative. Venous blood samples were collected into three different anti-coagulant tubes (ACD, Heparin, and EDTA) from

each of the five participants (three males and two females). These samples were stored in a refrigerator overnight per protocol.

The anticoagulated blood was washed in 15 ml tubes with incomplete parasite medium to prepare for culturing. The washing process involved placing the blood in a 15 ml tube, adding the incomplete medium, mixing it gently, and centrifuging at 2000 rpm for 5 minutes. The supernatant was removed, and this washing process was repeated three times to remove white blood cells, platelets, antibodies, and plasma. Only the red blood cells were retained and were stored and used for the culturing of the parasites.

3.6 Research Materials and Equipment

Small and large culture flasks (Cellstar, USA), Serological pipettes (Sarstedt, Germany), Ninewell Culture plates (Sarstedt, Germany), Citrate phosphate buffer tubes (BD Vacutainer, Belgium) EDTA tubes (BD Vacutainer, Belgium), Heparin tubes (BD Vacutainer, Belgium), Butterfly needles (Greiner bio-one, Germany), 21G needles for gassing (Bioline), 0.2µm filters, Ethanol (BDH, England, Special Gas Mixture (5% Carbon Dioxide, 92.5% Nitrogen, 2.5 % Oxygen) (Air Liquide, England), Giemsa stain (Sigma, USA), Methanol (BDH, England) Microscope slides (Bioline), Immersion oil (SIGMA, Germany).

3.7 Workflow

The workflow was divided into four phases, which is planning that involved ensuring the availability of all required reagents and consumables through ordering as the first phase. The second phase involved culturing of Laboratory strain parasites for the experiment. This phase, the 3D7 *P. falciparum* Laboratory strain was removed from cryo-preservation and put into *in vitro* cultures in O⁺ blood group type and after obtaining enough parasites, they were re-cryopreserved

at the same concentrations and volumes and were ready as the test parasites. In brief, the 3D7 Laboratory Strain was to set up in three 50mL Culture flasks and cultured until an estimated 5% parasitaemia. The parasite cultures were then sub-cultured by transferring into 6 large flasks (75mL culture flasks) that resulted in the production more parasites which were then harvested and 100 μ L aliquots were placed into cryo-vials and treated with glycerolyte, and frozen at -80°C as the third phase. The final phase was the experimental set up that was conducted using a six-well plate, with each plate representing one participant. Each participant's blood was performed in duplicates as shown (Figure 3.1). These experiments were repeated twice.

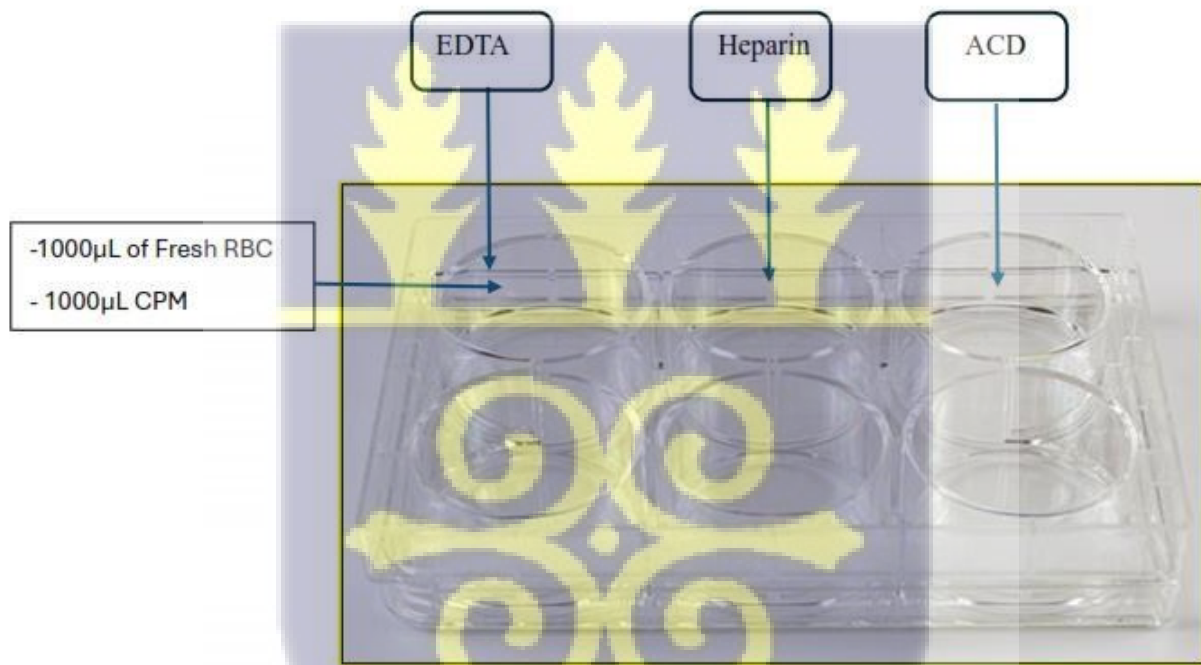


Figure 3.1: Experimental Set -up with plate layout



Figure 3.2: Image of experimental set-up

a. Each set up as shown above was done for each participant, where in each well was placed 1000uL of the respective packed RBC collected on each of the anticoagulants and 1000uL of the complete parasite medium, gassed using the special gas mixture and placed in the Incubator set at 37°C. These cultures were maintained and parasitaemia recorded daily. The morphology of infected and uninfected RBCs was recorded and photographed. These cultures were maintained until they crashed.

3.8 Monitoring of Parasites rate of multiplication, and Morphology of Parasites and RBCs

Microscopy was employed to monitor parasite stages, parasite multiplication rates, and parasite morphology, along with observing the morphology of red blood cells. Parasite growth rates and morphology were assessed in blood cells exposed to three different anticoagulants. Daily

monitoring of the cultures was done and this included media changes and slide preparations to observe parasite growth and red blood cell (RBC) changes. The culture flasks and wells of the plates were maintained in a 37°C incubator throughout the experiment. The newly established cultures were maintained for three cycles (approximately two weeks), as *Plasmodium falciparum* completes one cycle from the ring stage to schizont within three days

3.9 Data Analysis

Parasitaemia percentage progression across the 14-day study period in ACD, Heparin and EDTA plates was calculated at each study time point using the formula: (number of parasitised RBCs/total number of RBCs) *100%. The means were then plotted as a line graph. The difference in the level of parasite growth among the various plates in the respective anticoagulants was assessed using the one-way ANOVA and presented as a table. Statistical analysis was carried out using Microsoft Excel and parametric tests were used after normality test revealed a normal data distribution.

3.10 Data management plan

Each participant was assigned a unique identification number for this study and this information including age, blood group type and sex was stored in an excel sheet while ensuring confidentiality of participants. Parasite growth changes were also stored as a Comma Separated Values (CSV) file. Microscopic images of the parasite morphology were taken using the Olympus Teaching Microscope and uploaded unto google drive.



CHAPTER FOUR

4.0 RESULTS

4.1 Parasite growth measured as parasitaemia levels of cultures to estimate the best anticoagulant for parasite cultures

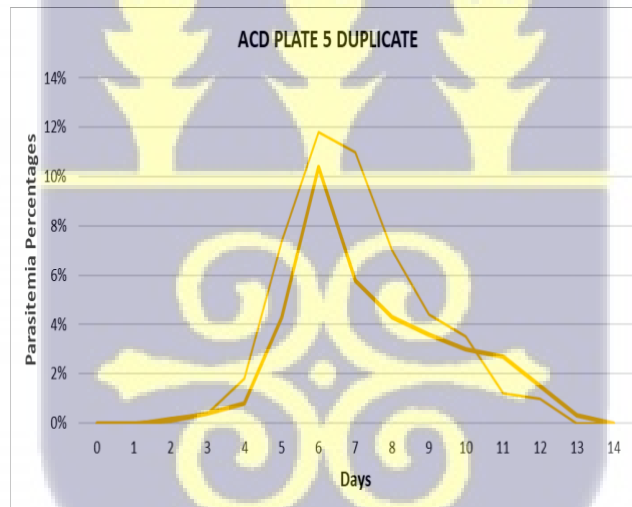
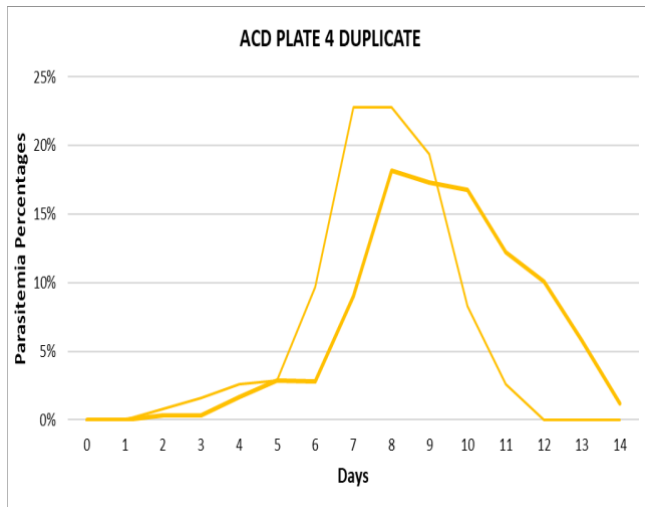
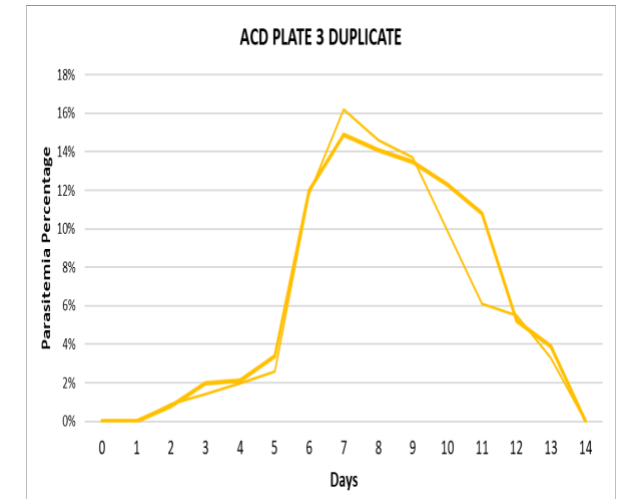
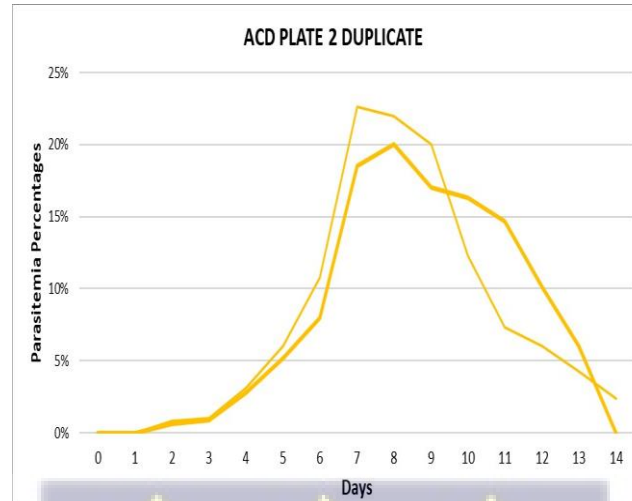
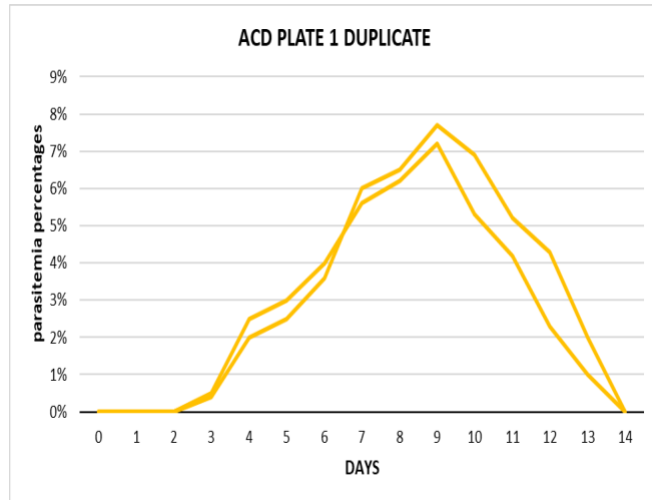
To evaluate the dynamics of parasite proliferation *in vitro*, parasitaemia levels were monitored daily by microscopy for 14 days using RBCs collected on ACD, Heparin and EDTA anticoagulants from five blood donors (Plate 1 to Plate 5), each set up was performed in duplicates. The temporal patterns of parasitaemia progression revealed consistent trends of initial growth followed by decline, with varying peak intensities across plates.

