UNIVERSITY OF CAPE COAST

HIGH PREVALENCE OF *PLASMODIUM FALCIPARUM* DRUG RESISTANCE MARKERS IN GHANA, AN ARTEMISININ-BASED COMBINATION THERAPY (ACT) SETTING

BY

RICHMOND AFOAKWAH

Thesis submitted to the Department of Biomedical and Forensic Sciences of the School of Biological Sciences, University of Cape Coast in partial fulfillment of the requirements for award of Doctor of Philosophy degree in Parasitology

MAY, 2013

DECLARATIONS

CANDIDATE'S DECLARATION

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Name:

RICHMOND AFOAKWAH

Signature:...................... Date: 19-08-2013

SUPERVISORS' DECLARATION

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Name:

DR JOHNSON NYARKO BOAMPONG

Signature: .... Date: 19/8/13

Co-Supervisor's Name:

DR ALEXANDER P. EGYIR-YAWSON

Signature: .... Date: 19/08/2013

ii

ABSTRACT

Resistance to the artemisinins has been reported in less than a decade after their global deployment. The molecular markers for artemisinin resistance, however, have not been clearly characterized. The aim of this study is to determine and describe the genetic polymorphisms of the *Pfcrt, Pfmdr1,* and *PfATPase6* genes as associated to artemisinin resistance. A total of 1,318 subjects were recruited for the study. A 12.75% prevalence of malaria was recorded. Malaria transmission was not found to be seasonal. PCR-RFLP was employed to analyze mutations at codon positions 76 of the *Pfcrt* gene as well as positions 86 and 184 of the *Pfmdr1* gene which have been associated with artemisinin resistance. These mutations were found in very high prevalence among 246 cases of 1,318 subjects recruited for the study. Mutations in the *PfATPase6* gene were analyzed by PCR-RFLP and Sequencing. The SNPs of *PfATPase6* gene that are said to confer resistance to artemisinins were not found in this study, like in many others, suggesting a growing irrelevance of the *PfATPase6* gene as a marker for artemisinin resistance. Two novel SNPs in the *PfATPase6* gene were, however, discovered confirming the highly diverse nature of this gene. With the current artemisinin drug pressure and the observed high prevalence of SNPs associated with artemisinin resistance, it is only a matter of time for a stable drug resistance to be recorded in Ghana. A national programme to monitor the development of resistance to artemisinin is, thus, crucially needed.

ACKNOWLEDGMENTS

My sincerest gratitude goes to the University of Cape Coast (UCC) for awarding me a scholarship to undertake this PhD programme. Such an opportunity in our part of the world is indeed very rare. I am therefore very grateful that you found me worthy of funding. I am particularly grateful to Prof (Mrs) Mary Botchey, the then Head of the defunct Human Biology Department, UCC, for nominating me for the scholarship. Thank you so much, Prof, for believing in me.

Words will not be enough to thank my principal supervisor, Dr Johnson Nyarko Boampong. He has taught, nurtured and mentored me both consciously and unconsciously all throughout the period of my postgraduate studies. I appreciate his contribution of time, stimulating ideas and productive criticisms which has shaped this thesis into what it is. His characteristic selflessness and zeal to work has been undeniably contagious and motivational in the development of my professional career.

I am also very grateful for the enormous contribution of my co-supervisor, Dr Alexander Prince Egyir-Yawson. I am particularly grateful for his provision of a conducive environment for the successful completion of the laboratory proceedings of this research work. His openness and readiness to assist in any way he could undoubtedly helped in the early completion of this work.
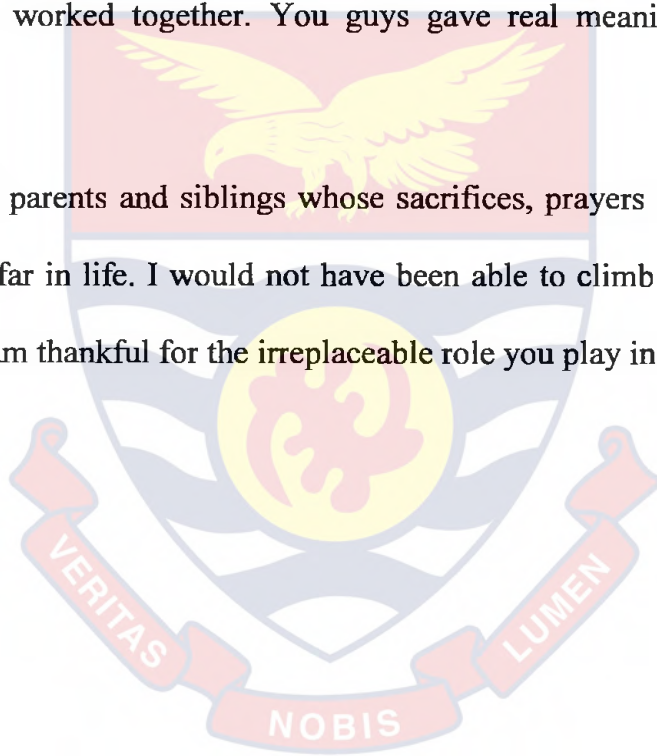
I am also thankful to the Medical Superintendents and Laboratory staff of the Central Regional Hospital, Effia-Nkwanta Regional Hospital, Ridge Hospital, Kumasi South Hospital, Ho Regional Hospital and Sunyani Regional Hospital for

iv

permitting and assisting me take samples from their facilities. I am eternally grateful.

The completion of this work could not have been possible without the support, understanding and love of my wife, Naa and son, Nana Yaw, considering the long weeks I had to leave you for my laboratory work in Accra. You stood by me through thick and thin. I appreciate your continuous encouragement. Thank you.

And to my comrades Ekene, Orish and Joe, I appreciate the cordiality and unity with which we worked together. You guys gave real meaning to 'work and happiness'.

I also thank my parents and siblings whose sacrifices, prayers and concern have carried me this far in life. I would not have been able to climb this high without your support. I am thankful for the irreplaceable role you play in my life.

DEDICATION

To my lovely wife, Mrs Elsie Gifty Afoakwah.

TABLE OF CONTENTS

CONTENTS

CHAPTER THREE: MATERIALS AND METHODS

x

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

LIST OF PLATES

PLATE

CHAPTER ONE

INTRODUCTION

Background to the study

Malaria is the most important parasitic disease of man caused by parasites of the genus *Plasmodium*. Human malaria is caused by four different species of *Plasmodium*, namely *P. falciparum, P. ovale, P. vivax,* and *P. malariae*. The most virulent of these four parasites is *P. falciparum.* It has been discovered recently that *P. knowlesi,* a malaria parasite of macaque monkeys, cause human malaria (White, 2008; Cox-Singh et al., 2008). There are about 3 billion people at risk of infection in 106 malaria endemic countries. The number of cases of the disease rose from 233 million in 2000 to 244 million in 2005 but decreased to 225 million in 2009. The number of deaths due to malaria is also reported to have decreased from 985 000 in 2000 to 781 000 in 2009. In Ghana, however, there is limited evidence of decrease in malaria cases between 2000 and 2009 (WHO, 2010a)

Malaria control requires an integrated approach comprising prevention, including vector control, and treatment with antimalarials (WHO, 2006b). Ideally, prevention would be the most efficient way to control malaria, but in the absence of a potent vaccine, chemotherapy remains the mainstay in controlling the disease (Fidock, Eastman, Ward, & Meshnick, 2008). The

1

arsenal of antimalarial drugs is limited and most of these have become obsolete because the parasites have developed resistance to them. *P. falciparum* has developed resistance to almost all currently used antimalarials – amodiaquine, chloroquine, mefloquine, quinine and sulfadoxine-pyrimethamine (WHO, 2006b). *P. falciparum* resistance to antimalarials has been associated, among other factors, with single nucleotide polymorphisms (SNPs) in a number of *P. falciparum* genes. For example, chloroquine resistance is associated with polymorphisms in the *P. falciparum* chloroquine resistance transporter (*pfcrt*) gene (Fidock, et al., 2000), while polymorphisms in the *P. falciparum* multidrug resistance 1 (*pfmdr1*) gene have been shown by transfection to modulate higher levels of chloroquine resistance (Djimde, Doumbo, Steketee, & Plowe, 2001a). Sulphadoxine and pyrimethamine resistance is associated with polymorphisms in the dihydropteroate synthase (*dhps*) and the dihydrofolate reductase (*dhfr*) genes respectively (Wang, et al., 1997; Pearce, Drakeley, Chandramohan, Mosha, & Roper, 2003).

Drug resistance arises from rare, spontaneous and random point mutations in the genome or gene duplications which are independent of drug selection pressure (White, 2009; WHO, 2010b). Once formed, the continuous use of parasite-resistant drug confers selective advantage to parasites that carry the resistant gene(s) (WHO, 2006b). The resistant parasites are selected for and begin to multiply, eventually resulting in a parasite population that is no longer susceptible to treatment (Targett et al., 2001; Drakeley et al., 2004; Pukrittayakamee et al., 2004).

2

Owing to the resistance developed by the parasites against chloroquine and sulfadoxine-pyrimethamine, artemisinin-based combination therapy (ACT) is recommended for use in the whole sub-Saharan Africa (WHO, 2006b) for a better efficacy and to delay the occurrence of resistance. Ghana adopted artesunate-amodiaquine as first-line treatment for uncomplicated malaria in 2004 (WHO, 2008).

Artemisinins are extracted from the sweet wormwood, *Artemisia annua* (Eckstein-Ludwig et al., 2003). The artemisinins are the most potent antimalarial drugs available (Hein and White, 1993). They rapidly kill all asexual stages of *P. falciparum* (ter Kuile, White, Hollaway, Pasvol, & Krishner, 1993), however, they are rapidly eliminated (WHO, 2006b). The ACT treatment policy therefore requires the artemisinin being combined with a longer lasting partner for efficient clearance of the parasites. Currently the recommended ACTs include artemether + lumefantrine, artesunate + amodiaquine, artesunate + mefloquine, and artesunate + sulfadoxine-pyrimethamine (WHO, 2006b).

Clinical resistance (Sahr, Willoughby, Gbakima, & Bockarie, 2001; Luxemburger et al., 1998), treatment failures (Ittarat et al., 2003; Jackson, Chappuis, Loutan, & Taylor, 2006) as well as *in vitro* resistance (Jambou, Legrand, Niang, Khim, & Mercereau-Puijalon, 2005; Chaijaroenkul, Bangchang, Mungthin, & Ward, 2005; Cojean, Hubert, Le Bras, & Durand, 2006; Ferreira et al., 2007) to artemisinins have been reported. Recent studies

3

conducted in Cambodia and Thailand showed an increase in proportion of patients who were still parasitaemic on day 3 after administration of various ACTs (Denis et al., 2006; Alker et al., 2007; Noedl et al., 2008; Dondorp et al., 2009), signaling a change in the pattern of parasite susceptibility and a possible first stage of artemisinin resistance (WHO, 2010b). Similar findings have been reported on the Myanmar-Thailand and China-Myanmar borders (Phyo et al., 2012; Wang et al., *2012)*, albeit, the situation in these areas is less severe than on the Cambodia-Thailand border.

In falciparum malaria, artemisinins are thought to inhibit the sarco-endoplasmic reticulum calcium-ATPase (SERCA)-type, PfATPase 6 protein (Woodrow & Krishna, 2006). This may, however, not be the only target (Valderramos, Scaanfeld, Uhlemann, Fidock & Krishna, 2010; Afoakwah, Boampong, Acheampong, & Nwaefuna, 2011). *PfATPase6* Ser769Asn has been proposed as the molecular marker for artemether resistance but this proposal is based entirely on findings from *in vitro* tests (Jambou et al., 2005), with no confirmation from field studies (Zhang et al., 2008; Tahar, Ringwald, & Basco, 2009).

Amplification of the *Pfmdr1* gene is associated with relatively small but significant reductions in susceptibility to artemisinins *in vitro*, which could explain the cross-resistance observed between amino-alcohols and artemisinins *in vitro* (Price et al., 2004; Chavchich et al., 2010).

4

Laboratory-induced artemisinin resistance in the *P. chabaudi* model has been demonstrated in a chloroquine resistant strain (Afonso et al., 2006), possibly suggesting that chloroquine resistance in these models might be a prerequisite for the subsequent development of artemisinin resistance (Imwong et al., 2010).

So far, none of the known markers correlate with the artemisinin resistance phenotype observed at the Cambodia–Thailand border. It is becoming quite obvious that resistance to artemisinin is multifactorial, possibly involving cross resistance as well as amplification and point mutations in some specific genes.

Detecting artemisinin resistance will require an integrated approach involving therapeutic efficacy studies, in vitro tests, use of molecular markers and measurement of drug concentrations (WHO, 2010b) of relatively large sample sizes (Noedl, 2005).

Statement of the problem

Most known antecedents to artemisinin resistance exist in Ghana and most Sub-sahara African countries. For example, before the advent of artesunate-amodiaquine as the first-line anti-malarial drug in Ghana, artemisinins were taken as mono-therapy. In 2010, WHO reported that 25 countries, including Ghana, were still allowing the market of artemisinin-based

5

monotherapies and 39 pharmaceutical companies, 2 of which are in Ghana, were manufacturing them (WHO, 2010b).

Again, chloroquine resistance has long been reported with a study reporting more than 70% treatment failure with chloroquine in Ghana (WHO, 2010b).

Treatment failures with ACTs have been reported in Ghana with some studies reporting as high as 13.8% treatment failure for Artemether-lumefantrine and 14.0% treatment failure for Artesunate-amodiaquine (WHO, 2010b).

If these indications are anything to go by, then it is only a matter of time for stable artemisinin resistance to develop in Ghana and other African countries.

Rationale

Most malaria endemic countries have adopted ACT as the first-line antimalarial drug for uncomplicated malaria. Prior to the adoption of ACT as the first line antimalarial drug, artemisinin compounds were already in use as monotherapy in most malaria endemic countries including Ghana. However, data on the resistance level of the parasites to artemisinin compounds prior to the ACT-treatment-policy adoption, and even after the adoption of the policy, are scanty. Results of this research will, therefore, add to the baseline data, on

6

P. falciparum resistance to artemisinins that can influence anti-malarial drug policy formulation in these malaria endemic countries, especially Ghana.

Hypothesis

The single nucleotide polymorphisms of the *Pfcrt*, *Pfmdr1*, and *PfATPase6* genes which have been associated with artemisinin resistance are highly prevalent in Ghana after eight years of adopting the ACT treatment policy.

Objectives

The main goal of the study is to determine and describe the genetic polymorphisms of the *Pfcrt, Pfmdr1,* and *PfATPase6* genes as associated to artemisinin resistance.

Specific objectives

- To determine the baseline characteristics of the study participants and how they influence malaria

- To find the prevalence of malaria in the study sites

- To ascertain the association between malaria and anaemia

7

- To determine sensitivity and specificity of the methods used in detecting the malaria parasites

- To ascertain the seasonality of malaria in the study sites

- To determine the occurrence of the *Pfcrt*, *Pfmdr1* and *PfATPase6* mutations in Ghana.

- To determine seasonal variations, if any, in the prevalence of the *Pfcrt*, *Pfmfr1* and *PfATPase6* mutations.

- To investigate the distribution of *Pfcrt*, *Pfmfr1* and *PfATPase6* mutations in the selected regions of Ghana.
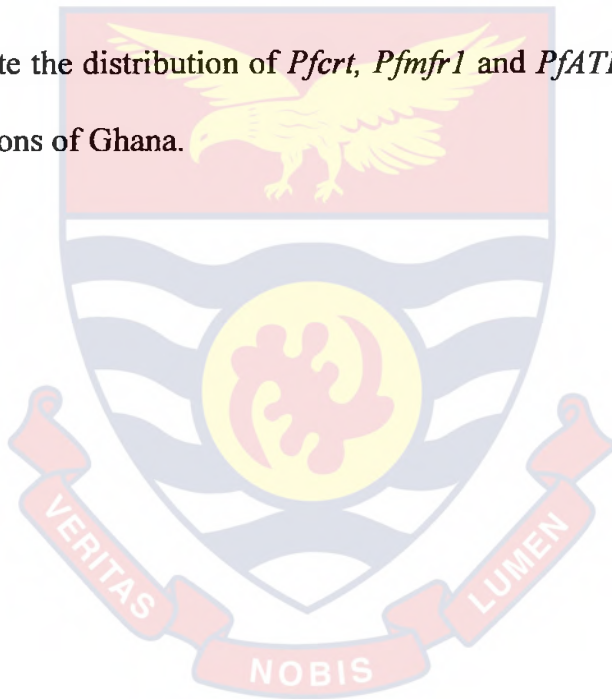
CHAPTER TWO

LITERATURE REVIEW

Biology of the malaria parasite

Parasite

Malaria is the most common serious infectious and parasitic disease worldwide (Nester, Anderson, Roberts, Pearsall, & Nester, 2004). It is caused by parasites of the genus *Plasmodium*. The parasite is classified as follows; Kingdom Animalia, Phylum Alveolata, Subphylum Apicomplexa, Class Haematozoa, Family Haemosporida, and Genus *Plasmodium*. Protozoan parasites of the phylum Apicomplexa contain three genetic elements, namely the nuclear genome, mitochondrial genome and an 35-kilobase circular extrachromosomal DNA, which encodes a vestigial plastid, the apicoplast (White, 2009). Malaria is a disease of mammals, reptiles and birds. *Plasmodium falciparum, P. ovale, P. vivax and P. malariae* are the four main different species of *Plasmodium* which cause human malaria. Occasionally, humans may be infected with the monkey malaria parasite, *P. knowlesi* (WHO, 2006b; White, 2008; Cox-singh et al., 2008 Daneshvar et al., 2010). *P. falciparum* is the most virulent of all five human malaria parasites, accounting for much of the recorded malaria morbidity and mortality (WHO, 2008).

9

Human malaria parasites are transmitted to and from man by mosquitoes of the genus *Anopheles*. At temperatures below 16°C, or above 33°C, and at altitudes greater than 2000m development of *Plasmodium* in the mosquito is haltered (White, 2009). Hence malaria transmission does not occur under such conditions. The best conditions for parasite development and vector survival, and consequent disease transmission include high humidity, ambient temperature between 20 and 30°C as well as optimal rainfall. These conditions are best found in the tropics and subtropics, making malaria endemic in these areas.

Anopheline mosquitoes are the only mosquito species capable of transmitting malaria. Vectoral capacity of the various species of Anopheles varies greatly (White, 2009). There are about 400 species of anopheline mosquitoes, but only about 80 are capable of transmitting malaria (Gillies, 1988).

A. gambiae complex are the most efficient vectors of malaria in the world (Coetzee, 2004). There are six named and one unnamed morphologically similar species in the *A. gambiae s.s* (Hunt, Coetzee, & Fettene, 1998). This species complex is extremely anthropohilic and endophilic, explaining their success as malaria vectors. It is found all over tropical Africa and prefers breeding sites represented by sunny and clean water pools, devoid of vegetation (Esposito and Habluetzel, 1997)

10

A. funestus complex and *A. minimus* complex are also important

vectors. Male mosquitoes do not transmit malaria since they do not feed on

blood. The females require the protein in blood to develop their eggs.

Life cycle

Plasmodium sp. requires two hosts, a vertebrate and an invertebrate, to

complete its life cycle as shown in fig 2. Infection of the vertebrate begins with

the bite of an infected female mosquito. During blood feeding by the mosquito,

plasmodial sporozoites are inoculated into the bloodstream of the host. Up to

about 100 sporozoites may be injected. However, only a few (about 10) are

required to establish an infection (Ponnudurai, Lensen, van Gemert, Bolmer &

Meuwissen, 1991; Rosenburg and Wirtz, 1990). Within 15 to 45 minutes of the

bite, all sporozoites disappear from the bloodstream. They are either cleared by

the body's defenses or they attach to and invade liver cells by binding to the

hepatocyte receptor for the serum proteins thrombospondin and properdin

(Cerami et al., 1992).

Invasion of the hepatocytes begins an exogenous asexual reproduction of

the parasite. This phase lasts an average of 5.5 days in *P. falciparum*, 8 in *P.

vivax*, 9 in *P. ovale*, 15 in *P. malariae* and 6 in *P. knowlesi* (White, 2009). In the

hepatocyte the parasite undergoes considerable asexual multiplication. In this

process, the sporozoite develops into a large (30–70 µm), multinuclear schizont

(Kayser, Bienz, Eckert, & Zinkernagel, 2005). Following cytoplasmic division

11

of the schizont 30 000, 10 000, 15 000 and 2000 merozoites are produced respectively in *P. falciparum, P. vivax, P. ovale* and *P. malaria* (White, 2009). The number of merozoites formed per hepatic schizont is not yet known for *P. knowlesi* in man. Rapture of hepatocytes release merozoites into the bloodstream. In infections with *P. vivax* and *P. ovale*, sporozoites develop into schizonts as described above, but some remain dormant as hypnozoites, which may develop into schizonts following activation after months or years. When infected hepatocytes rapture, merozoites are released into the bloodstream and they immediately invade erythrocytes in which the parasites reproduce asexually. Merozoites invasion of erythrocytes requires species-specific ligand-receptor interactions, which explains why certain *Plasmodium* sp prefer certain cell types: *P. malariae* infects mainly older erythrocytes, *P. vivax* and *P. ovale* prefer reticulocytes and *P. falciparum* infects both younger and older erythrocytes (Keyser et al., 2005).

The invasion process involves merozoite binding, apical reorientation, tight-junction formation and final parasite entry as seen in fig 1. The rapidity with which the merozoite invades red cells is influenced by shape and surface area to volume ratio of the erythrocytes among other factors (Boampong, Manno, Koshino, & Takakuwa, 2007). Once inside the erythrocyte, the parasite develops from merozoite through ring to mature trophozoite followed by asexual division (schizogony) to form schizonts, each of which contains numerous merozoites. Merozoites are released into the bloodstream as schizonts

cells and the erythrocytic cycle repeats itself at approximately 24 hours for *P. knowlesi*, 48 hours for *P. falciparum, P. vivax* and *P. ovale*, or 72 hours for *P. malariae*. Most erythrocytic merozoites divide to form more merozoites. A few develop into sexual forms called gametocytes.
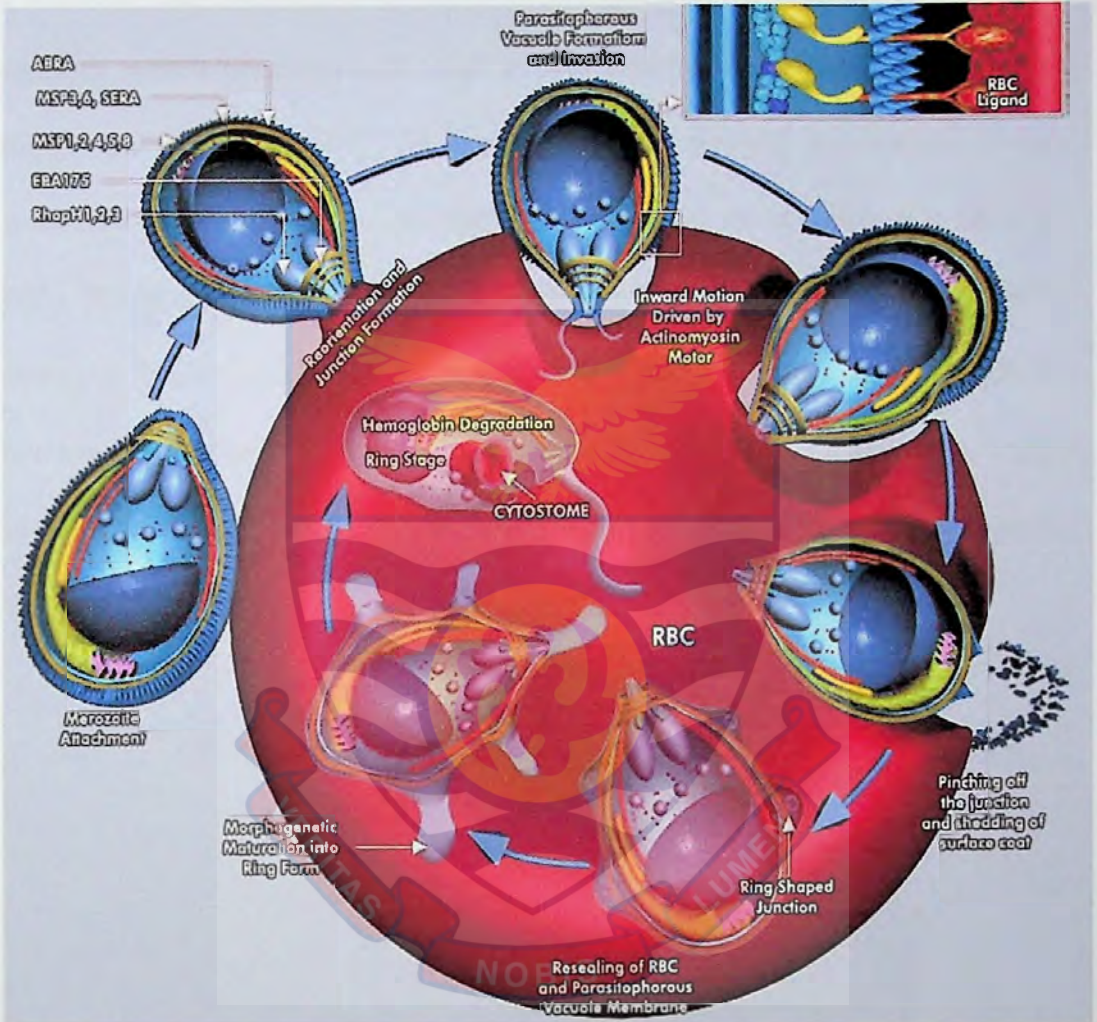
Fig 1: Invasion pathway of *Plasmodium sp* (© QIAGEN)

The motile gametocytes are the stages which transmit the malaria infection. Gametocytes are of two forms micro- (male form) and macro- (female

13

form). When the anopheline mosquito takes a blood meal from an infected person, it ingests the gametocytes. In the mosquito midgut, each microgametocytes, containing eight nuclei, exflagellates (divides) into eight microgametes and the macrogametocyte transforms into a macrogamete. Fusion of a micro- and macrogamete result in the formation of a motile zygote, the ookinete, which penetrates the wall of the midgut and encysts as an oocyst (Biggs and Brown, 2001). In the oocyst the parasite undergoes nuclear proliferation (sporogony) to form thousands of motile sporozoites (Kayser et al., 2005). When the oocyst breaks open the sporozoites are freed into the coelomic cavity of the mosquito, from where they migrate to the salivary glands. Development of the parasite in the mosquito takes 8 to 35 days depending on the ambient temperature, species of the parasite and species of the vector (White, 2009).