Table 4.1: Parasitaemia levels recorded each day during culturing. This table consists of data from Heparin, EDTA and ACD tubes run in duplicates

PATICIPANTS PLATE ID	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14
1 ACD A	0.00	0.00	0.40	2.40	2.90	3.50	5.50	6.00	6.30	7.90	7.00	4.50	3.00	0.00
1 ACD B	0.00	0.00	0.40	2.00	2.50	3.70	5.00	5.80	6.10	7.10	6.00	3.00	2.00	0.00
1 HEPARIN A	0.00	0.00	0.50	1.00	2.00	3.00	3.70	4.00	4.20	5.00	5.40	5.20	1.00	0.00
1 HEPARIN B	0.00	0.00	0.40	1.00	2.00	2.90	3.90	4.20	4.50	4.70	5.00	1.00	0.20	0.00
1 EDTA A	0.00	0.00	1.00	2.00	2.20	2.70	3.20	3.70	4.20	5.20	4.00	1.00	0.70	0.00
1 EDTA B	0.00	0.00	1.00	2.50	2.60	3.10	3.20	3.50	4.50	4.70	3.20	2.90	1.00	0.00
2 ACD A	0.00	0.00	0.40	0.56	2.50	5.40	8.00	17.00	22.00	21.00	17.00	12.00	7.00	0.50
2 ACD B	0.00	0.00	0.40	0.60	2.30	5.20	7.00	15.00	20.00	19.00	14.00	7.00	5.50	0.00
2 HEPARIN A	0.00	0.70	2.70	3.00	5.00	8.00	7.90	7.50	6.00	5.00	3.00	0.50	0.00	0.00
2 HEPARIN B	0.00	0.50	0.90	2.90	4.00	6.00	7.20	6.00	5.70	4.50	1.00	0.00	0.00	0.00
2 EDTA A	0.00	1.90	1.50	2.90	4.00	7.50	6.00	5.00	4.00	2.50	0.00	0.00	0.00	0.00
2 EDTA B	0.00	1.90	1.50	2.00	4.00	5.00	6.50	5.60	5.00	2.50	0.00	0.00	0.00	0.00
3 ACD A	0.00	0.50	1.50	2.20	6.00	12.00	16.00	14.00	13.00	12.00	6.00	5.00	3.50	0.00
3 ACD B	0.00	0.50	2.00	2.53	6.00	12.00	15.00	13.00	13.00	13.00	11.00	5.00	4.00	0.00

3 HEPARIN A	0.00	0.00	2.00	2.20	6.00	6.50	8.50	8.00	7.90	6.00	4.00	2.00	0.00	0.00
3 HEPARIN B	0.00	0.00	1.90	2.00	2.50	6.00	7.90	11.00	10.50	8.00	6.00	3.50	0.50	0.00
3 EDTA A	0.00	0.20	0.20	1.00	1.50	4.00	3.50	1.00	0.70	0.00	0.00	0.00	0.00	0.00
3 EDTA B	0.00	0.20	1.00	1.00	1.80	3.00	3.00	0.90	0.20	0.00	0.00	0.00	0.00	0.00
4 ACD A	0.00	0.30	0.50	1.00	1.00	5.50	10.00	17.00	22.00	20.00	14.00	0.00	0.00	0.00
4 ACD B	0.00	0.00	0.20	0.50	1.00	4.00	9.00	14.00	17.00	17.00	17.00	14.00	5.00	0.00
4 HEPARIN A	0.00	2.00	2.90	2.90	6.00	10.00	17.00	16.00	13.00	2.00	0.00	0.00	0.00	0.00
4 HEPARIN B	0.00	2.00	2.00	2.00	2.00	6.00	10.00	14.00	13.00	6.00	2.50	0.00	0.00	0.00
4 EDTA A	0.00	0.90	0.90	1.00	2.00	9.00	5.50	4.00	1.70	1.00	0.80	0.50	0.00	0.00
4 EDTA B	0.00	0.20	0.90	1.00	2.00	7.50	5.50	4.00	1.00	0.20	0.00	0.00	0.00	0.00
5 ACD A	0.00	0.20	0.50	1.70	4.00	6.00	11.50	11.00	7.00	4.50	3.90	3.00	0.50	0.00
5 ACD B	0.00	0.20	0.50	1.00	3.00	5.70	10.00	6.00	4.50	3.90	3.50	2.00	0.00	0.00
5 HEPARIN A	0.00	0.50	1.00	5.00	7.00	6.00	4.00	2.00	0.90	0.70	0.00	0.00	0.00	0.00
5 HEPARIN B	0.00	0.50	0.70	5.00	4.90	4.00	3.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00
5 EDTA A	0.00	0.50	0.90	1.00	2.00	4.00	2.00	1.90	1.00	0.20	0.00	0.00	0.00	0.00
5 EDTA B	0.00	0.50	0.50	1.00	2.00	2.50	2.70	4.00	4.00	2.00	1.00	0.00	0.00	0.00





 Replicate 1
 Replicate 2



ACD Plates over a 14-Day Period

Figure 4.1: Parasitaemia Progression in Red Blood Cells collected on ACD over a 14-Day Period from five different blood Donors This figure shows the parasitaemia levels in five different blood donors RBCs collected into ACD anticoagulants and used for the parasite cultures (ACD Plate 1–5) over 14 days, with two trials per plate, illustrating initial growth phases, varying peak parasitaemia levels (ranging from ~7% to 22.8%) occurring between Days 6–9, followed by a gradual decline, with most trials reaching 0% by Day 14. Light yellow lines represent means of replicate 1 while deep yellow represent means of replicate 2.



4.1.1 Parasites Culture progression measured as Parasitaemia on RBCs collected on ACDanticoagulant.

In ACD plate run 1 (Blood donor 1), parasitaemia remains at 0% for the first two days, indicating no detectable parasitic activity initially. By Day 3, parasitaemia began to rise, with replicate 1 reaching 0.4% and replicate 2 reaching 0.5%. The levels then increased steadily over the next several days with the highest parasitaemia observed on Day 9 in both replicates (7.7% and 7.2% respectively). After day 9, a gradual decline in parasitaemia levels began, with both replicates showing a consistent decline to 0% by day 14, suggesting a complete reduction or removal of detectable parasites by the end of the observation period.

For plate run 2 (Blood Donor 2), parasitaemia levels increased from day 2 with replicate 1 reaching 0.8% and replicate 2 reaching 0.6%. The levels rose gradually, achieving a peak at day 8 (22% in replicate 1 and 20% in replicate 2). This was followed by a decline to 7.3% in replicate 1 and 14.7% in replicate 2 by Day 11. Similar to plate one, parasitaemia levels dropped to initial levels (0%) by day 14 that is the culture was allowed to crash.

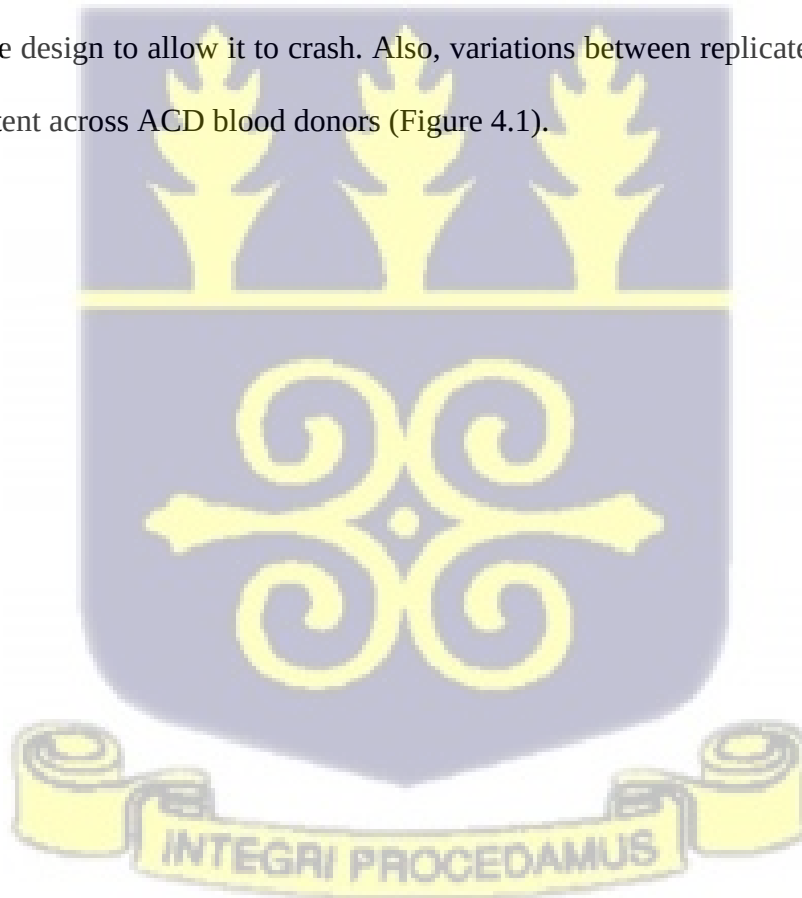
The parasite growth in ACD by blood donor 3 demonstrated a rapid increase in parasitaemia, reaching 11.8% in replicate 1 and 12.0% in replicate 2 by day 6. Both replicates peaked at Day 7, with replicate 1 reaching 16.2% and replicate 2, 14.9% with a sharp decrease in parasitaemia levels by Day 14 (Figure 4.1).

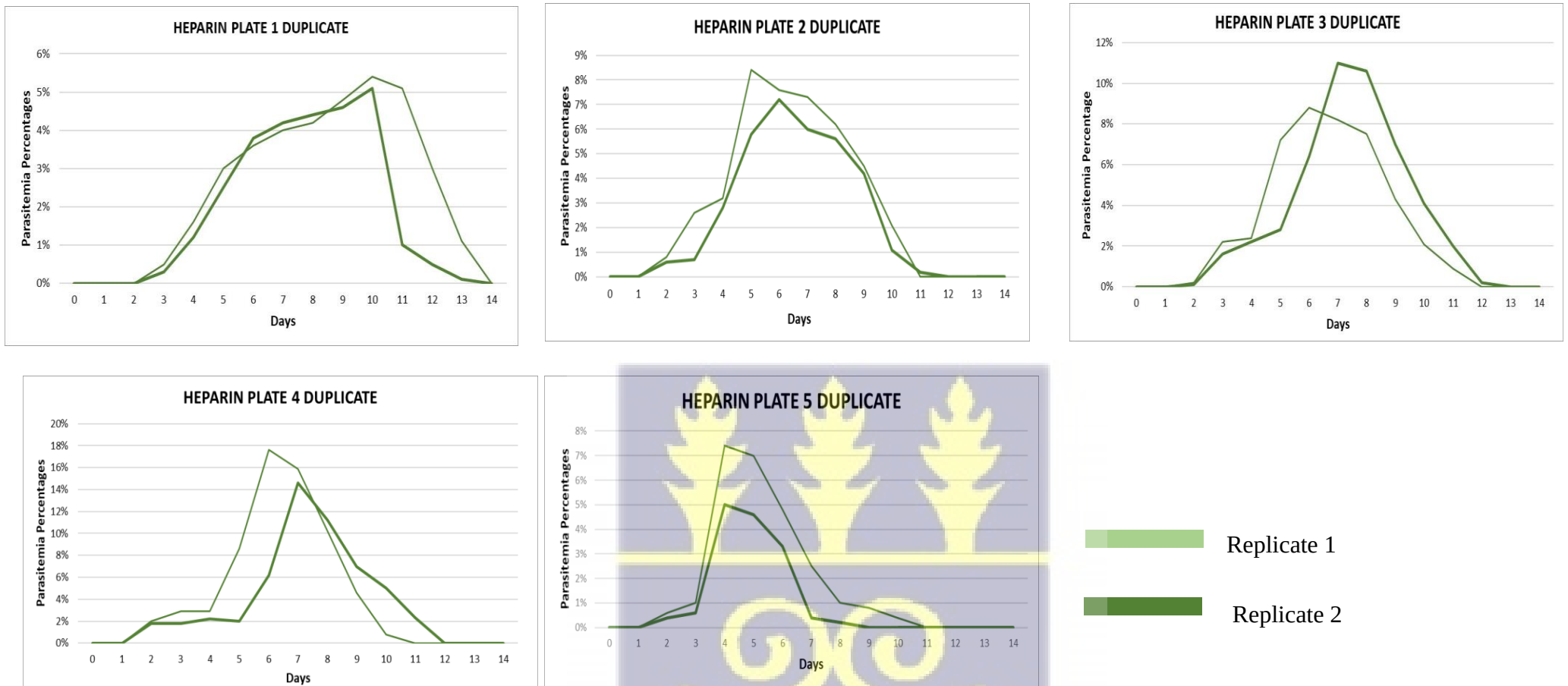
Interestingly the parasite growth pattern observed in ACD Donor 4, by day 6, replicate 1 had reached a parasitaemia of 9.7%, while replicate 2 reached 2.8%. Similar to what was observed in donor 3 (plate 3), a peak was recorded at 22.8% and 18.2% for replicate 1 and 2 respectively at day 7. By Day 10, replicate 1 dropped to 8.3%, while replicate 2 remains higher at 16.8%.

Replicate 1 crashed by Day 12, while replicate 2 gradually declined, ending at a parasitaemia of 1.2% on Day 14 when the culture was stopped.