14
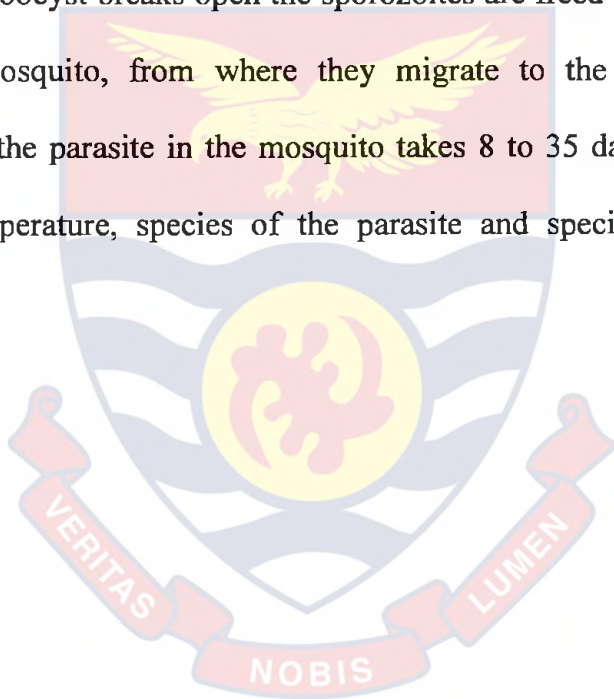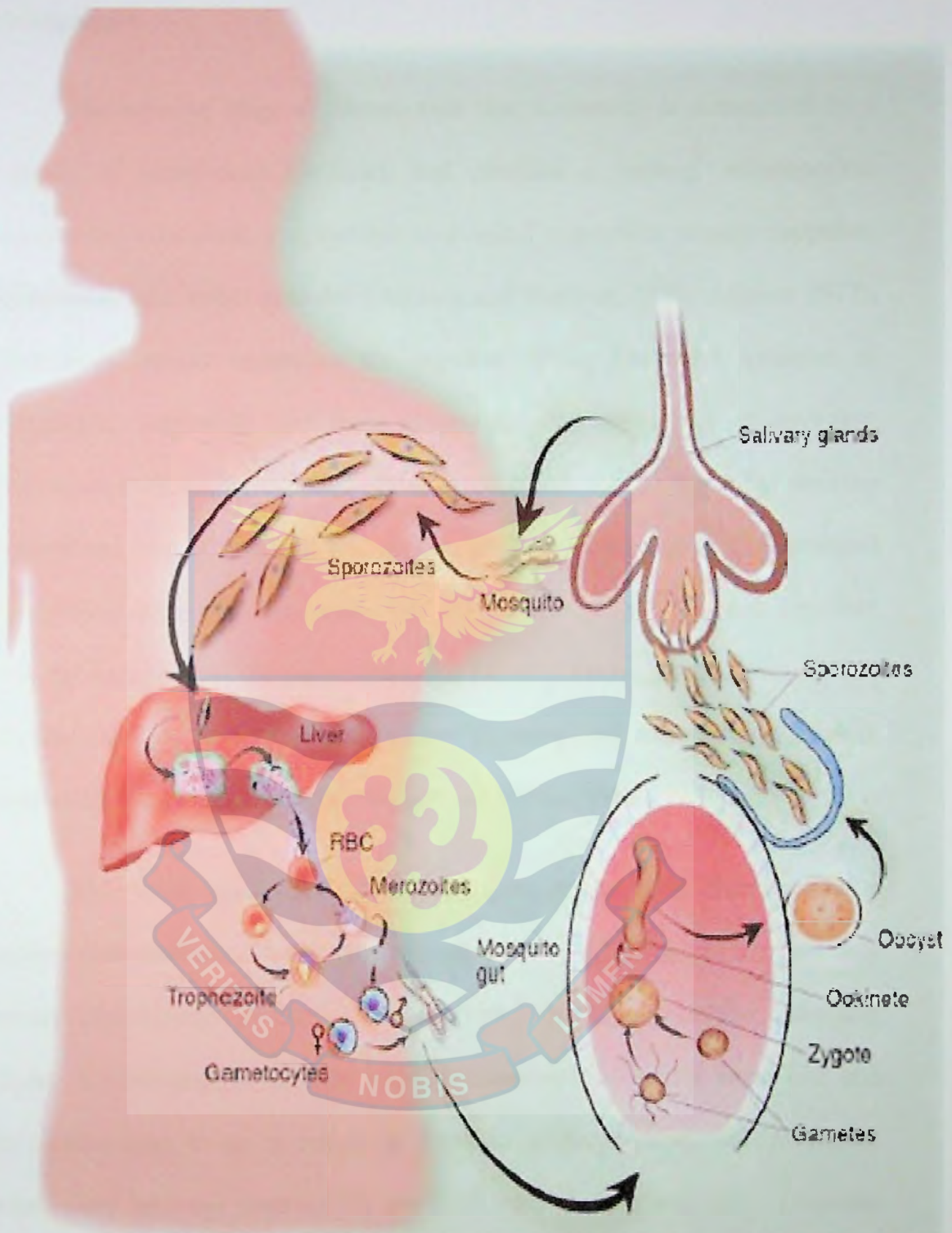
Fig 2: Life Cycle of *Plasmodium* sp. (© Ken Beauchamp *J. Clin. Invest*)

15

Pathogenesis

The invasive stage of *Plasmodium* (the merozoite) is surrounded by a complex of membranes (pellicle), and contains a nucleus, mitochondria, endoplasmic reticulum, a cytostome and apical organelles namely rhoptries, micronemes and dense granules (Aikawa and Sterling, 1974; Aikawa 1977). Contents of apical organelles are expelled during merozoite invasion of erythrocyte, suggesting that these organelles play some role in invasion. Microneme contents are expelled first and occur with initial contact between the parasite and host (Carruthers and Sibley, 1999). The rhoptries are discharged immediately after the micronemes and the release of their contents correlate with the formation of the parasitophorous vacuole. Dense granule contents are released after the parasite has completed its entry, and therefore, are usually implicated in the modification of the host cell (Wiser, 1999).

Merozoites rapidly and specifically enter erythrocytes. This specificity implies receptor-ligand interaction, exemplified by *P. vivax*, which can only invade erythrocytes of Duffy-positive (FyFy) phenotype (Miller, Manson, Clyde, & McGiniss, 1976). The initial interaction between the merozoite and the erythrocyte is as a result of random collision involving reversible interactions between proteins on the merozoite surface and the erythrocyte (Wiser, 1999). Following the binding, the parasite reorients itself so that its apical end is superimposed on the erythrocyte and a tight junction forms between the merozoite apical end and Erythrocyte membrane. Apical membrane

16

antigen-1 (AMA-1), which is localized at the apical end, has been implicated in merozoite reorientation (Mitchell, Thomas, Margos, Dluzewski, & Bannister, 2004).

The junction formation is initiated by microneme discharge which exposes the receptor-binding domains of parasite ligands (Wiser, 1999). In *P. falciparum,* Erythrocyte Binding Antigen–175 (EBA-175) binds to sialic acid residue on glycophorins of the RBCs (Ockenhouse et al., 2001). In *P. vivax,* Duffy Binding Protein (DBP) binds to Duffy antigens of the RBCs (Miller, Baruch, Marsh, & Doumbo, 2002). Both EBA-175 and DBP are microneme proteins. Entry into the erythrocyte is neither by uptake nor phagocytosis (Wiser, 1999). A parasite protease cleaves band 3 on the erythrocyte surface (Braun-Breton, & Pereira, 1993), thereby disrupting the underlying cytoskeleton. The impetus for entering the erythrocyte is provided by the merozoite (Wiser, 1999).

The paroxysms of fever and chills that characterize malaria are related to the rapture of erythrocytes and release of merozoite and parasite products (Biggs and Brown, 2001), including Glycosylphosphotidylinositol (GPI)-linked proteins. A glycolipid material, associated with the GPI, has properties of bacterial endotoxins (Bate, Taverne, & Playfair, 1988; Bate, Taverne, & Playfair, 1990) and hence induces release of cytokines in a similar way as bacterial endotoxins (Clark, Virelizier, Carswell, & Wood, 1981; Kwiatkowski, Cannon, Manogue, Dinarello, & Greenwood, 1989).

17

The greater pathogenecity of *P. falciparum* is contributed by several factors. It has the ability to amplify to high parasitaemia levels since it invades erythrocytes of any age (Biggs and Brown, 2001) leading to profound anaemia (McAdam and Sharpe, 2005). Parasitized erythrocytes express parasite-derived proteins called *Plasmodium falciparum* Erythrocyte Membrane Protein 1 (PfEMP1) (Miller et al., 2002). PfEMP1 are variant surface antigen (VSA) proteins encoded by a family of about 60 'var' genes (Su et al., 1995). PfEMP1, together with other proteins, form knobs on the surface of the erythrocytes (Chen, Schlichtherle, & Wahlgren, 2000). Specific domains of PfEMP1 attach to the complement receptor CR1, heparin sulphate-like glycosaminoglycans (HS-like GAGs), immunoglobulin M (IgM), blood group A antigen, and other red cell molecules (Miller et al., 2002) as shown in fig 3. This causes the adherence of uninfected erythrocytes onto infected ones, a phenomenon referred to as rosetting. Infected erythrocytes may also bind onto themselves in a process called clumping. Parasite-infected erythrocytes may, again, bind to dendritic cells, causing downregulation of the host's immune response (Miller et al., 2002).

Another important difference between *P. falciparum* and other human malarias is the way in which *P. falciparum* modifies the surface of the erythrocytes so that asexual parasites and gametocytes can adhere (cytoadherence) to the endothelium and thus, disappear from circulation. This process, known as sequestration, is the hallmark of falciparum malaria (Biggs and Brown, 2001). Sequestration occurs in the capillaries and postcapillary

18

venules of vital organs such as the brain, lungs, heart, kidney, liver, pancreas, intestines as well as in the intervillous spaces of the placenta (Prommano et al., 2005; Miller et al., 2002; White and Ho, 1992; Luse and Miller, 1971). It is mediated by the binding of the parasite-derived ligand, PfEMP1, to a variety of host endothelial receptors (Baruch et al., 1995). The GPI-linked proteins released upon erythrocyte lysing induce the production of high levels of cytokines, including Tumour Necrosis Factor (TNF), interferon γ (IFN-γ) and interleukin − 1 (IL-1) (Chen et al., 2000). The cytokines cause dyserythropoiesis, increase fever, induce nitric oxide production, leading to tissue damage, and also induce the expression of endothelial receptors for PfEMP1 (McAdam and Sharpe, 2005). Many putative endothelial cytoadherence receptors have been described, including thrombospondin (TSP), hyaluronic acid (HA), endothelial-leukocyte adhesion molecule 1 (ELAM-1), P-selectin (P-Sel), vascular cell adhesion molecule 1 (VCAM-1), platelet endothelial cell adhesion molecule (PECAM), chondroitin sulphate A (CSA) and CD36 (Miller et al., 2002; Biggs and Brown, 2001; Wiser, 1999).

Cytoadherence and the related phenomena of rosetting and clumping lead to microcirculatory obstruction in falciparum malaria (Dondorp, Pongponratn, & White, 2004). Microcirculatory obstruction results in activation of the vascular endothelium, endothelial dysfunction, together with ischaemia and hypoxia, leading to anaerobic glycolysis, lactic acidosis and cellular dysfunction (White, 2009). Ischaemia , due to poor perfusion, causes the manifestations of cerebral malaria, which is the main cause of death in malaria

19

in children (McAdam and Sharpe, 2005). *P. vivax, P. ovale* and *P. malariae* do not sequester, hence they do not cause microcirculatory obstruction and are rarely fatal (Biggs and Brown, 2001).
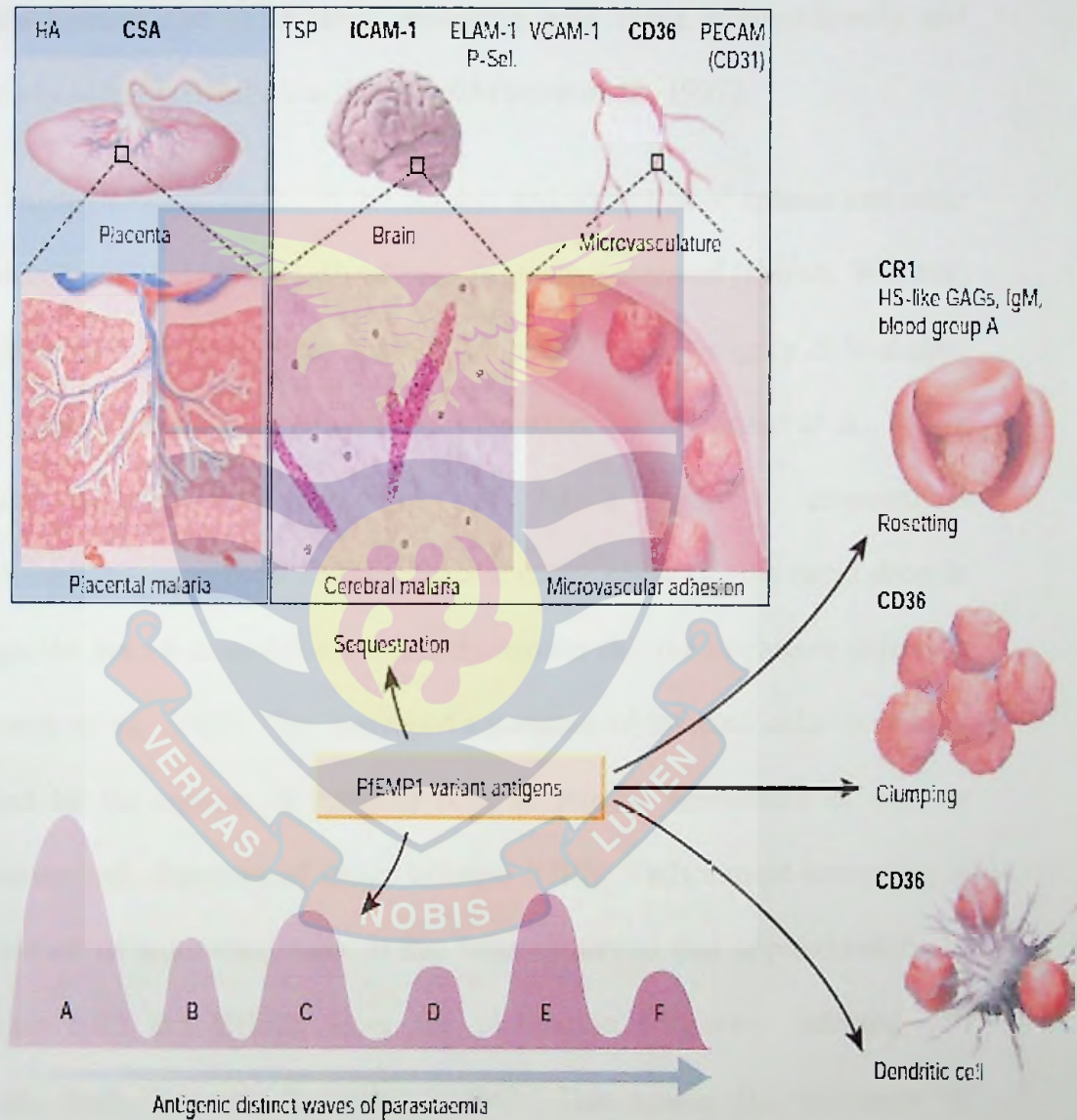
Fig 3: Pathogenesis of falciparum malaria (© Miller et al., 2002)

20

Uncomplicated falciparum malaria presents non-specific symptoms but severe form of the disease presents a number of syndromes. Anaemia and coma are the most important syndromes in childhood malaria. These syndromes have been found to be more prevalent in children with different ages. The median age of children presenting with severe malarial anaemia is typically 1 to 3 years old, while the median age of children presenting with coma is significantly and consistently older, typically 3 to 5 years old (Snow et al., 1997).