In the ACD donor 5 (Plate Five), by day 6, parasitaemia peaked with replicate 1 reaching parasitaemia levels of 11.8% and replicate 2 at 10.4%. By Day 10, replicate 1 dropped to 3.5%, while replicate 2 reached 3.0%. Parasitaemia levels in both replicates continue to decline, reaching near 0% by day 14. This pattern shows an initial growth phase, peaking around Days 5-6, followed by a steady decline in parasitic activity.

Overall, all ACD-treated cultures displayed an exponential growth phase occurring between days 4-8, followed by a progressive decline, with parasitaemia dropping to undetectable levels by day 14 which was the design to allow it to crash. Also, variations between replicates were minor and remained consistent across ACD blood donors (Figure 4.1).





Heparin Plates over a 14-Day Period

Figure 4.2: Parasitaemia Progression in parasites Cultured using RBCs collected on Heparin anticoagulants over a 14-Day Period from five different blood donors—This figure presents parasitaemia levels of parasites cultured in RBCs collected into Heparin-anticoagulants (Heparin Plate 1–5) over 14 days, with two trials per plate, showing initial growth phases, varying peak parasitaemia levels (ranging from ~5% to 17.6%) occurring between Days 4–10, followed by a gradual decline, with most trials reaching 0% by Day 14. Light green lines represent Replicate 1 and deep green represent Replicate 2.

4.1.2 Cultured Parasites growth patterns using RBCs collected on Heparin anticoagulant and measured as Parasitaemia

Parasitaemia dynamics in Heparin-treated cultures were monitored over a 14-day period using five RBCs collected from five Donors on a Heparin anticoagulant (Plate 1–5), each culture was performed in duplicate. The results demonstrated a consistent pattern of initial parasitaemia increase, peaking between Days 6 and 10, followed by a decline to 0% by Day 14 in all blood donors (Figure 4.2).

In plate 1 (Blood donor 1), parasitaemia peaked on day 10 at 5.4% and 5.1% for replicate 1 and 2 respectively, with a steady decline afterwards. By Day 13, parasitaemia was nearly back to baseline, reaching 1.1% in replicate 1 and 0.1% in replicate 2.

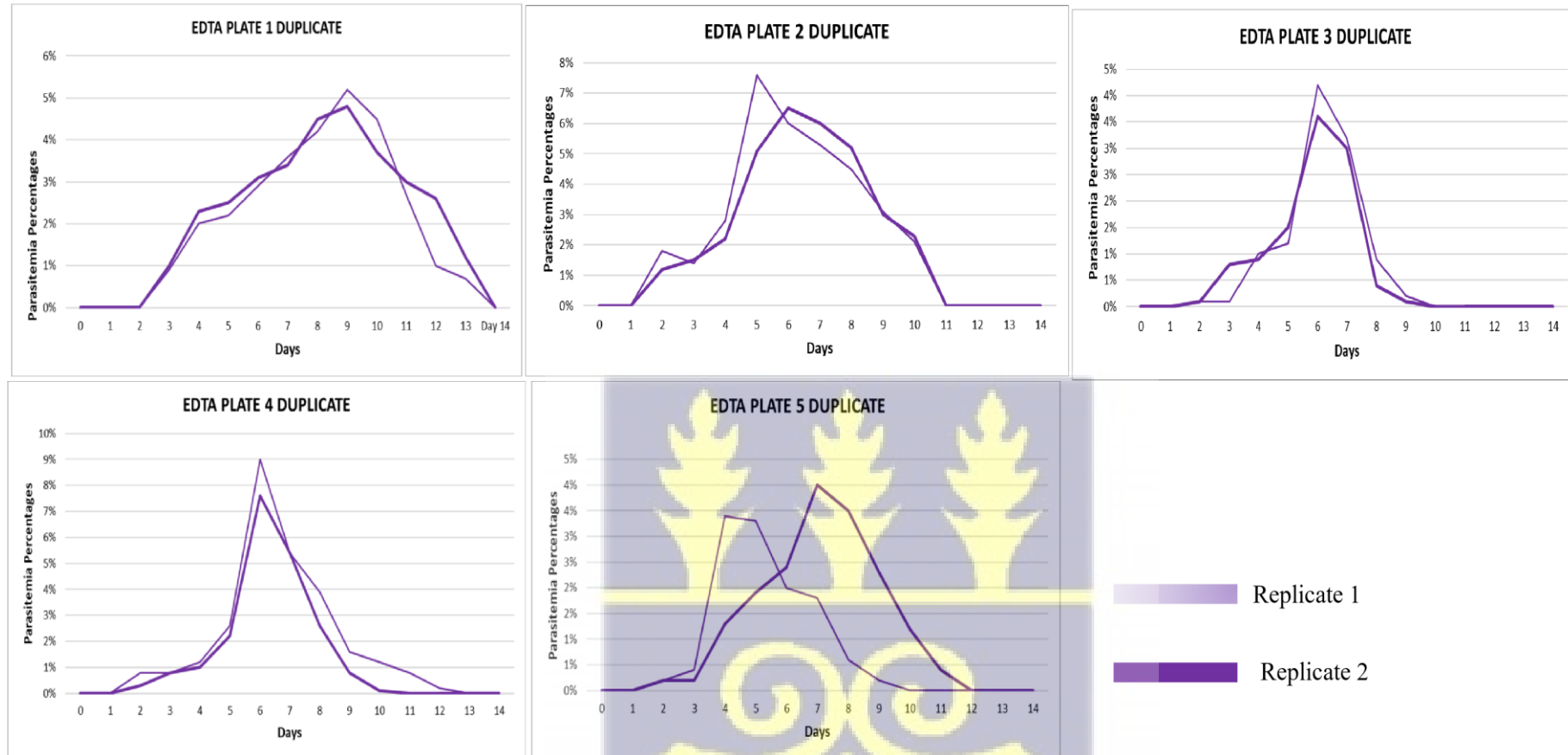
Plate 2 (blood Donor 2) showed an earlier peak around day 7 at 7.6% and 7.2% parasitaemia for replicates 1 and 2 respectively and by day 11, both cultures had crashed with a parasitaemia of 0% in both replicates 1 and 2. The parasite growth pattern in RBC donor 3, rose steadily from day 2 with replicate 1 reaching 7.2% by Day 5, while replicate 2 lagged slightly, reaching 2.8% by day 5. The highest parasitaemia levels was observed on day 8, with replicate 1 peaking at 8.8% and replicate 2 peaking at 11%, followed by a sharp drop by day 10 to day 14 when the cultures were allowed to crash.

Similar observations were made with respect to donor 4 (Plate 4) but in this donor there was a pronounced spike in parasitaemia reaching 17.6% in replicate 1 and 14.6% in replicate 2 on day 6 and 7. By day 9, replicate 1 had dropped to 4.6%, while replicate 2 dropped to 7%.

In plate 5 (Donor5), parasitaemia increased rapidly to 7.4% in replicate 1 and 5% in replicate 2 by Day 4. Replicates were well-aligned in terms of trend and magnitude, with a sharp decline observed

by day 11. Overall, parasite growth pattern observed in RBCs collected on Heparin anticoagulants showed parasitaemia peaks ranging from ~5% to 17.6%, typically occurring between Days 6–10.





EDTA Plates over a 14-Day Period

Figure 4.3: Parasitaemia Progression in parasites cultured using RBCs collected on EDTA anticoagulants over a 14-Day Period from five different blood donors —This figure illustrates parasitaemia levels in five EDTA-treated culture plates (EDTA Plate 1–5) over 14 days, with two duplicates per plate, showing an initial rise in parasitaemia, peak levels varying between ~3.4% and 9% occurring around Days 4–9, followed by a gradual decline, with all duplicates reaching 0% by Day 14.

4.1.3 Cultured Parasites growth patterns using RBCs collected on Heparin anticoagulant and measured as Parasitaemia

Parasites growth patterns measured as parasitaemia using RBCs collected in EDTA anticoagulants was evaluated over 14 days in five different blood donors (5 Plates), each in duplicate. All plates exhibited early increases in parasitaemia, with peak values occurring between Days 4–9, followed by a steady decline to 0% by Day 14 in all replicates when the cultures were allowed to crash (Figure 4.3).

In plate 1 (donor1), parasitaemia started coming up from day 3 at 0.90% and 1.00% for duplicate 1 and 2 respectively. Parasitaemia levels then gradually rose over the following days, reaching 5.20% and 4.80% by Day 9 for each duplicate. By Day 10, parasitaemia levels had dropped slightly for duplicate 1 and 2 at 4.50% and 3.70% respectively. The decline continued until Day 14, when the cultures were allowed to crash.

Similar patterns were observed in RBC donors 2 and 3 (Plate 2, 3 and 4) until the cultures were allowed to crash on day 14 (Figure 4.3)

Interestingly, in blood donor 5 an irregular pattern was observed, where the parasitaemia peaked around day 4 for replicate 1 at 3.4% whilst that of replicate 2 the peak was observed around day 7 at 4%, Figure 4.3).

4.1.4 The Mean Growth Patterns of Parasites Cultured using RBCs Collected on Different Anticoagulants

Similar parasite growth patterns were observed when all the parasitaemia were put together for all the cultures on the three anti-coagulants (Figure 4.4). Starting from the same parasitaemia, the growth rate started slowing and steadily rose to peaks which were slightly different for each anticoagulant over the 14-day period.

The mean percentage parasitaemia of parasites cultured using RBCs collected on EDTA began with undetected levels of parasitaemia (0% on Days 0 and 1). Parasitaemia levels started rising by day 2, and peaked at 4.13% on day 6.

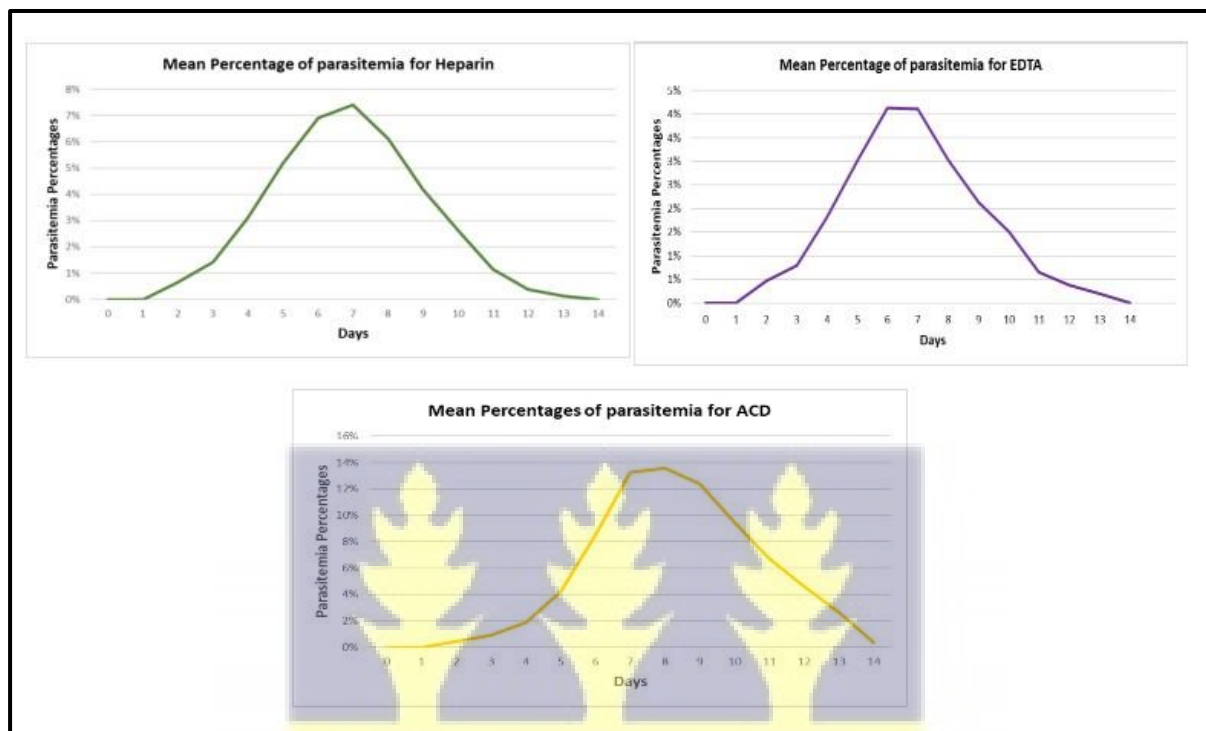
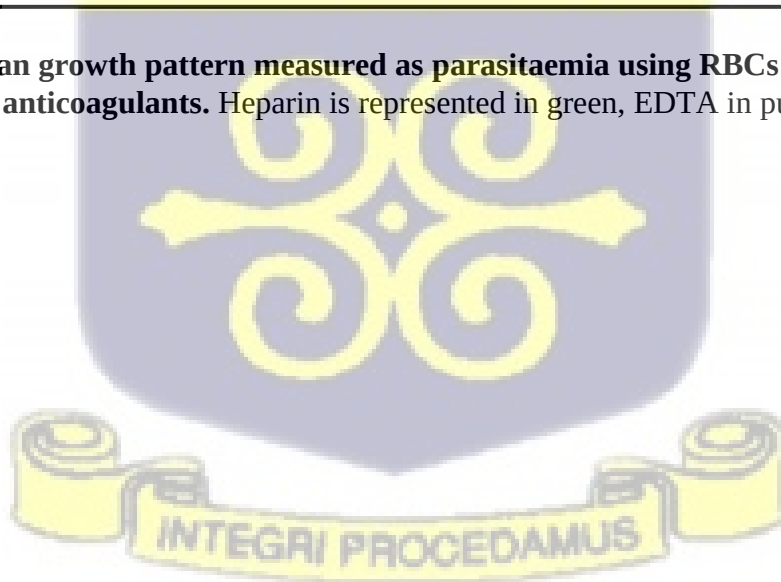


Figure 4.4: Mean growth pattern measured as parasitaemia using RBCs collected on three different anticoagulants. Heparin is represented in green, EDTA in purple and ACD in yellow.