During malarial infection the number and activation of splenic and other macrophages for phagocytosis of red cells is greatly increased (Brown, Webster, Teja-Isavadharm & Keeratithakul, 1990; Mohan, Dubey, Ganguly & Mahajan, 1995; Ladhani, Lowe, Cole, Kowuondo & Newton, 2002; Jenkins *et* al., 2006). Increased extravascular haemolysis of RBCs with a concomitant dyserythropoeisis have been found to be the major cause of the rapid drop in haemoglobin during acute infection and the slower decline in chronic infection (Lamikanra et al., 2007). The increased clearance of infected cells is readily explained by the rupture of infected cells to release merozoites as well as opsonisation and clearance of intact infected RBCs. Rather more intriguing is the clearance of uninfected cells. It has been estimated that approximately 10 uninfected cells are cleared from the circulation for every infected cell (Jakeman, Saul, Hogarth & Collins, 1999). This makes the clearance of uninfected cells critically important for the development of malarial anaemia.

21

The increased clearance of uninfected erythrocytes is due to extrinsic and intrinsic changes to the RBCs that enhance their recognition and phagocytosis. Deposition of parasite-derived antigens on the surface of uninfected RBCs reduces deformability leading to enhanced clearance in the spleen. Severe reduction in red cell deformability has been recognized to be a strong predictor for mortality measured on admission, both in adults and children with severe malaria (Dondorp et al., 1997; Dondorp et al., 2002). Second, the deposition of immunoglobulin and complement on uninfected RBCs may enhance receptor-mediated uptake by macrophages. Deposition of malaria-specific immune complexes on the surface of RBCs has been found to be very frequent in children with malaria (Facer, Bray & Brown, 1979; Facer, 1980). The clearance of these immune-complex-coated uninfected RBCs is, however, highly regulated by a number of plasma and membrane proteins including Complement receptor 1 (CR1 or CD35), decay accelerating factor (DAF or CD55) and the membrane inhibitor of reactive lysis (MIRL or CD59). CR1 may enhance binding of C3b in immune complexes, CR1 and CD55 enhance inactivation of C3 convertases and CD59 interferes with the assembly of the terminal components of complement that form the membrane attack complex (Devine, 1991).

It has been shown that the amount of red cell surface IgG is increased but red cell surface CR1 and CD55 is reduced in children with severe malaria compared with asymptomatic and symptomatic controls (Waitumbi, Opollo, Muga, Misore, & Stoute, 2000). The reduction in these proteins (CR1, CD55

22

and CD59) may, thus, increase the susceptibility of children to malarial anaemia (Kai & Roberts, 2008). Population studies in Europe and Africa showed the CR1 expression was strongly age dependent and increases of both CR1 and CD55 were seen after 4 years of age and low levels of CR1 and CD55 expression were seen in a cases of severe malarial anaemia compared with slightly older children with cerebral malaria (Waitumbi, Donvito, Kisserli, Cohen, & Stoute, 2004).

Genetic polymorphisms also affect the expression levels, sequence and domain structure of CR1 in Africans and other populations (Xiang, Rundles, Hamilton, Wilson, 1999; Cockburn et al., 2004). Moreover, CR1 is a ligand for the variant antigens expressed at the surface of infected RBCs allowing the formation of rosettes of infected and uninfected RBCs (Udomsangpetch et al., 1989; Rowe, Moulds, Newbold, & Miller, 1997). In Melanesian populations, low levels of CR1 expression have been associated with reduced rosette formation and protection from severe malaria (Cockburn et al., 2004). It is possible therefore, that age-related and genetically determined reduction of levels of CR1 expressed on RBCs are associated with an increased susceptibility to anaemia but protection from other forms of severe malaria and may provide an example of how innate resistance to one syndrome of malaria may be at the expense of susceptibility to other pathophysiological pathways involved in malaria infection.

23

Global burden of malaria

As illustrated in fig 4, malaria is endemic in about 106 countries worldwide. In 2009 there were an estimated 225,000,000 malaria cases and 781,000 malaria deaths worldwide. The global number of cases was estimated to have increased between 2000 and 2005 in line with population growth and decreased subsequently due to the impact of malaria control. The largest percentage reductions since 2005 were estimated to have occurred in the European Region (86%) followed by the Region of the Americas (42%). The vast majority of cases in 2009 (78%) were in the African Region, followed by the South-East Asia (15%) and Eastern Mediterranean Regions (5%) (WHO, 2010a).

The global number of malaria deaths is estimated to have decreased from 985,000 in 2000 to 781,000 in 2009. The largest percentage decreases were seen in the Region of the Americas (48%) and the largest absolute decline was observed in the African Region. It is estimated that 91% of deaths in 2009 were in the African Region, followed by the South-East Asia (6%) and Eastern Mediterranean Regions (2%). About 85% of deaths globally were in children under 5 years of age. The number of deaths in the South-East Asian Region is higher than previously estimated owing to increased estimates in India and Indonesia (WHO, 2010a).

Aside morbidity and mortality, malaria has been found to exert constraints on the economic growth of affected countries. It is estimated to be

24

responsible for a 1.3% annual reduction of economic growth in affected countries (Sachs and Malaney, 2002). General poverty, lack of access to treatment, low quality housing, political instability and inexistence of active malaria control programmes increase malaria transmission (Miller et al., 2002; WHO, 2005). Since poverty is endemic in the tropics and subtropics (Sachs and Malaney, 2002), it is no surprise that malaria is also endemic in these regions.

Even though malaria prevalence exists in the tropical and subtopical world, the burden varies in different geographical areas. An interplay of host, parasite, geographical and social factors account for the variation in malaria burden (Miller et al., 2002). The parasite and vector are both sensitive to low temperature, explaining the concentration of malaria in tropical and subtropical regions.
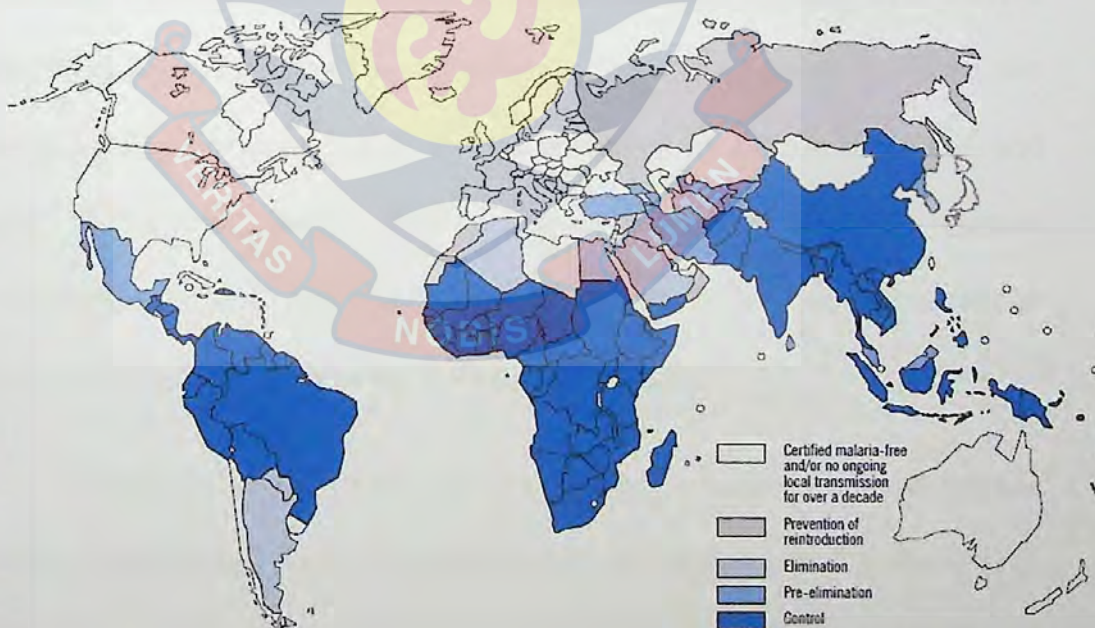
Fig 4: Global malaria burden. (© WHO, World malaria report 2008)

25

Malaria is usually a 'rainy season diseaese' coinciding with increased mosquito abundance. In some areas, however, parasite rates are relatively constant throughout the year, but the majority of cases still do occur during the rainy season (White, 2009). Prolonged drought, such as the sub-sahelian drought, can reduce rainfall and hence mosquito population and malaria transmission. Deforestation, population migration and chages in agricultural practices have profound effects on malaria transmission (White, 2009)

Host resistance to malaria

Malaria has been the strongest known force for evolutionary selection of the human genome (Kwiatkowski, 2005). The mark of malaria, especially falciparum malaria, has been the huge mortalities recorded annually, especially in children. However, some individuals living in malaria endemic areas survive untreated attacks, signifying some protection against the disease. There are two general mechanisms contributing to host resistance to malaria (McAdam and Sharpe, 2005). First, inherited alterations in erythrocytes make people resistant to the disease. Second, repeated or prolonged exposure to *P.* sp. stimulates an immune response that reduces the severity of the illness.

Erythrocyte abnormalities in haemoglobin, enzymes and membrane proteins confer some level of resistance to consequences of malaria. The geographical distributions of hemoglobin S (HbS), hemoglobin C (HbC) and alpha+-thalassemia strongly suggest balancing selection with malaria. The

26

greatest protection is, perhaps, conferred by sickle cell trait (Hill, 1992). Children who are carriers of haemoglobin S (HbAS) have been found to have 90% protection against severe malaria (Hill, 1992). Erythrocytes with haemoglobin S sickle at low oxygen tension and thus resist invasion by merozoites. If invasion is successful, the parasite utilizes the available oxygen causing the erythrocyte to sickle, facilitating their clearance by the reticuloendothelial system. Sequestration of parasitized erythrocytes in microvasculature, where oxygen tension is low, increases the sickling of the parasitized HbAS cells, leading to inhibition of parasite growth and protection against severe malaria (Biggs and Brown, 2001). The geographical distribution of the sickling trait is similar to that of *P. falciparum*, suggesting an evolutionary selection of the trait by the parasite (McAdam and Sharpe, 2005).

The thalassaemias and Glucose-6-phosphate dehydrogenase (G6PD) deficiency confer a weaker protection against malaria (Weatherall, 1997; Ruwende et al., 1995).

Absence of some erythrocyte surface proteins may also contribute to resistance to malaria. For example, *P. vivax* is unable to invade Duffy antigen-negative erythrocytes. The Duffy antigen is required by *P. vivax* to invade erythrocytes. People with African origin are resistant to infection by *P. vivax* due to the absence of the Duffy antigen on the erythrocytes of such Africans (Miller et al., 1976).

27

In contrast to *P. vivax*, *P. falciparum* can invade erythrocytes through many redundant pathways at equal or reduced efficiency (Hadley et al., 1987; Dolan, Miller, & Wellems, 1990; Okoyeh, Pillai, & Chitnis, 1999). Sialic acid-dependent pathways involving RBCs and parasite receptors have been identified (Miller et al., 2002). In these pathways parasite receptors such as the EBA-175 and BAEBL33 bind to sialc acid residues on glycophorins A, B, and C/D (Sim, Chitnis, Wasniowska, Hadley, & Miller, 1994).

The erythrocyte-membrane sialoglycoprotein glycophorin A and B determine the ABO blood group of humans. Individuals possessing only glycophorin A belong to blood group A, those possessing only glycophorin B, blood group B, those possessing both glycophorins, blood group AB, and those possessing non, blood group O. The A and B antigens have been identified as receptors on uninfected RBCs to the parasite derived PfEMP1 ligand on infected RBCs (Barragan, Kremser, Wahlgren, & Carlson, 2000). Thus, rosetting occurs readily in individuals with A and/or B antigens (i.e. blood groups A, B and AB) putting them at a higher risk of getting severe malaria than their counterparts with blood group O. *P falciparum* forms larger and more stable rosettes in non-O blood groups than in blood group O (Udomsangpetch, Todd, Carlson, & Greenwood, 1993; Carlson & Walhgren, 1992). Although rosettes form in group O RBCs, they are smaller and weaker than in non-O RBCs (Carlson & Walhgren, 1992; Barragan et al., 2000). Again, the percentage of infected RBCs forming rosettes has been found to be significantly lower in fresh clinical isolates derived from group O than in non-O patients

(Barragan et al., 2000). These phenomena may offer a survival advantage to blood group O patients over groups A, B and AB patients in severe malaria (Afoakwah et al., unpublished data).

Another erythrocyte membrane protein that has been implicated in malaria resistance is band 3 (Kwiatkowski, 2005). A 27 base pair deletion in the band 3 gene results in a form of ovalocytosis (Jarolim et al., 1991) that is common in Southeast Asia. This Southeast Asian ovalocytosis confers protection both against malaria infection (Foo, Rekhraj, Chiang, & Mak, 1992; Cattani, Gibson, Alpers, & Crane, 1987) and cerebral malaria (Genton et al., 1995; Allen et al., 1999)

Clinical disease

Clinical manifestations of malaria depend on the previous immune status of the host. In areas of intense *P. falciparum* malaria transmission, adults usually show asymptomatic parasitaemia due to development of immunity (premunition). Hence, the presence of parasites in a febrile adult living in a high transmission area does not necessarily mean malaria is the cause of sickness (Smith, 2007). Childhood febrile disease is, thus, the most common clinical manifestation of *P. falciparum* infection since children have very little or no immunity resulting from lack of continuous exposure. It is the endpoint most commonly used to measure the public health burden of the disease (Olotu et al., 2010).

29

Malaria presents two forms of disease; uncomplicated and complicated/severe malaria. The fundamental feature of malaria is fever. In uncomplicated malaria the fever is preceded by non-specific symptoms like headache, abdominal discomfort, fatigue, malaise, nausea, lethargy, myalgia and loss of appetite. These symptoms are difficult to distinguish from other febrile illnesses. Symptoms for uncomplicated malaria do not differ in all five *Plasmodium sp*, but the rate of progression and severity of symptoms may differ. *P. vivax* tends to cause severe symptoms early in the course of the infection, *P. malariae* and *P. ovale* both have a more gradual onset, while the onset of *P. falciparum* ranges from gradual to fulminant (White, 2009). The fever in malaria usually regularizes to a 2-day cycle (tertian malaria) in *P. falciparum, P. vivax* and *P. ovale*, and a 3-day cycle (quartan malaria) in *P. malaria. P. falciparum*, however, may remain erratic and never regularize to a tertian pattern (Kitchen, 1949). As infection continues there is hepatosplenomegaly and patient looses weight. If no treatment is given, the natural infection stabilizes for several weeks or months and gradually resolves (White, 2009).

If untreated, uncomplicated malaria may progress to complicated/severe malaria. Almost all severe cases of malaria are caused by *P. falciparum*. Rarely *P. vivax* and *P. ovale* produce serious complications, debilitating relapse and even death (Svenson, Maclean, Gyorkos, & Keystone, 1995). The major complications of severe malaria, as revised by WHO, include cerebral malaria (involving drowsiness, impaired consciousness, recurrent convulsions and/or

30

unrousable coma), pulmonary oedema, acute renal failure, severe anaemia, bleeding, acidosis, and hypoglycemia (WHO, 2000a). Severe malaria can develop rapidly and progress to death within a few hours (WHO, 2000a). Age less than 5 years, age greater than 65 years, female gender (especially when associated with pregnancy), nonimmune status, comorbidities, lack of antimalarial prophylaxis, delay in treatment and severity of the illness at admission have been identified as the major risk factors for severe malaria and death (Blumberg, Lee, Lipman, & Beards, 1996; Schwartz, Sadetzki, Murad, & Raveh, 2001; Bruneel et al., 2003).

Infants do not develop complicated malaria frequently. If they do, however, the mortality is quite high (White, 2009). Passive transfer of maternal immunity (McGregor, 1984) through breastfeeding and high haemoglobin F content of the infants' haemoglobin, which retards parasite development (Pasvol, Weatherall & Wilson, 1977), is responsible for the observed infrequent malaria in infants.

Diagnosis of malaria

Appropriate therapeutic intervention of malaria is essential to avoid non-target effects, delay the advent of resistance, save cost on alternative drugs (Wongsrichanalai, Barcus, Muth, Sutamihardja & Wernsdorfer, 2007) and prevent the progression of uncomplicated disease to a complicated one (Castelli and Carosi, 1997). This is unachievable without accurate diagnosis of the

31

disease. Limited attention is paid malaria diagnosis in the fight against the disease. Only a very small percentage of the total investment in malaria research and development is dedicated to malaria diagnosis (Malaria Research and Development Alliance, 2005). Due to drug resistance and its consequent requirement of more expensive drugs unaffordable to poor countries (Barnish, Bates & Iboro, 2004), WHO has recently recommended confirmatory diagnosis before treatment of malaria (WHO, 2010a).

Clinical diagnosis is the least expensive most commonly used method and it is the basis for self treatment (Wongsrichanalai et al., 2007). The extremely wide spectrum of clinical symptoms of malaria makes accurate clinical diagnosis challenging, especially in malaria endemic countries where parasitaemia does not always correspond with morbidity. The accuracy of this diagnostic method is affected by factors like the level of endemicity, malaria season, and age. Clinical diagnosis, as reported in numerous studies, has resulted in alarming rates of overdiagnosis (van der Hoek, Premasiri & Wickremasinghe, 1997; Stephens, Phanart, Rooney & Barnish, 1999; Bell et al., 2001; Dicko et al., 2005; Mwangi, Mohammed, Dayo, Snow & Marsh, 2005; Othnigue, Wyss, Tanner & Genton, 2006; Reyburn, Ruanda, Mwerinde & Drakeley, 2006). Treatment based on clinical diagnosis is acceptable only in young children living in high transmission areas (WHO, 2000b; Chandramohan, Jaffar & Greenwood, 2002). Prompt parasitological confirmation is, hence, recommended in all patients suspected of malaria before treatment is started (White, 1997b).

32

Light microscopy of thick and thin stained blood smears remain the standard method for diagnosing malaria (Moody and Chiodini, 2000). It involves relatively less equipment and can be used to quantify parasitaemia, identify *Plasmodium* species, differentiate asexual parasite stages from gametocytes (Trampuz, Jareb, Muzlovic & Probhu, 2003). It can detect parasitaemia as low as 5 parasites/µl (Bruce-Chwatt, 1984; Payne, 1988; Warrell and Giles, 2002). A more realistic threshold of 50-100 parasites/µl is applicable under field conditions (Milne, Kyi, Chiodini & Warhurst, 1994) and in remote settings where quality equipment and skilled microscopists are lacking, an even higher threshold is probable (Wongsrichanalai et al., 2007). However, the diagnostic accuracy of light microscopy is highly variable, depending on the quality of reagents used to stain the blood smear, the condition of the microscope, the time spent reading the smear, the skill with which the blood smear was prepared, workload and the experience of the microscopist (Trampuz et al., 2003; Wongsrichanalai et al., 2007). Significant reporting delays, misdiagnosis and incorrect species identification are, hence, common in laboratories that depend on microscopy (Kain & Keystone, 1998), even in developed countries (Milne et al., 1994; Thomson, Lohman, Crawford, Dubash & Richardson, 2000; Johnston et al., 2006). Artefacts, including bacteria, fungi, stain precipitation, dirt and cell debris, are commonly generated on poorly prepared blood films (Houwen, 2002) resulting in false positive results. Decreasing parasite densities and sequestration of parasites increases the chance of false negative results (McKenzie, Sirichaisinthop, Miller, Gasser &

33

Wongsrichanalai, 2003; Maguire et al., 2006). Hence, a blood film may only be declared negative if the recommended 100 – 400 fields have been carefully observed by an expert microscopist (WHO, 1991).

An array of alternate diagnostic tests have, therefore, been produced to be used independent of or in conjunction with light microscopy to give more accurate diagnosis of malaria. Such tests include detection of malaria antibodies by indirect immunofluorescence antibody assay (IFA) and enzyme-linked immunosorbent assay (ELISA) (Sulzer, Wilson & Hall, 1969; Spencer, Collins, Chin & Skinner, 1979), as well as detection of malaria antigens through immunochromatographic assay (which forms the basis of malaria rapid diagnostic test (RDT) kits) (Shiff, Minjas & Premji, 1994; Moody and Chiodini, 2002), DNA probes and polymerase chain reaction (PCR) (Bruce-Chwatt, 1984; Snounou, Viriyakosol, Jarra, Thaithong & Brown, 1993).

Different RDTs have been produced to detect plasmodia specific and non-specific antibodies. They utilize a monoclonal antibody to a parasite antigen on an immunochromatographic strip to detect the presence of parasites in peripheral blood (Wongsrichanalai et al., 2007; Bronzan, McMorrow & Kachur, 2008). RDTs require neither capital investment nor electricity. They are simple to perform and are easy to interpret (Wongsrichanalai et al., 2007). Currently available RDTs target the histidine-rich protein 2 (HRP2), *Plasmodium* lactate dehydrogenase (pLDH) and aldolase. HRP2 is specific for *P. falciparum* while pLDH and aldolase are common to all human species of

34

Plasmodium. HRP2 often persists in the patient's blood for weeks after successful parasite clearance. pLDH on the other hand is readily cleared from the patient's blood following successful treatment, making pLDH a more appropriate target for monitoring treatment (Moody, Hunt-Cooke, Gabbett & Chiodini, 2000). However, plasmodial gametocytes also produce pLDH, hence an pLDH-base RDT test may remain positive even after clearance of asexual parasite forms (Miller, McDaniel & Wongsrichanalai, 2001). False positives are, therefore commonly reported in malaria RDTs due to the above-mentioned factors. Cross-reactivity with rheumatoid factor may also result in false positive results (Laferl, Kandel & Pichler, 1997; Grobusch, Alpermann, Schwenke, Jelinek & Warhust, 1999; Mishra, Samantaray, Kumar & Mirdha,1999).

To be considered useful diagnostic tool, RDTs must achieve a sensitivity greater than 95% (WHO, 2000b). Numerous studies, as shown in Table 1 below, have reported very high sensitivity for malaria RDT kit (Richardson, Ciach, Zhong, Crandall & Kain, 2002; Farcas, Zhong, Lovegrove, Graham & Kain, 2003; Grobusch et al., 2003; Palmer et al., 2003; Forney et al., 2003; Iqbal, Muneer, Khalid, & Ahmed, 2003; Pattanasin et al., 2003; Buchachart et al., 2004; Fernando, Karunaweera, & Fernando, 2004; Mboera et al., 2006; Acheampong et al., 2011) offering a great promise in extending rapid diagnosis to areas where traditional microscopy is impracticable (WHO, 2003). However, the implementation of RDT as independent diagnostic tool based on result of the over 100 published RDT trials may be problematic. Comparative assessment of these trials is difficult because they do not share common guidelines, clinical

35

and epidemiological characteristic of study populations vary, reference standards vary, and batch numbers and manufacturers of RDT kits are different.

PCR is increasingly being used as the gold standard for malaria diagnosis in research and reference laboratories (Bronzan et al., 2008). It is capable of detecting parasites below the detection threshold for microscopic identification (10 parasites/µl of blood). It can detect parasitaemia as low as 1 parasite/µl of blood. With an expert microscopist, however, sensitivity of microscopy may not be different from that of PCR (Warrel and Giles, 2002). The usefulness of PCR is in species identification, especially in cases where parasite morphology is distorted. It is also useful in identifying human cases of *Plasmodium sp* that are typical of animals (Bronzan et al., 2008).

Table 1: Reported sensitivities and specificities of some previous studies

STUDY	SENSITIVITY (%)	SPECIFICITY (%)
Richardson et al., 2002	97	96
Palmer et al., 2003	98	100
Grobusch et al., 2003	95	97
	91	99
	98	99
	76	100
Farcas et al., 2003	94	99
Iqbal et al., 2003	85	99

36

Pattanasin et al., 2003	90	96
	88	92
Forney et al., 2003	98	93
Buchachart et al., 2004	96	93
Fernando et al., 2004	100	100
Mboera et al., 2006	90	97
Acheampong et al., 2011	79	95

Control

Control of malaria has two facets; prevention of the infection and treatment of infected persons. Both facets aim at curtailing transmission of the parasite. The need for effective malaria control remains great since malaria eradication has eluded most endemic countries. Most malaria control programmes have not yielded the expected outcomes due to development of drug resistance among parasites and insecticide resistance among mosquitoes. This has heightened the need for an effective malaria vaccine (Biggs and Brown, 2001). Until an effective vaccine is developed, malaria control will continuously be based on prevention of infection and treatment of infected persons. So far global malaria prevention strategies rely on vector control through Insecticide Treated Net (ITN) usage and Indoor Residual Spraying (IRS) as well as Intermittent Preventive Treatment (IPT) of risk groups,

predominantly young children under 5 years and pregnant women in endemic areas (WHO, 2010a).

The objectives of malaria vector control are twofold: to protect people against infective anopheline mosquito bites by reducing vector longevity, vector density and human-vector contact; and to reduce the intensity of local malaria transmission at community level and hence the incidence and prevalence of infection and disease (WHO 2010).

Insecticide treated nets (ITNs)

Mosquito nets offer a physical protective barrier, preventing mosquito bites. But untreated mosquito nets have not demonstrated protective efficacy (White, 2009). Hence, the nets are often treated with pyrethroid insecticides. The insecticides repel and/or kill the mosquitoes that come into contact with the nets, adding a chemical barrier to the physical one (WHO/GMP, http://www.un.org/millenniumgoals). The use of ITNs have proved remarkably effective in some areas. It reduced the overall child mortality by 60% in Gambia (Alonso et al., 1991). When used by a majority of the target population, ITNs provide protection for all people in the community including those who do not themselves sleep under nets (Binka et al., 1998; Hawley et al., 2003). Thus, the protection afforded by sleeping without a net in a village where ITNs are used extensively, may be greater than sleeping under an ITN in a village where no one else uses them (White, 2009).

38

A single impregnation of a mosquito net will provide protection for about 1 year (Lindsay and Gibson, 1988; Alonso et al., 1991) after which the net should be retreated. Currently, WHO recommends that ITNs be replaced by long-lasting insecticidal nets (LLINs). LLINs are designed to maintain their biological efficacy against vector mosquitoes for at least three years in the field under recommended condition or use (WHO/GMP, http://www.un.org/millenniumgoals).

Indoor residual spraying (IRS)

Indoor Residual Spraying (IRS) is the application of long-acting chemical insecticides on walls and roofs of houses and domestic animal shelters in a given area to kill adult mosquitoes that land on the treated surfaces (WHO, 2011). IRS had been very effective in reducing or interrupting malaria transmission in the 1940s and 1950s (Russel, 1955; MacDonald, 1957), helping to eradicate the disease from Europe, the former USSR and several countries in Asia and the Caribbean (WHO, 2006a). The IRS story in Africa has been different. Not much success has been achieved. From 1950s to 1970s malaria eradication pilot projects were initiated in Benin, Burkina Faso, Burundi, Cameroon, Kenya, Liberia, Madagascar, Nigeria, Rwanda, Senegal, Uganda and Tanzania (Lividas, Mouchet, Gariou, & Chastang, 1958; Garret-Jones, 1964; De Zuleta et al., 1964; Kouznetsov, 1977; Beales, Orlov, & Kouynetsov, 1989). High levels of vector control was achieved by these projects. In most cases, however, transmission could not be interrupted. In Botswana, Namibia, South

39

Africa, Swaziland and Zimbabwe, IRS has successfully altered distribution of malaria vectors with consequent alteration in epidemiological pattern of the disease in these contries (Hansford, 1972; Sharp, Sueur, & Becker, 1990; SAMC, 2000). Currently 12 insecticides, belonging to four chemical groups (one organochlorine, six pyrethroids, three organonphosphates and two carbamates), have been recommended by WHO. The choice of insecticide for IRS of a particular area must be informed by insecticide susceptibility/resistance, vector behavior, safety for humans and the environment and cost effectiveness (WHO, 2006a).