This dataset represents the mean percentage of parasitaemia over 14 days across all duplicate plates. Parasitaemia begins at 0% on Day 0 and Day 1, with a gradual increase starting on Day 2, reaching 0.45%. This upward trend continues, with parasitaemia rising steadily each day, reaching 4.2% by Day 5.

The growth rate accelerates through Day 6, reaching 8.49%, and peaks around Day 7-8, with parasitaemia at 13.24% and 13.57%, respectively. After this peak, parasitaemia begins to decline gradually, dropping to 9.46% by Day 10 and 6.7% by Day 11. The decline continues steadily until Day 14, where parasitaemia falls to 0.36%, nearly returning to baseline levels. Similar patterns were observed also in parasite cultured using RBCs collected on ACD (Figure 4.4)

Table 4.2: Differences in Percentages Parasitaemia by Anticoagulant Type Using ANOVA

CALCULATION STEP	CALCULATION DETAIL	VALUE
OVERALL MEAN	$(5.26 + 4.53 + 1.59) / 3$	3.79%
SUM OF SQUARES BETWEEN (SSB)		
SSB FOR ACD	$5 \times (5.26 - 3.79)^2$	10.8
SSB FOR HEPARIN	$5 \times (4.53 - 3.79)^2$	2.74
SSB FOR EDTA	$5 \times (1.48 - 3.79)^2$	26.68
TOTAL SSB	$14.1 + 0.128 + 11.5$	40.22
SUM OF SQUARES WITHIN (SSW)		
SSW FOR ACD	Variance within ACD group	430.91
SSW FOR HEPARIN	Variance within Heparin group	136.45
SSW FOR EDTA	Variance within EDTA group	30.66
TOTAL SSW	$430.91 + 136.45 + 30.66$	598.02

DEGREES OF FREEDOM		
DF BETWEEN	$k - 1$	2
DF WITHIN	$N - k$	12
MEAN SQUARES		
MSB (MEAN SQUARE BETWEEN)	SSB / Df Between	20.11
MSW (MEAN SQUARE WITHIN)	SSW / Df Within	49.835
F-STATISTIC	MSB / MSW	0.404
CRITICAL F-VALUE	F-distribution table ($\alpha = 0.05$, $df = 2$, 12)	3.88
CONCLUSION	Since $0.404 < 3.88$, fail to reject the null hypothesis.	No significant difference

Mean Calculation: The overall mean percentage of parasitaemia is calculated for all groups. **Sum of Squares Between =SSB): Sum of Squares Within= (SSW): Degrees of Freedom: Mean Squares and F-Statistic:** The MSB and MSW are calculated, leading to the F-statistic. The F-statistic (0.404) is less than the critical value (3.88), leading to the conclusion that there is no significant difference among the groups. ANOVA results ($F = 0.404$, $p > 0.05$) indicated no significant difference in mean parasitaemia.

4.2 Morphology of parasites and RBCs after cultures

Morphological examination of both the infected and uninfected RBCs from the cultures showed that all stages of the parasite observed were not affected by the use of the ACD as an anticoagulant (Figure 4.6). Both the infected and Uninfected RBCs looked healthy and maintained their shapes and sizes at the initial stages of the cultures as expected.

On the other hand, the RBCs collected on heparin had few of the parasites in the trophozoite stages showing slightly distorted morphology but the uninfected RBCs maintained their morphology (Figure 4.6 C (ii)).

The parasites cultured using the RBCs collected on EDTA also had some of the slides showing some of the ring and few trophozoite stages also exhibiting shrunken cytoplasm. In the EDTA anticoagulant, the red blood cells also maintained the normal biconcave round shapes throughout the culturing period of (14 days, with only a few showing leave-like shapes with pikes at one side or on the surface of the red blood cells from day13 and 14 indicating that some of the red blood cells were stressed but that was expected because the design was to allow it to crash over the period (Figure 4.7 B (iii) and D (iv & v)). These were confirmed using Figure 2.3



Morphological Images of Infected and Uninfected RBCs Collected on ACD Anticoagulant for Parasite Cultures

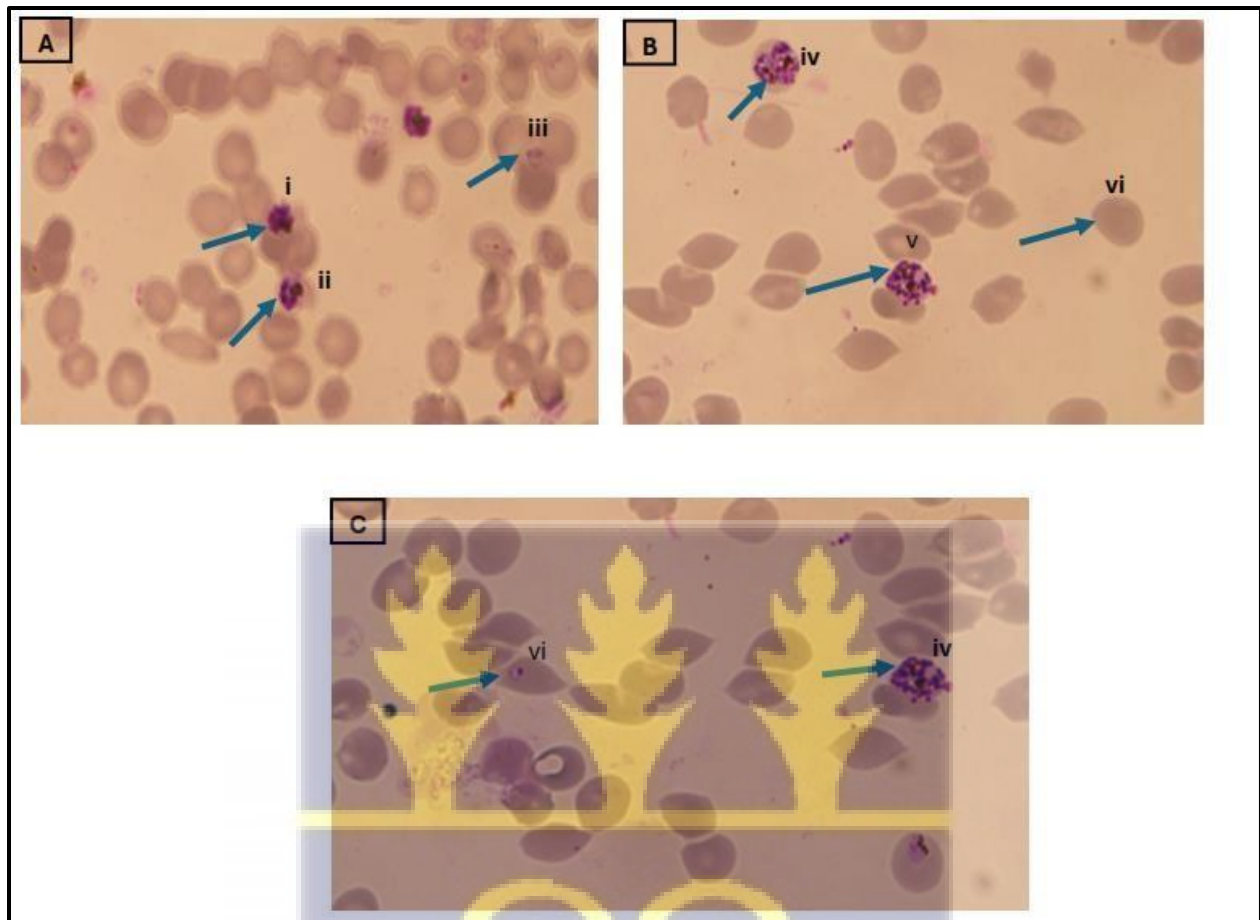


Figure 4.5: Micrographs of Parasites Cultured Using RBCs Collected on ACD Anticoagulant. A (i & ii) are ring stages developing into early trophozoites during early stages of the cultures with iii showing 3 RBCs with a ring stage in the center B (iv & v) are typical matured schizonts ready to burst to release rings, C (iv & v) are cultures showing rings and schizonts, with vi being an uninfected RBC

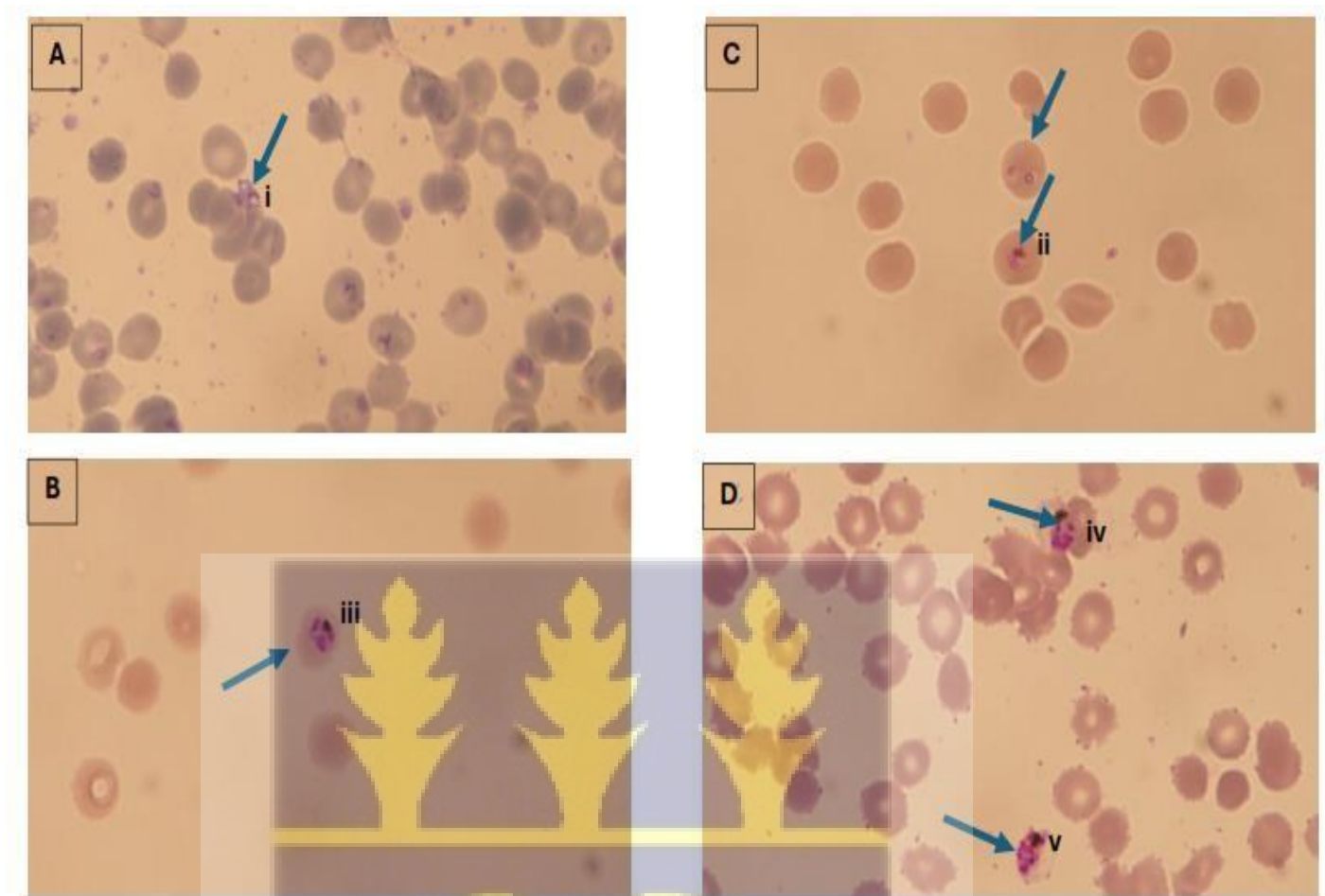


Figure 4.6: Micrographs of Parasites Cultured Using RBCs Collected on Heparin and EDTA Anticoagulant- A and C are micrographs Infected and uninfected RBCs from heparin anticoagulant and B and D are from EDTA anticoagulant A(i) ring High percentage of ring stages in a culture invading new red blood cells from Heparin Anticoagulant. C(ii) Ring stages multiplying in a red blood cell at the top and beneath an early trophozoite slightly distorted from Heparin Anticoagulant culture. B(iii) is Ring stage of *P. falciparum* developing into early trophozoites from EDTA Anticoagulant cultures showing shrunken cytoplasm. D (iv, v) are late trophozoites developing into schizonts with some red blood cells showing thin spikes on the surfaces from EDTA Anticoagulant culture on day 13 of the cultures.