The use of IRS in malaria control has significantly declined in recent years despite numerous scientific evidences confirming its efficacy. This is due to lack of government commitment, concern about insecticide resistance and community acceptance (WHO, 2006a). WHO has, however, recommended the introduction and/or scaling up coverage of IRS as a primary malaria control intervention in countries where available data indicates that it can be effective towards achieving malaria targets

Intermittent preventive treatment (IPT)

Mass drug administration in helminthes has proven very useful. It has been described as the cornerstone of global efforts to reduce morbidity and mortality caused by parasitic worms (Humphries, Hguyena, Boakye, Wilson, & Cappelloa, 2012). The story is different in malaria. The use of antimalarial

40

drugs to prevent malaria in non-immune visitors to malaria endemic areas has been a well accepted practice. However, the use of drugs to prevent malaria in long term residents in endemic areas is controversial. Mass drug administration of therapeutic doses of antimalarial drug to whole populations at risk may result in the development of resistance. Chloroquine resistance might have arisen as a result of the use of chloroquine impregnated salt in an attempt to control malaria by mass prophylaxis (Verdrager, 1986).

Intermittent preventive treatment (IPT) involves administration of full therapeutic course of an antimalarial drug to the whole of a population at risk, whether or not they are known to be infected, at specified times with the aim of preventing mortality or morbidity (Greenwood, 2006). In malaria endemic areas, pregnant women and children under 5 years are the most vulnerable groups. IPT is therefore recommended in pregnancy (IPTp) and infancy (IPTi). IPT has been shown to be efficacious to reduce the burden of malaria in pregnant women (Schultz et al., 1994; Praise et al., 1998; Verhoeff et al., 1998; Shulman et al., 1999) and children (Schellenberg et al., 2001; Massaga et al., 2003; Schellenberg et al., 2005; Chandramohan et al., 2005; Macete et al., 2006). Drugs suitable for IPT use must have a long half-life and a very good safety profile. Sulphadoxine-pyrimethamine is the best option for IPT use (WHO, 2008).

41

Antimalarial drugs

Available antimalarial drugs fall into three broad categories: quinoline-containing drugs (quinine, chloroquine, amodiaquine, mefloquine, halofantrine, lumefantrine, piperaquine, pyronaridine, primaquine and tafenoquine); folate antagonists (pyrimethamine, proguanil, chlorproguanil and trimethoprim); and endoperoxides (artemisinin, dihydroartemisinin, artemether, artemotil and artesunate). Several antibacterial drugs like sulphonomides and sulphones, tetracycline, clindamycin, macrolides and chloramphenicol also have antiplasmodial activity although in general their action is slow, and they are normally used in combination with antimalarial drugs (White, 2009).

Quinolines are weak bases and readily concentrate in the acid food vacuole of the parasite (Krogstad and Schlesinger, 1987). It has been suggested that the quinolines kill the parasites by causing swelling of the food vacuole, increasing granularity and ultimate cell lysis (Foote and Cowman, 1994). This is associated with inhibition of haem polymerization, but the detailed mechanism of parasite death caused by the various quinolines are yet to be established. Chloroquine binds to ferriprotoporphyrin IX, a product of haemoglobin degradation (Chou, Chevli, & Fitch, 1980), and thus inhibit haem demerization.

Chloroquine, a 4-aminoquinoline, has been widely used for the treatment and prevention of malaria. Resistance has now rendered it practically ineffective against *P. falciparum* infections in most parts of the world. It, however, remains considerably effective for the treatment of *P. vivax, P. ovale* and *P. malariae*

42

infections. Chloroquine interferes with parasite haem detoxification (Hay, Guerra, Tatem, Noor, & Snow, 2004; Mendis, Sina, Marchesini, & Carter, 2001).

Chloroquine is swiftly and almost completely absorbed from the gastrointestinal tract when taken orally. Absorption is also very rapid following intramuscular and subcutaneous administration (Beg et al., 2002; Mohapatra, Padhiary, Mishra, & Sethy, 2002). Chloroquine is extensively distributed into body tissues, including the placenta and breast milk, and has an enormous total apparent volume of distribution. The relatively small volume of distribution of the central compartment means that transiently cardiotoxic levels may occur following intravenous administration unless the rate of parenteral delivery is strictly controlled. Some 60% of chloroquine is bound to plasma proteins, and the drug is eliminated slowly from the body via the kidneys, with an estimated terminal elimination half-life of 1–2 months. Chloroquine is metabolized in the liver, mainly to monodesethylchloroquine, which has similar activity against *P. falciparum* (WHO 2006b).

Chloroquine is generally well tolerated. It has a low safety margin and is very dangerous in overdosage. Larger doses of chloroquine are used for the treatment of rheumatoid arthritis than for malaria, so adverse effects are seen more frequently in patients with arthritis (WHO, 2006b). The principle limiting adverse effects in practice are the unpleasant taste, which may upset children, and pruritus, which may be severe in dark-skinned patients (Tanios, Kogelman,

43

McGovern, & Hassoun, 2001). Other less common side effects include headache, various skin eruptions and gastrointestinal disturbances, such as nausea, vomiting, diarrhoea, convulsions, keratopathy, retinopathy, myopathy, reduced hearing, photosensitivity and loss of hair. Blood disorders, such as aplastic anaemia, are extremely uncommon (Oh et al., 2001). Acute overdosage is extremely dangerous and death can occur within a few hours. The patient may progress from feeling dizzy and drowsy with headache and gastrointestinal upset, to developing sudden visual disturbance, convulsions, hypokalaemia, hypotension and cardiac arrhythmias. There is no specific treatment, although diazepam and epinephrine (adrenaline) administered together are beneficial (Mehta et al., 2001; Naqvi, Ahmad, Akhtar, Naqvi, & Rizvi, 2003).

Amodiaquine is a Mannich base 4-aminoquinoline. It's mode of action is similar to that of chloroquine. It is effective against some chloroquine-resistant strains of *P. falciparum,* although there is cross-resistance (WHO, 2006b). Amodiaquine hydrochloride is readily absorbed from the gastrointestinal tract. It is rapidly converted in the liver to the active metabolite desethylamodiaquine, which contributes nearly all of the antimalarial effect (Prakash, Singh, Kumar, & Saxena, 2003). Both amodiaquine and its active metabolite are detectable in urine several months after administration.

The adverse effects of amodiaquine are similar to those of chloroquine. It is associated with less pruritus and is more palatable than chloroquine, but is associated with a much higher risk of agranulocytosis and, to a lesser degree, of

hepatitis when used for prophylaxis (Makkar, Mukhopadhyay, Monga, Monga, & Gupta, 2002). It is not clear whether the risks are lower when amodiaquine is used to treat malaria. Following overdose cardiotoxicity appears to be less frequent than with chloroquine. Large doses of amodiaquine have been reported to cause syncope, spasticity, convulsions and involuntary movements (WHO, 2006b).

The folate antagonists inhibit folic acid synthesis in the parasites. Folic acid synthesis is essential to malaria parasites since they are unable to scavenge pyrimidines from their host (Biggs and Brown, 2001). Inhibiting folate synthesis depletes pyrimidines, methionine and serine leading to cell cycle arrest and subsequent parasite death (Foote and Cowman, 1994). Antifolates are grouped into two; the dihydrofolate reductase (DHFR) inhibitors (pyrimethamine and proguanil) and the dihydropteroate synthase (DHPS) inhibitors (sulphanomide and sulphone antibiotics, sulphadoxine and dapsone). There is marked synergy in antimalarial activity between the two classes of compounds (White, 2009).

Artemisinin, also known as qinghaosu, and its derivatives are endoperoxide-containing sesquiterpene lactone. Four derivatives of artemisinin are widely used artemether, artemotil, artesunate and dihydroartemisinin (White, 2009). The mechanism of action of these drugs remain controversial. In the body, the artemisinins are converted into free radicals, which are reported to react with and damage specific *Plasmodium* membrane-associated proteins

45

(Biggs and Brown, 2001). The role of the free radicals in the mechanism of action of the artemisinins has, however, been challenged (Olliaro, Haynes, Meunier, & Yuthavong, 2001). The sarcoplasmic endoplasmic reticulum adenosine triphosphatase 6 (PfATPase6) has been proposed as the primary target of the artemisinins (Eckstein-Ludwig *et* al., 2003), but sufficient evidence has, again, not been found to support this postulate (Afoakwah *et* al., 2011).

The artemisinins are the most potent antimalarials currently in use (Hein and White, 1993). They rapidly kill all of the asexual stages of *Plasmodium* sp (ter Kuile et al., 1993) and gametocytes of *P. falciparum* (Meshnick, Taylor, Koachonwongspaisan, 1996) reducing the infectivity of man to *Anopheles* sp. They have also been found to possess an excellent safety profile (White, 2009) and are considered the best drugs for severe malaria (Dondorp et al., 2005). They rapidly reduce parasitaemia by killing young circulation ring-stage *P. falciparum* parasites, reducing considerably the number of parasites that mature to sequester in and block capillaries (White, 1997a). Artemisinins, however, have very short elimination half lives. Artemisinin and artemether have half lives of 1 hour (Ashton et al., 1998) while artesunate and dihydroartemisinin have half lives of 45 minutes (Hein et al., 2004; Newton et al., 2002). The artemisinins are available as tablets, rectal suppositories and parenteral formulations (Biggs and Brown, 2001). They may be prescribed as monotherpies or in combination therapies with other antimalarials. For effective treatment and prevention of development of resistance to the artemisinins, WHO recommends that uncomplicated malaria in endemic regions be treated

46

with artemisinin-based combination therapies (ACTs) (WHO, 2006b). However, artemisinin monotherapies are still being manufactured and sold in endemic areas (WHO, 2011) threatening the sustenance of ACT treatment policy.

Antimalarial combination therapy

Antimalarial combination therapy is the simultaneous use of two or more blood schizontocidal drugs with independent modes of action and different biochemical targets in the parasite. Thus, multiple-drug therapies that include a nonantimalarial drug to enhance the antimalarial effect of a blood schizontocidal drug (e.g. chloroquine and chlorpheniramine) are not considered combination therapy. Similarly, sulphadoxine-pyrimethamine (SP), sulfalene-pyrimethamine, proguanil-dapsone, chlorproguanil-dapsone and atovaquone-proguanil, which fit the criteria of synergistic fixed-dose combinations are operationally considered as single products since the drug targets in the parasite are linked and neither of the individual components would be given alone for antimalarial therapy (WHO, 2001; WHO, 2006b). The rationale for combining antimalarials with different modes of action is two-fold: to increase efficacy; and to prevent development of drug resistance (WHO, 2006b). The drugs in combination use their various modes of action to clear parasites hence, increasing the treatment efficacy of the combined therapy than the monotherapies of the drugs. Again, in the rare event that mutant parasites that are resistant to one of the drugs arises *de novo*, they will be cleared by the other

47

drug(s) thereby preventing or delaying the development of resistance to the drugs in combination.

Antimalarial drug combinations in use include chloroquine + sulphadoxine-pyrimethamine (CQ+SP), amodiaquine + sulphadoxine-pyrimethamine (AQ+SP), mefloquine + sulphadoxine-pyrimethamine (MSP), quinine + tetracycline and the artemisinin based combination therapies (ACTs). The prevailing high prevalence of resistance has compromised the use of most of these combination therapies. Studies have shown that the efficacy of CQ+SP depends on the level of resistance to the individual components (Darlow et al., 1982; Bojang et al., 1998). There is no convincing evidence that the CQ+SP combination is more effective than sulphadoxine alone. AQ+SP, however, has been shown to be more effective than SP alone (Qilin et al., 1988; Schapira, & Schwalbach, 1988; Dinis & Schapira, 1990). The use of MSP for treating uncomplicated falciparum malaria in Thailand resulted in the rapid development of mefloquine resistance (Nosten et al., 1991; White, 1992) making recommendation of MSP combine therapy difficult. Adherence is a practical challenge in quinine + tetracycline treatment policy. Drug regimens require eight-hourly doses of quinine for 3 to 7 days, and six-hourly doses of tetracycline for 7 days. Tetracycline is, also, contra-indicated in pregnant women, breastfeeding women and children less than 8 years of age (WHO, 2001).

Artemisinin-based combination therapies are the best antimalarial combination therapy produced so far. They have proven to be highly efficacious (Falade et al., 2005; Mutabingwa et al., 2005; Piola et al., 2005; Mulenga et al., 2006; Dorsey et al., 2007). The most common combinations are artemether-lumefantrine, artesunate-amodiaquine, artesunate-sulfadoxine-pyrimethamine, artesunate-mefloquine, and dihydroartemisinin-piperaquine. The choice of ACT to be deployed in a particular area depends on safety, tolerability, adherence, availability, coformulation cost, and effectiveness (Whitty, Chandler, Ansah, Leslie, & Staedke, 2008). The effectiveness of the ACT relies heavily on the effectiveness of the partner drug. In a 3-day ACT regimen, the artemisinin component is present in the body during only two asexual parasite life cycles. This exposure to 3 days of artemisinin treatment reduces the number of parasites in the body by a factor of hundred million. However, complete parasite clearance is achieved by an effective partner drug persisting at parasiticidal concentrations until all the parasites have been killed. Hence, the partner drug needs to be slowly eliminating. As a result of this, the artemisinin component is protected from resistance by the partner drug and vice versa (WHO, 2006b).