4.3 Best anticoagulant for cultures

Based on the parasitaemia percentage progression, Acid Citrate Dextrose (ACD) had the highest mean parasitaemia followed by Heparin, with EDTA being the lowest (Figure 4.4). Also, the integrity of RBCs were maintained with the morphology of the parasites being more prominent in the ACD cultures compared to EDTA and Heparin plates. Based on this, I recommend ACD anticoagulant tubes over heparin and EDTA tubes.



CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Anticoagulant Type and *in vitro* parasite growth rates

The first objective of the study was to assess the differences in *in vitro* growth rates of *P. falciparum* parasites cultured using RBCs collected on different anticoagulants (ACD, Heparin, and EDTA) by microscopy. The observed growth patterns of all three anticoagulants used, indicated ACD to be consistently supportive of parasite growth *in vitro* and it showed the highest peak parasitaemia among all the blood donors. In some of the donors the cultures were able to reach a percentage parasitaemia of 22.8% with an average of 13.57% by day 8.

In contrast, Heparin showed moderate support for parasite development, with average parasitaemia peaking at 7.4% by day 7. EDTA, on the other hand, exhibited the lowest growth potential, with an average peak parasitaemia of only 4.13%, followed by rapid decline. Despite these variations, no statistically significant differences (ANOVA, $F = 0.404$, $p > 0.05$) were observed between the groups. This could be attributed to a high intra-group variability observed within replicates. These findings suggest that although the growth rates differ visually and temporally, the statistical overlap indicates variability may depend more on other microenvironmental or methodological factors than solely on the anticoagulant type.

Similar patterns have been noted in previous works, where anticoagulants were shown to influence *P. falciparum* viability and development. A study (Jensen, 2019) observed that ACD preserved red cell structure better, thus promoting sustained parasitic growth in cultures of Babesia and Plasmodium species. Likewise, other studies, reported that heparin seems to reduce parasite invasion efficiency at higher concentrations, possibly due to its interference with merozoite

attachment (Hmoud, 2019a). Meanwhile, EDTA has been long suspected of disrupting essential ionic interactions necessary for parasite growth due to its chelating effect on divalent cations like Ca^{2+} and Mg^{2+} (Lardinois et al., 2021). This study's findings align with this hypothesis, as EDTA-treated cultures peaked the lowest and cleared fastest. Therefore, although statistical differences were not significant, the biological trends reinforce that ACD offers the most conducive environment for parasite proliferation.

5.1.2 Morphology of parasites and Red Blood Cells after cultures

The second objective focused on evaluating parasite and red blood cell morphology under each anticoagulant condition using microscopy. Morphological observations revealed clearer developmental stages—rings, trophozoites, and schizonts—in ACD-treated samples, indicating healthier and more synchronous parasite development. Trophozoites appeared more distinct and schizogony was better defined, which may be due to ACD's ability to maintain pH stability and preserve membrane integrity.

The blood stage cycle of *Plasmodium falciparum* is a 48-hour cycle within red blood cells. It involves the parasite replicating asexually, leading to the rupture of red blood cells and the release of new parasites to infect more cells. This cycle is responsible for the characteristic fever cycles in humans.

Heparin cultures showed viable parasites, but trophozoites appeared slightly distorted or asynchronous at peak parasitaemia, possibly indicating mild cellular stress. In EDTA-treated samples, however, parasite morphology was often compromised; rings were less defined, and trophozoites exhibited shrunken cytoplasm or premature rupture. This supports previous research by Rowe et al. (1994), who noted that EDTA-induced changes in ionic concentrations could destabilize parasite development.

Additional comparative studies, such as those by (Solarte et al., 2007) , also emphasize how anticoagulants influence not just growth but also the morphology of intracellular parasites. (Solarte et al., 2007) demonstrated morphological degradation of *P. vivax* oocysts in mosquitoes fed on EDTA-treated blood, while Werimo observed higher incidence of abnormal forms when EDTA or heparin was used. Your findings reinforce these earlier observations and provide updated insight specific to *P. falciparum*, highlighting ACD's capacity to maintain optimal conditions for parasite development while minimizing morphological anomalies.

5.1.3 Best anticoagulant for cultures

The final objective sought to determine which anticoagulant most effectively supports *P. falciparum* *in vitro* cultivation. Based on both parasitaemia trends and morphological integrity, ACD emerges as the most effective anticoagulant. It not only supported higher parasitaemia for a prolonged duration but also preserved parasite development through all erythrocytic stages. Although Heparin showed intermediate support, its variability and reported interference with invasion processes (Hmoud, 2019b) suggest limitations in its utility. EDTA proved the least favourable due to its detrimental effects on ionic balance and membrane integrity, which likely inhibited parasite survival and led to faster clearance. These conclusions are supported by Pseudo thrombocytopenia (PTCP), a relative common finding in clinical laboratories, can lead to diagnostic errors, overtreatment, and further (even invasive) unnecessary testing. Clinical consequences with potential life-threatening events (e.g., unnecessary platelet transfusion, inappropriate treatment including splenectomy or corticosteroids) are still observed when PTCP is not readily detected. The phenomenon is even more complex when occurring with different anticoagulants. In this review, a case of multi-anticoagulant PTCP was presented, where we studied different parameters including temperature, amikacin supplementation, measurement

methods, and type of anticoagulant. Prevalence, clinical risk factors, pre-analytical and analytical factors, along with clinical implications, will be discussed. The detection of an anticoagulant-dependent PTCP does not necessarily imply the presence of specific disorders. Conversely, the incidence of PTCP seems higher in patients receiving low molecular weight heparin, during hospitalization, or in men aged 50 years or older. New analytical technologies, such as fluorescence or optical platelet counting, will be soon overturning traditional algorithms and represent valuable diagnostic aids. A practical laboratory approach, based on current knowledge of PTCP, is finally proposed for overcoming spuriously low (Lardinois et al., 2021), who caution against EDTA in parasite-based assays due to its potential to alter cellular interactions and chelate critical ions.

While the lack of statistically significant differences in growth rates across the anticoagulants may temper definitive conclusions, trend-based interpretations and morphological observations provide strong support for ACD as the superior anticoagulant for *P. falciparum* cultivation. Further studies with larger sample sizes and enhanced controls may help clarify subtle differences and better elucidate the molecular mechanisms by which anticoagulants affect parasite biology. Ultimately, these findings contribute to refining laboratory methodologies for malaria research, ensuring better reproducibility and more physiologically relevant outcomes.

5.2 CONCLUSION

5.2.1 Introduction

The primary objective of this study was to investigate the effects of three commonly used anticoagulants—Heparin, Acid Citrate Dextrose (ACD), and EDTA—on the morphology of *plasmodium falciparum*, red blood cells and *in vitro* growth rate of *Plasmodium falciparum* parasites. Given the importance of *P. falciparum* cultures in malaria research, including drug

susceptibility testing and resistance studies, the choice of anticoagulant plays a crucial role in obtaining accurate and reliable data.

This study has provided a detailed examination of the effects of ACD, Heparin, and EDTA on the growth and viability of *Plasmodium falciparum*. The findings indicate that while ACD supports the highest initial growth and sustains long-term viability, whereas Heparin offers a more moderate. EDTA imposes significant restrictions on parasite growth, suggesting its potential utility in studies focused on inhibition mechanisms.

The lack of statistically significant differences among the anticoagulants highlights the need for further research to explore the nuances of *P. falciparum* growth dynamics. By addressing the recommendations outlined in this chapter, future studies can enhance our understanding of malaria parasites and contribute to the development of more effective treatments and research methodologies.

Ultimately, the insights gained from this research will be instrumental in advancing our knowledge of malaria biology and improving strategies for combating this global health challenge. As malaria continues to pose a significant threat to public health worldwide, ongoing research efforts are essential to develop innovative solutions that can effectively address this persistent issue.

5.2.2 Summary of Findings

The study assessed the effects of three widely used anticoagulants—Acid Citrate Dextrose (ACD), Heparin, and EDTA—on the *in vitro* growth rate and morphology of *Plasmodium falciparum* parasite and red cells. Overall, findings revealed that ACD provided the most supportive environment for parasite viability, showing a trend of higher growth rates and better-preserved parasite morphology compared to Heparin and EDTA. Cultures treated with ACD displayed

relatively consistent parasite stages and maintained red blood cell integrity, which suggests that ACD may create more favorable conditions for the parasite's development.

Heparin was observed to support moderate growth, though its effects were not as pronounced as ACD.

EDTA, on the other hand, was the least supportive for *P. falciparum* growth, aligning with previous studies that suggest EDTA may disrupt cellular functions necessary for parasite survival. However, ANOVA analysis showed that the differences in growth rates and morphological changes between ACD, Heparin, and EDTA were not statistically significant. This indicates that while ACD is optimal, Heparin and EDTA are not significantly different in their impact on the parasite (Optimal conditions for all the cultures are all parasites were kept in an airtight gas chamber filled with oxygen, carbon dioxide and nitrogen and was kept in an incubator 37 degrees Celsius and slides were prepared each morning to check growth of the cultures under the microscope). This finding is notable because it implies that Heparin and EDTA may still serve as viable anticoagulants in *Plasmodium falciparum* cultures when ACD is unavailable.

Microscopic observations further supported these findings. Parasites cultured in ACD-treated samples clearly progressed through various erythrocytic stages—from rings and trophozoites to mature schizonts—with minimal morphological abnormalities. Specific images captured rings multiplying within red blood cells, early trophozoites forming from rings, and late trophozoites maturing into schizonts, all of which indicated robust development. In contrast, EDTA-treated cultures showed reduced parasite development, with fewer advanced stages such as schizonts, suggesting a disruption in the parasite's life cycle. Heparin-treated samples displayed moderate development, with some schizont formation and ring invasion, but overall growth was less vigorous than in ACD cultures.

Heparin was observed to support moderate growth, though its effects were not as pronounced as ACD. EDTA, on the other hand, was the least supportive for *P. falciparum* growth, aligning with previous studies that suggest EDTA may disrupt cellular functions necessary for parasite survival. However, ANOVA analysis showed that the differences in growth rates and morphological changes between ACD, Heparin, and EDTA were not statistically significant. This indicates that while ACD is optimal, Heparin and EDTA are not significantly different in their impact on the parasite. This finding is notable because it implies that Heparin and EDTA may still serve as viable anticoagulants in *P. falciparum* cultures when ACD is unavailable or impractical.

Finally, the study evaluated the effects of three anticoagulants—Acid Citrate Dextrose (ACD), Heparin, and EDTA—on the *in vitro* growth and morphology of *Plasmodium falciparum*. While the ANOVA results ($F = 0.404$, $p > 0.05$) indicated that the differences in mean parasitaemia across the three groups were not statistically significant, trend-based analysis revealed notable differences in parasite behaviour and morphology. ACD consistently supported higher parasitaemia levels, better morphological preservation, and more complete parasite developmental stages, as observed under microscopy. Heparin showed moderate support for parasite growth, while EDTA was the least favourable.

5.2.3 Implications of Findings

The findings of this study underscore that while ACD was observed to be the most supportive anticoagulant for *Plasmodium falciparum* cultures, both Heparin and EDTA showed only marginally lower performance in terms of supporting parasite growth and preserving morphology. ANOVA analysis indicated no statistically significant differences between the anticoagulants, suggesting that despite ACD's apparent advantage, Heparin and EDTA may serve as viable alternatives. Specifically, ACD-treated cultures demonstrated a trend toward higher growth rates

and more intact parasite morphology, but the lack of statistical significance implies that the biological impact of using Heparin or EDTA instead of ACD may be minimal under standard experimental conditions.

The implications of these findings are considerable for *P. falciparum* research in settings where ACD may not be readily available or is cost-prohibitive. Researchers might opt to use Heparin or EDTA without substantially compromising the quality of the data obtained. This flexibility could be particularly valuable in regions or laboratories with limited resources, enabling a broader range of institutions to contribute to malaria research. However, for studies that require the highest levels of precision and consistency in parasite growth and morphology—such as those focused on detailed drug efficacy, molecular biology, and gene expression studies—ACD remains the preferable choice due to its slightly superior support for parasite viability.

Moreover, the study's findings contribute to a better understanding of how anticoagulant choice can affect *P. falciparum* cultures, offering researchers the opportunity to make more informed decisions based on the goals of their experiments. By identifying that Heparin and EDTA provide similar support to ACD, this study promotes greater accessibility to high-quality *P. falciparum* culturing techniques and opens up possibilities for scalable and reproducible research across a range of laboratory settings. This expanded flexibility could ultimately help drive more robust research into malaria biology and treatment, benefitting both high- and low-resource settings.