To increase the benefit of ACT deployment, and to have an impact on malaria, it will be necessary to deploy them as widely as possible. Deployment through the formal public health delivery system alone will not reach many of those who need treatment. In several countries, they must also be available through the private sector. Ultimately, effective treatment needs to be available at community or household level in such a way that there is no financial or

physical barrier to access. The strategy to secure full access must be based on an analysis of the national and local health systems, and will often require adjustment based on programme monitoring and operational research. The dissemination of clear national treatment guidelines, use of appropriate information, education and communication materials, monitoring both of the deployment process, access and coverage and provision of adequately packaged and presented antimalarials are needed to optimize the benefits of providing these new effective treatments widely (WHO, 2006b).

Antimalarial drug resistance

Wherever antimalarial drugs have been widely used, resistance has eventually followed. Antimalarial drug resistance has been defined by WHO as the ability of a parasite strain to survive and/or multiply despite the proper administration and absorption of the drug given in doses equal to or higher than those usually recommended but within the tolerance of the subject, where the form of the drug active against the parasite gains access to the parasite or the infected erythrocyte for the duration of the time necessary for its normal action (WHO, 2005). Antimalarial drug resistance is a major threat to health in endemic areas. In the last two decades of the twentieth century, the global death toll from malaria rose, while the mortality from other infectious diseases generally fell. This was attributed directly to drug resistance (White, 2009). However upon the introduction of the artemisinins in ACTs, to which the parasites have not developed stable resistance, the death toll from malaria has

50

continuously decreased (WHO, 2010a; WHO, 2011). This success in the malaria story is, however, not attributed to only the artemisinins. ITNs and IRS contributed substantially to it (WHO, 2010a).

P. falciparum has developed stable resistance to all currently used antimalarials including chloroquine, amodiaquine, mefloquine, quinine, and sulphadoxine-pyrimethamine (Simon, Le Bras, Gaudebout, & Girard, 1988; Nosten et al., 1991; White, 1992; White, 1999; Brockman et al., 2000; Trape, 2001). In recent years, artemisinin resistance has emerged in Cambodia and Thailand (Noedl et al., 2008; Dondorp et al., 2009) where the proportion of patients who were still parasitaemic on day 3 after treatment had increased. A similar situation has been reported on the Myanmar-Thailand and China-Myanmar borders (Phyo et al., 2012; Wang et al., 2012). Quinine resistance was first observed in 1910 (Nocht and Werner, 1910). However, the intensity of quinine resistance has not been high enough to compromise use of the drug. Antimalarial drug resistance was not considered important until *P. falciparum* developed stable resistance to chloroquine in most parts of endemic areas in the 1950s (WHO, 2006b). Chloroquine resistance arose simultaneously from Southeast Asia and South America spreading to all malaria endemic areas including Africa. *P. falciparum* has, again, developed resistance to the antifolate, pyrimethamine, and the sulphonamide, sulphadoxine, so that the synergistic combination of the two drugs is no more efficacious in much of East Asia, Southern and Central Africa and South America (White, 2009).

51

Resistance to antimalarials develops when a large population of the parasites is exposed to intense transmission under intense drug pressure (Verdrager, 1986). The resistance begins from the development of *de novo* mutations in the genome which are independent of drug selection pressure (White, 1999). Once the mutation(s) forms, the continuous use of parasite-resistant drug confers selective advantage to parasites that carry the resistant gene(s) (WHO, 2006b). This leads to transmission of the drug-resistant parasites through two mechanisms. First, the use of the parasite-resistant drug causes increase in the number of circulating gametocyte of the drug-resistant parasite (Targett et al., 2001; Drakeley et al., 2004; Pukrittayakamee et al., 2004). Secondly, the gametocytes carrying resistant genes have been shown to be more infectious to mosquitoes, and infect a higher proportion of mosquitoes than those carrying sensitive genes (Jambou, 2005). The said *de novo* mutation, even though very crucial, is not the only determining factor to development of stable resistance. Other parasite, host, environmental, drug and vector factors are also involved. The degree of resistance conferred by the mutation is very crucial. Thus some mutations need to be supported by other independent mutations to confer stable resistance. The level of parasite transmission in the community also is important in the development of drug resistance. In high transmission areas without the necessary drug pressure, a resistant strain of the parasite is not likely to be transmitted. The population in a high transmission area normally has immunity to the parasite. In these semi-immune individuals asymptomatic parasitaemia is very common. The parasites, both resistant and susceptible

52

strains, are therefore likely to be cleared before being transmitted to another individual. Exposure to subtherapeutic concentrations of the drug is another important factor in the development of stable resistance. Subtherapeutic concentrations of the drug successfully eliminate susceptible strains of the parasite while resistant ones are left unaffected to multiply and get transmitted. Thus non-immune patients infected with large numbers of parasites who receive inadequate treatment (because of poor drug quality, adherence, vomiting of an oral treatment, etc.) are a potent source of denovo resistance (White, 2009).

Antimalarials drug resistance is not necessarily the same as "treatment failure", which is a failure to clear malaria parasitaemia and resolve clinical symptoms despite the administration of an antimalarial. While drug resistance may lead to treatment failure, not all treatment failures are caused by drug resistance. Treatment failure can also be the result of incorrect dosing, problems of treatment adherence (compliance), poor drug quality, interactions with other drugs, compromised drug absorption, or misdiagnosis of the patient. These factors may accelerate the spread of true drug resistance by exposure of the parasite to inadequate drug levels (WHO, 2006b).

Genetic markers for artemisinin resistance

P. falciparum **multidrug resistant 1 (Pfmdr1) gene**

Multidrug resistance arises when cells which are resistant to one agent, become resistant to a wide range of structurally unrelated drugs (Juliano and

53

Ling, 1976). In mammals a multidrug resistant (mdr) transporter, also called *P-glycoprotein*, mediates this multidrug resistance. Multidrug resistance in cancer cell lines have been attributed to the mdr transporter. It effectively reduces drug concentrations in the cells. A similar mechanism was identified in chloroquine resistance in *P. falciparum* (Krogstad, Gluzman, Kyle, Oduola, & Martin, 1987), leading to the identification of the plasmodial homologue of the mammalian mdr transporter in *P. falciparum* (Foote, Thompson, Cowman, & Kemp, 1989).

Pfmdr1 is a typical member of the ATP-binding cassette (ABC) trasnsporter superfamily. It is localized to the parasite food vacuole membrane. This P-glycoprotein has been found to import antimalarial drugs and other substances into the food vacuole (Rohrbach et al., 2006). Like the mammalian homologue, *Pfmdr1* modulates the response to various antimalarial drugs by two mechanisms; gene amplification and single nucleotide polymorphisms (SNPs) (Foote, et al., 1989; Foote et al., 1990).

Amplification of *Pfmdr1* is the main contributor to mefloquine resistance (Duraisingh et al., 2000; Price et al., 1999). It has also been found to reduce artesunate susceptibility (Wilson et al., 1993). Expectedly, reduced copy number of *Pfmdr1* decreases *in vitro* susceptibility to mefloquine, lumefantrine and artemisinin (Barnes, Foote, Galatis, Kemp, & Cowman, 1992; Peel, Bright, Yount, Handy, & Baric, 1994; Rohrbach et al., 2006). In *in vivo* studies, amplification of the *Pfmdr1* has rather been associated with treatment failures

54

with mefloquine and artesunate-mefloquine (Price et al., 2004; Lim et al., 2009; Rogers et al., 2009) as well as quinine (Foote et al., 1989).

SNPs in *Pfmdr1* modify the transport of drugs by affecting substrate specificity (Sanchez, Rotmann, Stein, & Lanzer, 2008). Influx of the drug by *Pfmdr1* may reduce or efflux may increase causing a reduction in the concentration of the drug in the parasite. Two *pfmdr1* alleles have been identified in chloroquine resistant (CQR) field isolates: the K1 allele (containing the point mutation N86Y) and the 7G8 allele (containing Y184F, S1034C, N1042D and D1246Y) (Valderramos and Fidock, 2006). *In vitro* susceptibility to mefloquine, lumefantrine, artemisinin, artesunate and dihydroartemisinin have been shown by transfection to be reduced by all the point mutations of the K1 and 7G8 alleles (Duraisingh, Roper, Walliker, & Warhusrst, 2000; Reed, Saliba, Caruana, Kirk, & Cowman, 2000; Sidhu, Verdier-Pinard, & Fidock, 2002; Anderson et al., 2005). In *in vivo* studies, *Pfmdr1* asn-86, 184-phe, asp-1246 have been associated with artemether lumefantrine treatment failure (Sisowath et al., 2005; Dokomajilar, Nsobya, Greenhouse, Rosenthal, & Dorsey, 2006; Sisowath et al., 2007; Happi et al., 2009) and 86-tyr, tyr-184, 1246-tyr have been associated with amodiaquine and artesunate amodiaquine treatment failures (Holmgren et al., 2006; Dokomajilar et al., 2006; Holmgren et al., 2007).

Plasmodium falciparum **chloroquine resistant transporter**

The discovery of *P. falciparum* chloroquine resistant transporter (Pfcrt) was crucial in understanding the mechanism of resistance to chloroquine (Fidock et al., 2000; Valderramos and Fidock, 2006). Pfcrt is a transporter protein found in the membrane of the parasite food vacuole within which haemoglobin is degraded and detoxified and in which cloroquine concentrates and binds hematin thereby preventing synthesis of non toxic hemozoin (Uhlemann, Yuthavong, & Fidock, 2005). A single nucleotide polymorphism in the *Pfcrt* gene at position 76 resulting in a change in coding from lysine to threonine (K76T) has been shown by transfection and epidemiological studies to be the corner stone of chloroquine resistance (Su, Kirkman, Fujioka, & Wellems, 1997; Djimde et al., 2001b; Durand et al., 2001; Sidhu et al., 2002; Lakshmanan et al., 2005). In regions where chloroquine resistance persisted, replacement of chloroquine with other antimalarials resulted in reversion of the mutant 76T to the wild type K76 (Laufer et al., 2006), suggesting that chloroquine resistance may be at a fitness cost to the parasite (White, 2009). Other SNPs of the Pfcrt associated with chloroquine response are N75E/K, Q271E and R371I (Fidock et al., 2000; Wootton et al., 2002). In fact Pfcrt shows extraordinary sequence diversity among geographically distinct isolates with point mutations detected at 15 residues (72, 74, 75, 76, 97, 144, 148, 160, 194, 220, 271, 326, 333, 356, 371) (Valderramos and Fidock, 2006).

Pfcrt has been suggested to be important in the development of resistance to amodiaquine, quinine, halofantrine and artemisinin (Cooper et al., Sidhu et al., 2002, Johnson et al., 2004; Lakshamanan et al., 2005). High parasite susceptibility to mefloquine has been noticed in parasite lines carrying the mutant 76T allele (Sidhu et al., 2002). When the mutant 76T allele was reversed to the wild type K76, decreased susceptibility of parasites to mefloquine was recorded (Lakshmanan et al., 2005). A novel SNP at position 76 (K76I/N) in the Sudanese 106/1 clone of *P. falciparum* has also been found to significantly increase susceptibility to mefloquine and halofantrine (Cooper et al., 2002). Again chloroquine resistant lines carrying the K76T mutation have demonstrated high susceptibility to lumefantrine (Sisowath, et al., 2009). The Pfcrt S163R mutation which has been associated with mefloquine resistance has been found to be selected for by *P. falciparum* cell lines which are resistant to halofantrine (Johnson et al., 2004). Sidhu and his colleagues in 2002 replaced the endogenous pfcrt allele of a chloroquine sensitive line of *P. falciparum* with pfcrt alleles from chloroquine resistant lines of Asian, African or South-American origin and found increased susceptibility by a factor of 2 to 3 to artemisinin and dihydroartemisinin in the pfcrt-modified clones. Afonso et al. (2006) also successfully established a stable artemisinin and artesunate resistance in a chloroquine resistant strain of *P. chabaudi*. In the same study, attempts to establish stable artemisinin or artesunate resistance in a chloroquine-sensitive strain of *P*. chabaudi were unsuccessful leading to the hypothesis that chloroquine resistance may be a pre-requisite for artemisinin resistance.

57

P. falciparum adenosine triphosphatase 6 (PfATPase6)

Recent studies in antimalarials drug resistance have implicated other transporters most notable PfATPase6, the *P. falciparum* ortholog of the mammalian sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA). In the eukaryotic cell, calcium concentration is very regulated by influx and efflux of calcium through plasma membrane and various organelles. There is a steep calcium concentration gradient between the high concentrations in the extracellular space and intracellular organelles and the low concentration in the cytosol. Hence, the rapid release of calcium into the cytosol is used as a major signaling process in human protozoan parasites like *Trypanosoma brucei, Trypanosoma cruzi, Leishmania spp, Plasmodium spp, Toxoplasma gondii, Cryptosporidium parvum, Entamoeba histolytica, Giardia lamblia and Trichomonas vaginalis* (Moreno and Docampo, 2003). The endoplasmic reticulum is a repository of calcium in the cell and a primary source of calcium for signaling (Nagamune, Moreno, Chini, & Sibley, 2008). Calcium is rapidly cleared from the cytosol after its release to terminate signaling and prevent cell toxicity. Influx of calcium into the enoplasmic reticulum is mediated by SERCA. Hence inhibition of SERCA in the protozoans may offset the calcium homeostasis and result in cell toxicity.