5.3 RECOMMENDATIONS

Based on the findings and their implications, several recommendations can be made for future research:

- **Long Term Culture Studies:** Future studies should consider conducting extended long term culture experiments to assess the long-term effects of each anticoagulant on *P. falciparum* growth and viability.
- **Mechanistic Studies:** Further research should focus on understanding the mechanisms by which each anticoagulant influences *P. falciparum* growth. This could involve molecular and cellular analyses to elucidate the pathways affected by these anticoagulants, providing insights into parasite biology and potential targets for intervention. Understanding the molecular mechanisms could also inform the development of new therapeutic strategies.
- **Field Studies:** Conducting field studies to evaluate the effects of these anticoagulants in natural settings could help validate laboratory findings, will be crucial for translation into clinical applications.
- **Alternative Anticoagulants:** Exploring other anticoagulants not included in this study may reveal additional options that could support *P. falciparum* growth more effectively or sustainably.

5.4 Practical Application

The findings of this study have practical applications in both research and clinical settings. For researchers, the choice of anticoagulant can significantly impact the outcomes of experiments involving *P. falciparum*. Understanding the growth dynamics associated with each anticoagulant will enable researchers to select the most appropriate conditions for their specific experimental needs. This knowledge is essential for ensuring the reliability and reproducibility of research findings.

In clinical settings, insights gained from this study could inform the development of better culture systems for *P. falciparum*, which is essential for drug testing and vaccine development. By optimizing culture conditions, researchers can enhance the reliability and reproducibility of their

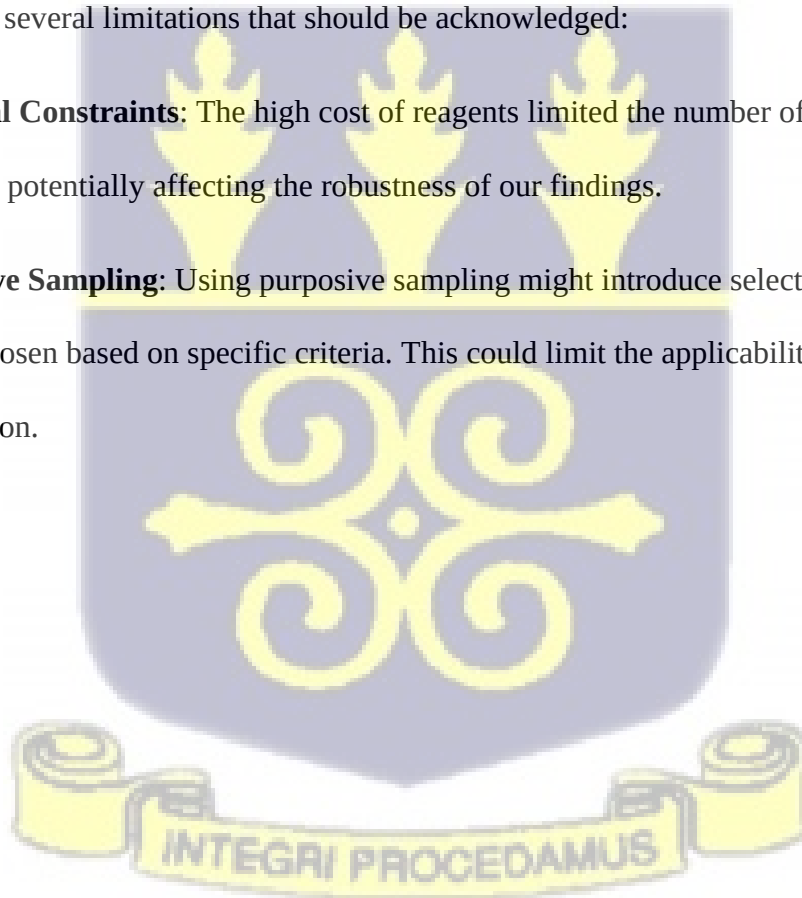
findings, ultimately contributing to more effective malaria treatments. Improved culture systems could also facilitate the identification of new antimalarial compounds and the development of vaccines, which are critical for combating malaria.

Furthermore, understanding the effects of different anticoagulants on *P. falciparum* growth could have implications for the design of clinical trials. Researchers could use this knowledge to select appropriate anticoagulants for patient samples, ensuring that the conditions are conducive to accurate assessments of treatment efficacy.

5.5 Limitations of the study

This study faced several limitations that should be acknowledged:

1. **Financial Constraints:** The high cost of reagents limited the number of experiments and trials conducted, potentially affecting the robustness of our findings.
2. **Purposive Sampling:** Using purposive sampling might introduce selection bias, as the samples were chosen based on specific criteria. This could limit the applicability of the results to a broader population.



REFERENCES

- Aheto, J. M. K. (2019). Predictive model and determinants of under-five child mortality: Evidence from the 2014 Ghana demographic and health survey. *BMC Public Health*, 19(1), 64. <https://doi.org/10.1186/s12889-019-6390-4>
- Aheto, J. M. K., Menezes, L. J., Takramah, W., & Cui, L. (2024). Modelling spatiotemporal variation in under-five malaria risk in Ghana in 2016–2021. *Malaria Journal*, 23(1), 102. <https://doi.org/10.1186/s12936-024-04918-x>
- Aird, W. C., Mosnier, L. O., & Fairhurst, R. M. (2014). Plasmodium falciparum picks (on) EPCR. *Blood*, 123(2), 163–167. <https://doi.org/10.1182/blood-2013-09-521005>
- Aninagyei, E., Doku, E. T., Adu, P., Egyir-Yawson, A., & Acheampong, D. O. (2018). Storage related haematological and biochemical changes in Plasmodium falciparum infected and sickle cell trait donor blood. *BMC Hematology*, 18(1), 30. <https://doi.org/10.1186/s12878-018-0128-x>
- Awine, T., Malm, K., Bart-Plange, C., & Silal, S. P. (2017). Towards malaria control and elimination in Ghana: Challenges and decision making tools to guide planning. *Global Health Action*, 10(1), 1381471. <https://doi.org/10.1080/16549716.2017.1381471>
- Banfi, G., Salvagno, G. L., & Lippi, G. (2007). The role of ethylenediamine tetraacetic acid (EDTA) as *in vitro* anticoagulant for diagnostic purposes. *Clinical Chemical Laboratory Medicine*, 45(5). <https://doi.org/10.1515/CCLM.2007.110>
- Basco, L. K. (2023). Cultivation of Asexual Intraerythrocytic Stages of Plasmodium falciparum. *Pathogens*, 12(7), Article 7. <https://doi.org/10.3390/pathogens12070900>

- Biswas, I., Panicker, S. R., Giri, H., Cai, X. S., & Rezaie, A. R. (2022). Antithrombin protects against Plasmodium falciparum histidine-rich protein II-mediated inflammation and coagulation. *Blood Advances*, 6(3), 931–945.
<https://doi.org/10.1182/bloodadvances.2021005836>
- Blasco, B., Leroy, D., & Fidock, D. A. (2017). Antimalarial drug resistance: Linking Plasmodium falciparum parasite biology to the clinic. *Nature Medicine*, 23(8), 917–928.
<https://doi.org/10.1038/nm.4381>
- Bonetti, G., Cancelli, V., Coccoli, G., Piccinelli, G., Brugnoli, D., Caimi, L., & Carta, M. (2016). Which sample tube should be used for routine glucose determination? *Primary Care Diabetes*, 10(3), 227–232. <https://doi.org/10.1016/j.pcd.2015.11.003>
- Borges-Pereira, L., Meissner, K. A., Wrenger, C., & Garcia, C. (2017) Plasmodium falciparum GFP-E NTPDase expression at the intraerythrocytic stages and its inhibition blocks the development of the human malaria parasite. University of São Paulo. 13(7030) https://www.researchgate.net/publication/314866403_
- Bowen, R. A. R., & Remaley, A. T. (2014). Interferences from blood collection tube components on clinical chemistry assays. *Biochemia Medica*, 31–44.
<https://doi.org/10.11613/BM.2014.006>
- Brown, A. C., & Guler, J. L. (2020). From Circulation to Cultivation: Plasmodium In Vivo versus In vitro. *Trends in Parasitology*, 36(11), 914–926. <https://doi.org/10.1016/j.pt.2020.08.008>
- Brown, A. C., Moore, C. C., & Guler, J. L. (2020). Cholesterol-dependent enrichment of understudied erythrocytic stages of human Plasmodium parasites. *Scientific Reports*, 10(1).
<https://doi.org/10.1038/s41598-020-61392-6>

- Buntsma, N. C., Gąsecka, A., Roos, Y. B. W. E. M., Van Leeuwen, T. G., Van Der Pol, E., & Nieuwland, R. (2022). EDTA stabilizes the concentration of platelet-derived extracellular vesicles during blood collection and handling. *Platelets*, 33(5), 764–771.
<https://doi.org/10.1080/09537104.2021.1991569>
- Cao, P., Collins, K. A., Zaloumis, S., Wattanakul, T., Tarning, J., Simpson, J. A., McCarthy, J., & McCaw, J. M. (2019). Modeling the dynamics of Plasmodium falciparum gametocytes in humans during malaria infection. *eLife*, 8. <https://doi.org/10.7554/elife.49058>
- CDC. (2010). The history of malaria, an ancient disease. *The History of Malaria, an Ancient Disease*.
- Cheng, K. K., Lam, T. H., & Leung, C. C. (2022). Wearing face masks in the community during the COVID-19 pandemic: Altruism and solidarity. *The Lancet*, 399(10336), e39–e40.
[https://doi.org/10.1016/S0140-6736\(20\)30918-1](https://doi.org/10.1016/S0140-6736(20)30918-1)
- Collins, K. A., Wang, C. Y. T., Adams, M., Mitchell, H., Rampton, M., Elliott, S., Reuling, I. J., Bousema, T., Sauerwein, R., Chalon, S., Möhrle, J. J., & McCarthy, J. S. (2018). A controlled human malaria infection model enabling evaluation of transmission-blocking interventions. *Journal of Clinical Investigation*, 128(4), 1551–1562.
<https://doi.org/10.1172/JCI98012>
- Daily, J. P., Minuti, A., & Khan, N. (2022). Diagnosis, Treatment, and Prevention of Malaria in the US: A Review. *JAMA*, 328(5), 460. <https://doi.org/10.1001/jama.2022.12366>
- Dans, M. G., Weiss, G. E., Wilson, D. W., Sleebs, B. E., Crabb, B. S., De Koning-Ward, T. F., & Gilson, P. R. (2020). Screening the Medicines for Malaria Venture Pathogen Box for invasion and egress inhibitors of the blood stage of Plasmodium falciparum reveals several inhibitory compounds. *International Journal for Parasitology*, 50(3), 235–252.
<https://doi.org/10.1016/j.ijpara.2020.01.002>

- Dao, F., Niangaly, A., Sogore, F., Wague, M., Dabita, D., Goita, S., Hadara, A. S., Diakite, O., Maiga, M., Maiga, F. O., Cazevieville, C., Cassan, C., Talman, A. M., Djimde, A. A., MarinMenendez, A., & Dembélé, L. (2025). Malian field isolates provide insight into Plasmodium malariae intra-erythrocytic development and invasion. *PLOS Neglected Tropical Diseases*, 19(1), e0012790. <https://doi.org/10.1371/journal.pntd.0012790>
- Dinarvand, P., Yang, L., Biswas, I., Giri, H., & Rezaie, A. R. (2020). Plasmodium falciparum histidine rich protein HRPII inhibits the anti-inflammatory function of antithrombin. *Journal of Thrombosis and Haemostasis*, 18(6), 1473–1483. <https://doi.org/10.1111/jth.14713>
- Fang, G., & Tang, B. (2020). Advanced delivery strategies facilitating oral absorption of heparins. *Asian Journal of Pharmaceutical Sciences*, 15(4), 449–460. <https://doi.org/10.1016/j.ajps.2019.11.006>
- Gazzinelli, R. T., Kalantari, P., Fitzgerald, K. A., & Golenbock, D. T. (2014). Innate sensing of malaria parasites. *Nature Reviews Immunology*, 14(11), 744–757. <https://doi.org/10.1038/nri3742>
- George, T., & Brady, M. F. (2024). Ethylenediaminetetraacetic Acid (EDTA). In *StatPearls*. StatPearls Publishing. <http://www.ncbi.nlm.nih.gov/books/NBK565883/>
- Giraldo, C. E., Álvarez, M. E., & Carmona, J. U. (2015). Effects of sodium citrate and acid citrate dextrose solutions on cell counts and growth factor release from equine pure-platelet rich plasma and pure-platelet rich gel. *BMC Veterinary Research*, 11(1), 60. <https://doi.org/10.1186/s12917-015-0370-4>

- Goheen, M. M., Clark, M. A., Kasthuri, R. S., & Cerami, C. (2016). Biopreservation of RBC s for *in vitro Plasmodium falciparum* culture. *British Journal of Haematology*, 175(4), 741–744. <https://doi.org/10.1111/bjh.13882>
- Graumans, W., Lanke, K., Van Gemert, G.-J., Alkema, M., Van De Vegte-Bolmer, M., Bousema, T., & Collins, K. A. (2022). The effect of anticoagulants in blood collection tubes on *Plasmodium falciparum* transmission in direct membrane feeding assays. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, 116(2), 187–189. <https://doi.org/10.1093/trstmh/trab095>
- Hemker, H. C. (2016). A century of heparin: Past, present and future. *Journal of Thrombosis and Haemostasis*, 14(12), 2329–2338. <https://doi.org/10.1111/jth.13555>
- Heuschen, A.-K., Abdul-Mumin, A., Abubakari, A., Agbozo, F., Lu, G., Jahn, A., & Müller, O. (2023). Effects of the COVID-19 pandemic on general health and malaria control in Ghana: A qualitative study with mothers and health care professionals. *Malaria Journal*, 22(1), 78. <https://doi.org/10.1186/s12936-023-04513-6>
- Hmoud, M. K. (2019a). *Exploring the erythrocyte invasion–blocking effect of modified heparin and heparin mimetics in the human malaria parasite Plasmodium falciparum* [PhD Thesis]. <https://keele-repository.worktribe.com/output/414945>
- Hmoud, M. K. (2019b). *Exploring the erythrocyte invasion–blocking effect of modified heparin and heparin mimetics in the human malaria parasite Plasmodium falciparum* [PhD Thesis]. <https://keele-repository.worktribe.com/output/414945>
- Jensen, J. B. (2019). *In vitro Cultivation of Protozoan Parasites* (1st ed.). CRC Press. <https://doi.org/10.1201/9781351073455>

- KARPATKIN, S., & FREEDMAN, M. L. (1978). Hypersplenic Thrombocytopenia Differentiated from Increased Peripheral Destruction by Platelet Volume. *Annals of Internal Medicine*, 89(2), 200–203. <https://doi.org/10.7326/0003-4819-89-2-200>
- Kobayashi, K., Takano, R., Takemae, H., Sugi, T., Ishiwa, A., Gong, H., Recuenco, F. C., Iwanaga, T., Horimoto, T., Akashi, H., & Kato, K. (2013). Analyses of Interactions Between Heparin and the Apical Surface Proteins of *Plasmodium falciparum*. *Scientific Reports*, 3(1), 3178. <https://doi.org/10.1038/srep03178>
- Kyu, H. H., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., Abbastabar, H., AbdAllah, F., Abdela, J., Abdelalim, A., Abdollahpour, I., Abdulkader, R. S., Abebe, M., Abebe, Z., Abil, O. Z., Aboyans, V., Abrham, A. R., Abu-Raddad, L. J., Abu-Rmeileh, N. M. E., ... Murray, C. J. L. (2018). Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*, 392(10159), 1859–1922. [https://doi.org/10.1016/S0140-6736\(18\)32335-3](https://doi.org/10.1016/S0140-6736(18)32335-3)
- Lancé, M. D., Sloep, M., Henskens, Y. M. C., & Marcus, M. A. E. (2012). Mean Platelet Volume as a Diagnostic Marker for Cardiovascular Disease: Drawbacks of Preanalytical Conditions and Measuring Techniques. *Clinical and Applied Thrombosis/Hemostasis*, 18(6), 561–568. <https://doi.org/10.1177/1076029612458147>
- Lantero, E., Aláez-Versón, C. R., Romero, P., Sierra, T., & Fernández-Busquets, X. (2020). Repurposing Heparin as Antimalarial: Evaluation of Multiple Modifications Toward In Vivo Application. *Pharmaceutics*, 12(9), 825. <https://doi.org/10.3390/pharmaceutics12090825>

- Lantero, E., Fernandes, J., Aláez-Versón, C. R., Gomes, J., Silveira, H., Nogueira, F., & FernàndezBusquets, X. (2020). Heparin Administered to Anopheles in Membrane Feeding Assays Blocks Plasmodium Development in the Mosquito. *Biomolecules*, 10(8), Article 8. <https://doi.org/10.3390/biom10081136>
- Lardinois, B., Favresse, J., Chatelain, B., Lippi, G., & Mullier, F. (2021). Pseudothrombocytopenia—A Review on Causes, Occurrence and Clinical Implications. *Journal of Clinical Medicine*, 10(4), 594. <https://doi.org/10.3390/jcm10040594>
- Lazrak, H. H., René, É., Elftouh, N., Leblanc, M., & Lafrance, J.-P. (2017). Safety of lowmolecular-weight heparin compared to unfractionated heparin in hemodialysis: A systematic review and meta-analysis. *BMC Nephrology*, 18(1), 187. <https://doi.org/10.1186/s12882-017-0596-4>
- Leitgeb, A. M., Blomqvist, K., Cho-Ngwa, F., Samje, M., Nde, P., Titanji, V., & Wahlgren, M. (2011). Low Anticoagulant Heparin Disrupts Plasmodium falciparum Rosettes in Fresh Clinical Isolates. *The American Society of Tropical Medicine and Hygiene*, 84(3), 390–396. <https://doi.org/10.4269/ajtmh.2011.10-0256>
- Lima, M., Rudd, T., & Yates, E. (2017). New Applications of Heparin and Other Glycosaminoglycans. *Molecules*, 22(5), 749. <https://doi.org/10.3390/molecules22050749>
- Lippi, G., & Plebani, M. (2012). EDTA-dependent pseudothrombocytopenia: Further insights and recommendations for prevention of a clinically threatening artifact. *Clinical Chemistry and Laboratory Medicine*, 50(8). <https://doi.org/10.1515/cclm-2012-0081>
- Mannuß, S. (2020). Influence of different methods and anticoagulants on platelet parameter measurement. *Journal of Laboratory Medicine*, 44(5), 255–272. <https://doi.org/10.1515/labmed-2020-0037>

- Marques, J., Valle-Delgado, J. J., Urbán, P., Baró, E., Prohens, R., Mayor, A., Cisteró, P., Delves, M., Sinden, R. E., Grandfils, C., De Paz, J. L., García-Salcedo, J. A., & FernàndezBusquets, X. (2017). Adaptation of targeted nanocarriers to changing requirements in antimalarial drug delivery. *Nanomedicine: Nanotechnology, Biology and Medicine*, 13(2), 515–525. <https://doi.org/10.1016/j.nano.2016.09.010>
- Marques, J., Vilanova, E., Mourão, P. A. S., & Fernàndez-Busquets, X. (2016). Marine organism sulfated polysaccharides exhibiting significant antimalarial activity and inhibition of red blood cell invasion by Plasmodium. *Scientific Reports*, 6(1), 24368. <https://doi.org/10.1038/srep24368>
- Mathison, B. A., & Pritt, B. S. (2017). Update on Malaria Diagnostics and Test Utilization. *Journal of Clinical Microbiology*, 55(7), 2009–2017. <https://doi.org/10.1128/JCM.02562-16>
- McNamara, C. (2017). Collection and Handling of Blood. In *Dacie and Lewis Practical Haematology* (pp. 1–7). Elsevier. <https://doi.org/10.1016/B978-0-7020-6696-2.00001-1>
- McQuaid, F., & Rowe, J. A. (2020). Rosetting revisited: A critical look at the evidence for host erythrocyte receptors in *Plasmodium falciparum* rosetting. *Parasitology*, 147(1), 1–11. <https://doi.org/10.1017/S0031182019001288>
- Meibalan, E., Barry, A., Gibbins, M. P., Awandu, S., Meerstein-Kessel, L., Achcar, F., Bopp, S., Moxon, C., Diarra, A., Debe, S., Ouédraogo, N., Barry-Some, I., Badoum, E. S., Fagnima, T., Lanke, K., Gonçalves, B. P., Bradley, J., Wirth, D., Drakeley, C., ... Bousema, T. (2021). *Plasmodium falciparum* Gametocyte Density and Infectivity in Peripheral Blood and Skin Tissue of Naturally Infected Parasite Carriers in Burkina Faso. *The Journal of Infectious Diseases*, 223(10), 1822–1830. <https://doi.org/10.1093/infdis/jiz680>

- Meibalan, E., & Marti, M. (2017). Biology of Malaria Transmission. *Cold Spring Harbor Perspectives in Medicine*, 7(3), a025452. <https://doi.org/10.1101/cshperspect.a025452>
- Mohanty, A. K., De Souza, C., Harjai, D., Ghavanalkar, P., Fernandes, M., Almeida, A., Walke, J., Manoharan, S. K., Pereira, L., Dash, R., Mascarenhas, A., Gomes, E., Thita, T., Chery, L., Anvikar, A. R., Kumar, A., Valecha, N., Rathod, P. K., & Patrapuvich, R. (2021). Optimization of Plasmodium vivax sporozoite production from Anopheles stephensi in South West India. *Malaria Journal*, 20(1), 221. <https://doi.org/10.1186/s12936-021-03767-2>
- Moon, R. W., Hall, J., Rangkuti, F., Ho, Y. S., Almond, N., Mitchell, G. H., Pain, A., Holder, A. A., & Blackman, M. J. (2013). Adaptation of the genetically tractable malaria pathogen *Plasmodium knowlesi* to continuous culture in human erythrocytes. *Proceedings of the National Academy of Sciences*, 110(2), 531–536. <https://doi.org/10.1073/pnas.1216457110>
- Mulloy, B., Hogwood, J., Gray, E., Lever, R., & Page, C. P. (2016). Pharmacology of Heparin and Related Drugs. *Pharmacological Reviews*, 68(1), 76–141. <https://doi.org/10.1124/pr.115.011247>
- Neveu, G., Dupuy, F., Ladli, M., Barbieri, D., Naissant, B., Richard, C., Martins, R. M., LopezRubio, J.-J., Bachmann, A., Verdier, F., & Lavazec, C. (2018). Plasmodium falciparum gametocyte-infected erythrocytes do not adhere to human primary erythroblasts. *Scientific Reports*, 8(1). <https://doi.org/10.1038/s41598-018-36148-y>
- Nureye, D., & Assefa, S. (2020a). Old and Recent Advances in Life Cycle, Pathogenesis, Diagnosis, Prevention, and Treatment of Malaria Including Perspectives in Ethiopia. *The Scientific World Journal*, 2020, 1–17. <https://doi.org/10.1155/2020/1295381>

- Nureye, D., & Assefa, S. (2020b). Old and Recent Advances in Life Cycle, Pathogenesis, Diagnosis, Prevention, and Treatment of Malaria Including Perspectives in Ethiopia. *The Scientific World Journal*, 2020(1), 1295381. <https://doi.org/10.1155/2020/1295381>
- Oduah, E., Linhardt, R., & Sharfstein, S. (2016). Heparin: Past, Present, and Future. *Pharmaceuticals*, 9(3), 38. <https://doi.org/10.3390/ph9030038>
- Ogretmen, Z., Hiz, M. M., Silan, F., Uludag, A., & Ozdemir, O. (2014). Association of endothelial nitric oxide synthase Glu298Asp gene polymorphism in psoriasis cases with hypertension. *Annals of Saudi Medicine*, 34(4), 340–345. <https://doi.org/10.5144/0256-4947.2014.340>
- Ramirez, M. I., Amorim, M. G., Gadelha, C., Milic, I., Welsh, J. A., Freitas, V. M., Nawaz, M., Akbar, N., Couch, Y., Makin, L., Cooke, F., Vettore, A. L., Batista, P. X., Freezor, R., Pezuk, J. A., Rosa-Fernandes, L., Carreira, A. C. O., Devitt, A., Jacobs, L., ... Dias-Neto, E. (2018). Technical challenges of working with extracellular vesicles. *Nanoscale*, 10(3), 881–906. <https://doi.org/10.1039/C7NR08360B>
- Rathmes, G., Rumisha, S. F., Lucas, T. C. D., Twohig, K. A., Python, A., Nguyen, M., Nandi, A. K., Keddie, S. H., Collins, E. L., Rozier, J. A., Gibson, H. S., Chestnutt, E. G., Battle, K. E., Humphreys, G. S., Amratia, P., Arambepola, R., Bertozzi-Villa, A., Hancock, P., Millar, J. J., ... Weiss, D. J. (2020). Global estimation of anti-malarial drug effectiveness for the treatment of uncomplicated *Plasmodium falciparum* malaria 1991–2019. *Malaria Journal*, 19(1), 374. <https://doi.org/10.1186/s12936-020-03446-8>
- Schneider, A. G., Journois, D., & Rimmelé, T. (2017). Complications of regional citrate anticoagulation: Accumulation or overload? *Critical Care*, 21(1), 281. <https://doi.org/10.1186/s13054-017-1880-1>

- Sherrard-Smith, E., Hogan, A. B., Hamlet, A., Watson, O. J., Whittaker, C., Winskill, P., Ali, F., Mohammad, A. B., Uhomoibhi, P., Maikore, I., Ogbulafor, N., Nikau, J., Kont, M. D., Challenger, J. D., Verity, R., Lambert, B., Cairns, M., Rao, B., Baguelin, M., ... Churcher, T. S. (2020). The potential public health consequences of COVID-19 on malaria in Africa. *Nature Medicine*, 26(9), 1411–1416. <https://doi.org/10.1038/s41591-020-1025-y>
- Solarte, Y., Del Rosario Manzano, M., Rocha, L., Castillo, Z., James, M. A., Herrera, S., & Arévalo-Herrera, M. (2007). Effects of Anticoagulants on Plasmodium vivax Oocyst Development in Anopheles albimanus Mosquitoes. *The American Journal of Tropical Medicine and Hygiene*, 77(2), 242–245. <https://doi.org/10.4269/ajtmh.2007.77.242>
- Tripathi, H., Bhalerao, P., Singh, S., Arya, H., Alotaibi, B. S., Rashid, S., Hasan, M. R., & Bhatt, T. K. (2023). Malaria therapeutics: Are we close enough? *Parasites & Vectors*, 16(1), 130. <https://doi.org/10.1186/s13071-023-05755-8>
- Van Schalkwyk, D. A., Burrow, R., Henriques, G., Gadalla, N. B., Beshir, K. B., Hasford, C., Wright, S. G., Ding, X. C., Chiodini, P. L., & Sutherland, C. J. (2013). Culture-adapted Plasmodium falciparum isolates from UK travellers: *In vitro* drug sensitivity, clonality and drug resistance markers. *Malaria Journal*, 12(1), 320. <https://doi.org/10.1186/1475-2875-12-320>
- Walencik, J., & Witeska, M. (2007). The effects of anticoagulants on hematological indices and blood cell morphology of common carp (Cyprinus carpio L.). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 146(3), 331–335. <https://doi.org/10.1016/j.cbpc.2007.04.004>
- Walker, N. F., Nadjm, B., & Whitty, C. J. M. (2010). Malaria. *Medicine*, 38(1), 41–46. <https://doi.org/10.1016/j.mpmed.2009.09.017>
- Warnock, L. B., & Huang, D. (2024). Heparin. In *StatPearls*. StatPearls Publishing.

<http://www.ncbi.nlm.nih.gov/books/NBK538247/>

Wax, P. M. (2013). Current Use of Chelation in American Health Care. *Journal of Medical Toxicology*, 9(4), 303–307. <https://doi.org/10.1007/s13181-013-0347-2>

Wharton-Smith, A., Baker, K., Roca-Feltrer, A., Rodrigues, M., Richardson, S., Bonnington, C. A., Rassi, C., Marasciulo, M., Enosse, S., Saute, F., Aide, P., Macete, E., & Candrinho, B. (2021). Assessment of the Feasibility, Acceptability, and Impact of Implementing Seasonal Malaria Chemoprevention in Nampula Province, Mozambique: Protocol for a Hybrid Effectiveness-Implementation Study. *JMIR Research Protocols*, 10(9), e27855. <https://doi.org/10.2196/27855>

White, N. J. (2022). Severe malaria. *Malaria Journal*, 21(1), 284. <https://doi.org/10.1186/s12936022-04301-8>

WHO. (2013). Malaria entomology and vector control. *Malaria Entomology and Vector Control*.

WHO. (2020). *World Malaria Report 2020: 20 Years of Global Progress and Challenges*.

WHO. (2022). *World malaria report 2022*.

Win, A. Y. N., Maung, T. M., Wai, K. T., Oo, T., Thi, A., Tipmontree, R., Soonthornworasiri, N., Kengganpanich, M., & Kaewkungwal, J. (2017). Understanding malaria treatment-seeking preferences within the public sector amongst mobile/migrant workers in a malaria elimination scenario: A mixed-methods study. *Malaria Journal*, 16(1), 462. <https://doi.org/10.1186/s12936-017-2113-4>

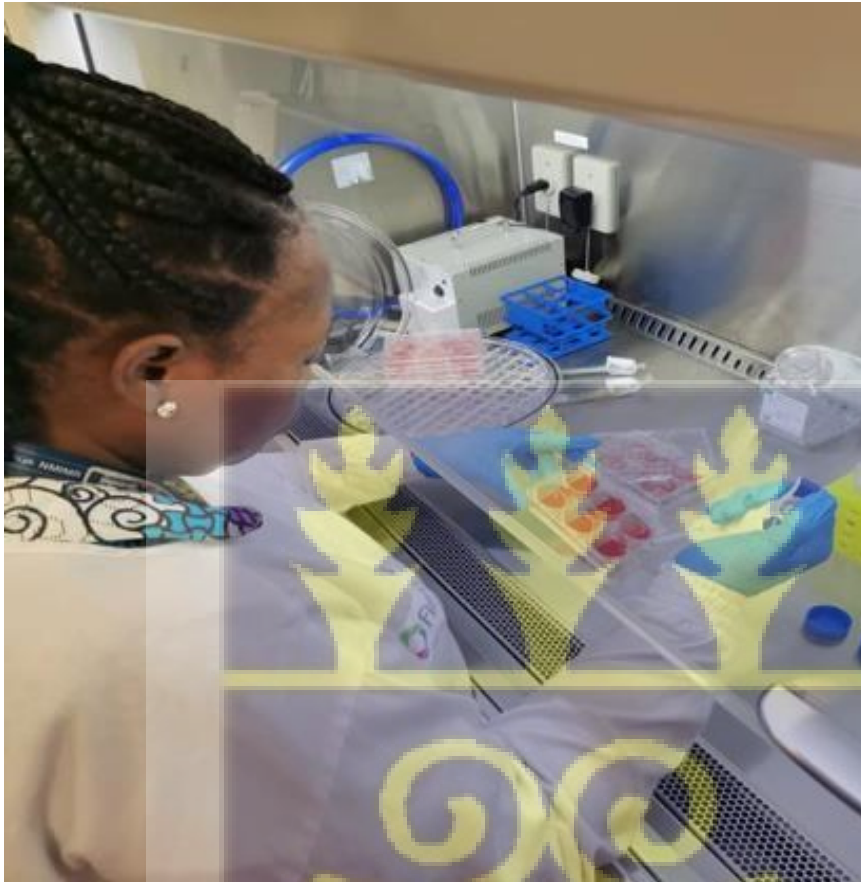
Wisgrill, L., Lamm, C., Hartmann, J., Preißing, F., Dragosits, K., Bee, A., Hell, L., Thaler, J., Ay, C., Pabinger, I., Berger, A., & Spittler, A. (2016). Peripheral blood microvesicles secretion is influenced by storage time, temperature, and anticoagulants. *Cytometry Part A*, 89(7), 663–672. <https://doi.org/10.1002/cyto.a.22892>

- Witeska, M., & Wargocka, W. (2011). Disodium EDTA used as anticoagulant causes hemolysis in common carp blood. *Turkish Journal of Veterinary & Animal Sciences*.
<https://doi.org/10.3906/vet-0908-51>
- Xiao, L.-X., Zhu, D. L., Chen, J., Lv, J., Liu, M.-J., Dai, X., Wang, D.-X., & Deng, W. (2024). Exploring the therapeutic role of early heparin administration in ARDS management: A MIMIC-IV database analysis. *Journal of Intensive Care*, 12(1), 9.
<https://doi.org/10.1186/s40560-024-00723-5>
- Zahraini, H., Indrasari, Y. N., & Kahar, H. (2021). Comparison of K2 and K3 EDTA Anticoagulant on Complete Blood Count and Erythrocyte Sedimentation Rate. *INDONESIAN JOURNAL OF CLINICAL PATHOLOGY AND MEDICAL LABORATORY*, 28(1), 75–79.
<https://doi.org/10.24293/ijcpml.v28i1.1735>
- Zhang, C., Yang, B.-C., Liu, W.-T., Li, Z.-Y., Song, Y.-J., Zhang, T.-C., & Luo, X.-G. (2019). Structure-based engineering of heparinase I with improved specific activity for degrading heparin. *BMC Biotechnology*, 19(1), 59. <https://doi.org/10.1186/s12896-019-0553-3>
- Zhou, Y., Grieser, A. M., Do, J., Itsara, L. S., Vaughan, A. M., & Ghosh, A. K. (2020). Purification and production of Plasmodium falciparum zygotes from *in vitro* culture using magnetic column and Percoll density gradient. *Malaria Journal*, 19(1), 192.
<https://doi.org/10.1186/s12936-020-03237-1>
- Zhou, Y., Hatzakis, K., MacMillen, Z., Laohajaratsang, M., Grieser, A. M., Itsara, L. S., Do, J., Davie, J. W., Ghosh, A. K., & Avril, M. (2024). Full maturation of *in vitro* Plasmodium falciparum oocysts using the AlgiMatrix 3D culture system. *Malaria Journal*, 23(1), 251.
<https://doi.org/10.1186/s12936-024-05079-7>

APPENDIX A

The appendix contains images of different stages of the experiment being carried out, from replacing culture media to staining blood smears onto slides and gassing in a gas chamber.

(A)

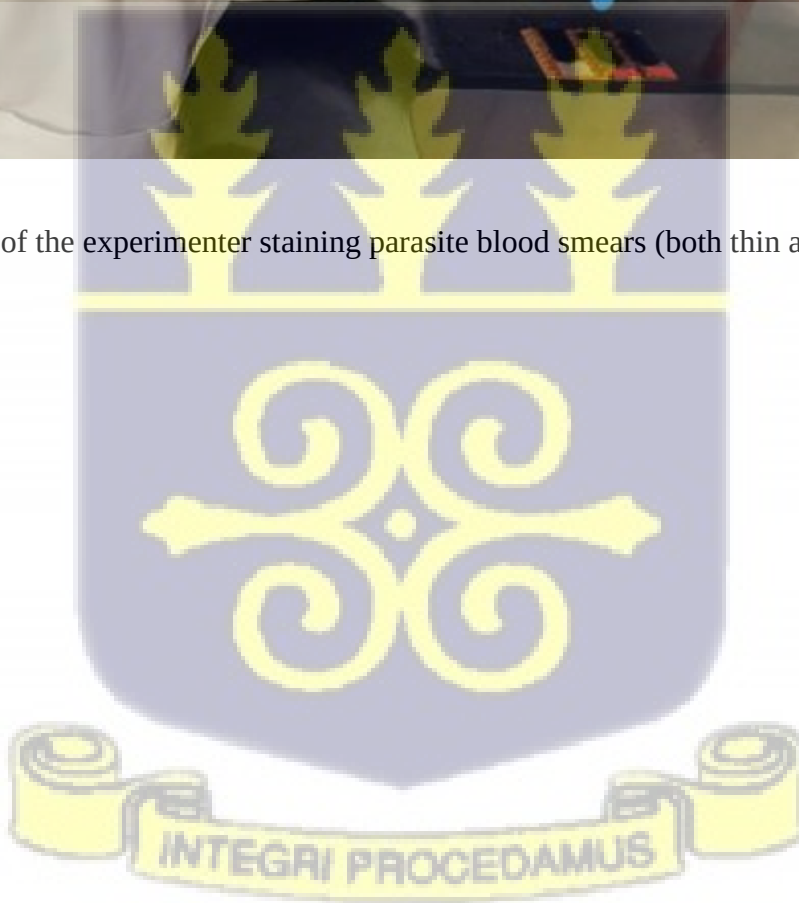


This is an image (A) of the experimenter, removing used media, preparing thick and thin smears and replacing with new media.

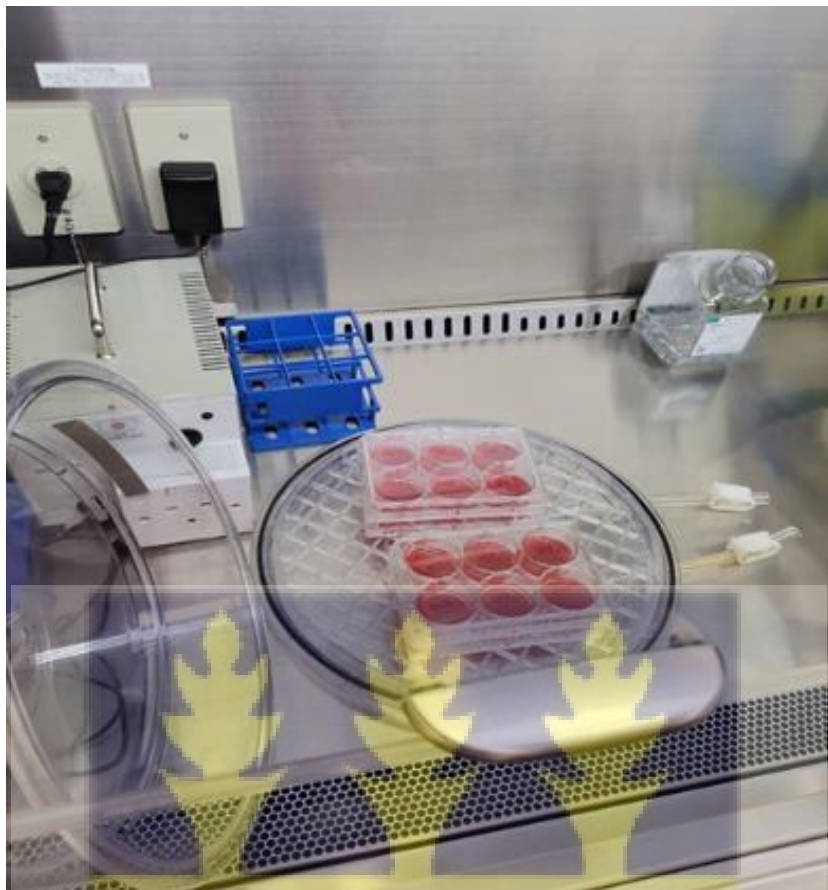
(B)



This is an image of the experimenter staining parasite blood smears (both thin and thick) on the same slides.



(C)



The image (C) is of the plates in a gas chamber, ready to be gassed.