Thapsigargin, a sesquiterpene lactone like artemisinin has been found to inhibit SERCA (Eckstein-Ludwig et al., 2003) leading to the hypothesis that artemisinin will inhibit PfATPase6, the only SERCA-type Ca^{2+} ATPase in *P.*

falciparum. PfATPase6 is very much diverse with about 41 SNPs (Afoakwah et al., 2011). Four of the 41 SNPs (L263E, E431K, A623E and S769N) have been shown to inhibit artemisinin action on the PfATPase6 (Jambou et al., 2005; Dahlstrom et al., 2008; Uhlemann et al., 2005). The L263E mutation has been recorded only by laboratory engineering (Uhlemann et al., 2005) and not yet in any wild parasite/field isolate. The question still remains to be answered whether the L263E mutation can ever develop in vivo, since laboratory culture settings arguably cannot be completely projected for internal mammalian conditions. The S769N, A623E and E431K mutations, on the other hand, have been found in field isolates (Jambou et al., 2005; Dahlström et al., 2008). Their occurrence however is very rare with the exception of the E431K mutant, which has been found in samples collected from sixteen Sub-Sahara African countries and China. The S769N mutation has only been found in French Guianan samples and the A623E mutation, in Senegalese and Zanzibari samples. It is just in one case that the A623E and E431K mutations have been found to be associated with reduced P. falciparum susceptibility to artemisinins (Jambou et al., 2005), and in this case they occurred together as a double mutant (even though their occurrence together could not be explained). It is still, thus, unclear if the E431K and A623E mutants can independently reduce *P. falciparum* susceptibility to artemisinins. The prevalence of these resistant SNPs, so far, seems relatively rare possibly because they may confer a loss of fitness to the parasite or they are just insignificant in the development of artemisinin resistance

CHAPTER THREE

MATERIALS AND METHODS

Study sites

The study was conducted in Ghana, situated on the coast of the Gulf of Guinea, at 7.4490° N, 0.9056° W. Ghana is bordered on the northwest and north by Burkina Faso, on the east by Togo, on the west by Cote d'Ivoire, and on the south by the Atlantic Ocean (Encyclopedia Britannica, 2009) as illustrated in Fig 5. The country has an area of 92,098 square miles (Encyclopedia Britannica, 2009) inhabiting a total human population of about 24,233,431 (http://www.statsghana.gov.gh/). The country has two seasons, a dry season and a wet season, with regional variations in rainfall. The dry portion of the savannah has a mean annual rainfall ranging from 1,100 to 1,200 mm, while that of the southern forest ranges from 1,200 to 2,188 mm and that of the coastal zone ranges from 750 to 1,000 mm. The country has three eco-epidemiological zones, which are the savanna, forest and coastal zones. All three zones are malaria endemic and patients have access to antimalarial drugs, especially artesunate + amodiaquine and artemether + lumefantrine, through the local health centers. Malaria transmission occurs all year round in Ghana with peak transmissions in the rainy seasons. *P. falciparum* is the main malaria species.

60
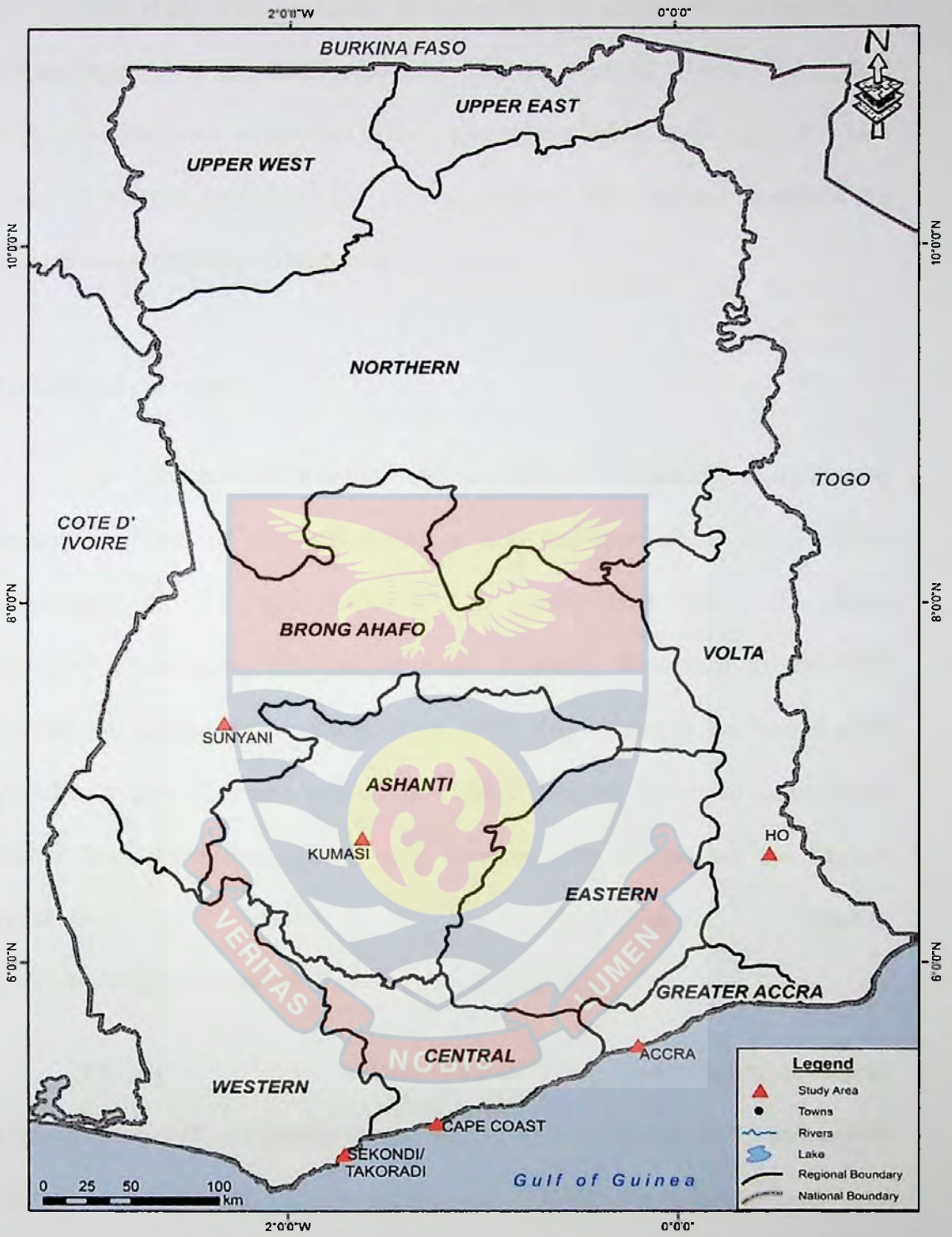
Fig 5: Map of Ghana showing study sites

This study was conducted in six of the ten administrative regions of Ghana, namely Brong-Ahafo, Ashanti, Western, Central, Greater Accra and Volta. The study was conducted in the regional hospitals of these regions, which serve as referral points for the various regions. The regional hospitals are located in administrative capitals of the regions.

Greater Accra region

The Greater Accra Region is the smallest of Ghana's ten administrative regions in terms of area, occupying a total land surface of 3,245 square kilometres or 1.4 per cent of the total land area of Ghana (http://en.wikipedia.org/wiki/Greater_Accra_Region). According to the 2010 census, the region has a population of 3,909,764, making it the second most populous region of Ghana behind the Ashanti Region. Owing to immigration and a high population growth rate, however, the region has the highest population density in the country (http://en.wikipedia.org/wiki/Greater_Accra_Region).

The region is relatively dry since it falls within the dry coastal equatorial climatic zone with temperatures ranging between 20°C and 30°C and annual rainfall ranging from 635 mm along the coast to 1,140 mm in the northern parts. There are two rainfall peaks notably in June and October. The first rainfall season between April and July is associated with the major cropping season in the region (http://ghanadistricts.com/region/). The Greater Accra region is

62

bordered on the north by the Eastern Region, on the east by the Volta Region, on the south by the Gulf of Guinea, and on the west by the Central Region. The prevalence of malaria in the region is 11.6% (http://www.statsghana.gov.gh/Prm.html).

Samples were taken from Ridge Hospital located in the Accra metropolitan area, which serves as the regional hospital for Greater Accra. The Accra metropolitan area, occupying a land area of 344 square kilometers, has a population of 1,981,000 with an annual population growth of 4.4%, making it the most densely populated metropolis in the country (Farvacque-Vitkovic, Raghunath, Eghoff, & Boakye, 2008).

Central region

The Central Region occupies an area of 9,826 square kilometres or 4.1% of Ghana's land area, making it the third smallest in area after Greater Accra and Upper East (http://ghanadistricts.com/region/). It shares common boundaries with Western Region on the west, Ashanti and Eastern Regions on the north, and Greater Accra Region on the east. On the south is the 168-kilometre length Atlantic Ocean (Gulf of Guinea) coastline (http://en.wikipedia.org/wiki/Central_Region_%28Ghana%29). The region has a population of 2,107,209 with a growth rate of 2.7% per annum. It is also the second most densely populated in the country, with a population density of 214 persons per square kilometer (http://www.ghana.gov.gh/census/phc2010.pdf).

63

Central region can be broadly divided into two ecological zones; the coast, which consists of undulating plains with isolated hills and cliffs characterised by sandy beaches and marsh in certain areas; and the hinterland, where the land rises between 250m and 300m

above sea level.

The Region lies within the dry equatorial zone and moist semi-equatorial zone. Annual rainfall ranges from 1,000mm along the coast to about 2000mm in the interior. The wettest months are May-June and September-October while the drier periods occur in December- February and a brief period in August. Mean monthly temperature ranges from 24°C in the coolest month (August) to about 30°C in the hottest months (March-April).

Along the coast can be found the coastal savannah with grassland and few trees while semi-deciduous forest predominates the inland areas. Much of the original dense forest vegetation has been cleared for the cultivation of cocoa and oil palm. Samples were collected from patients visiting the Central Regional Hospital in the Cape Coast metropolis.

Brong-Ahafo region

Brong-Ahafo, the second largest region of Ghana, covers an area of 39,557 square kilometres and shares boundaries with the Northern Region to the north, the Ashanti and Western Regions to the south, the Volta Region to the

64

east, the Eastern Region to the southeast and La Cote d'Ivoire to the west. It has 19 administrative districts, with Sunyani as the regional capital. The central and southern parts of the region lie in the forest zone and they are major cocoa and timber producing areas. The northern part of the region lies in the savannah zone and is a major grain and tuber producing region (http://www.modernghana.com/GhanaHome/regions/brongahafo.asp). The region has a population of 2,282,128, with a population growth rate of 2.2% (http://www.ghana.gov.gh/census/phc2010.pdf).

The region generally has a tropical climate, with high temperatures averaging 23.9°C and a double maxima rainfall pattern. Rainfall ranges, from an average of 1000mm millimetres in the northern parts to 1400 millimetres in the southern parts. Brong Ahafo has two main vegetation types, the moist semi-deciduous forest, mostly in the southern and southeastern parts, and the guinea savannah woodland, which is predominant in the northern and northeastern parts of the region (http://www.modernghana.com/GhanaHome/regions/brongahafo.asp).

The region has a 26.7% malaria prevalence (http://www.statsghana.gov.gh/Prm.html). Patients were recruited into the study from the Brong Ahafo regional hospital located in Sunyani.

Western region

The Western Region covers an area of 23,921 square kilometres, which is about 10 per cent of Ghana's total land surface. It is located in the south-western part of Ghana, bordered by Ivory Coast on the west, Central Region on the east, Ashanti and Brong-Ahafo Regions on the north and on the south by 192 km of coastline of the Atlantic Ocean. The southernmost part of Ghana, Cape Three Points, near Busua, is in the Ahanta West District of the region (http://www.modernghana.com/GhanaHome/regions/western). Western region of Ghana has a population of 2,325,597, with a 1.8% population growth rate (http://www.ghana.gov.gh/census/phc2010.pdf).

The region has about 75 per cent of its vegetation within the high forest zone of Ghana. The south-western areas of the region are noted for their rain forest, interspersed with patches of mangrove forest along the coast and coastal wetlands, while a large expanse of high tropical forest and semi-deciduous forest is also found in the northern part of the region. The Western Region has 24 forest reserves, which account for about 40 per cent of the forest reserves in the country. Prominent among them are the Bia Reserve, Cape Three Points National Park, and the Ankasa/Nini Suhyien Forest and Game Reserve.

The Western Region lies in the equatorial climatic zone that is characterised by moderate temperatures, ranging from 22°C at nightfall to 34°C during the day. The Region is the wettest part of Ghana, with a double maxima rainfall pattern averaging 1,600 mm per annum. The two rainfall peaks fall

66

between May-July and September-October. In addition to the two major rainy seasons, the region also experiences intermittent minor rains all year round. This high rainfall regime creates much moisture culminating in high relative humidity, ranging from 70 to 90 per cent in most parts of the region (http://www.modernghana.com/GhanaHome/regions/western.asp?).   Malaria prevalence   in   Western   region   is   18.5% (http://www.statsghana.gov.gh/Prm.html). Patients were recruited from Effia Nkwanta Hospital located in Secondi-Takoradi, which serves as the regional hospital.

Ashanti region

The Ashanti Region is centrally located in the middle belt of Ghana. The region shares boundaries with four of the ten political regions, Brong-Ahafo in the north, Eastern region in the east, Central region in the south and Western region in the South west.

The region occupies a total land area of 24,389 square kilometres representing 10.2 per cent of the total land area of Ghana. It is the third largest region after Northern (70,384 sq. kms) and Brong Ahafo (39,557 sq. kms) regions. The region has a population density of 194 persons per square kilometer, the third after Greater Accra and Central Regions. More than half of the region lies within the wet, semi-equatorial forest zone.

The region has an average annual rainfall of 1270mm and two rainy seasons. The major rainy season starts in March, with a major peak in May. The average daily temperature is about 27 degrees Celsius. Much of the region is situated between 150 and 300 metres above sea level. The region is drained by Lake Bosomtwe, the largest natural lake in the country, and Rivers Offin, Prah, Afram and Owabi. There are other smaller rivers and streams which serve as sources of drinking water for residents of some localities in the region (http://www.modernghana.com/GhanaHome/regions/ashanti.asp).

Prevalence of malaria in the Ashanti region is 16.1% (http://www.statsghana.gov.gh/Prm.html). Participants from the Ashanti region were sampled from the Kumasi South Hospital which serves as the regional hospital.

Volta region

The Volta Region is located along the southern half of the eastern border of Ghana, which it shares with the Republic of Togo. Greater Accra, Eastern and Brong Ahafo regions share boundaries with it on the west, on the north by the Northern Region, and on the south by the Gulf of Guinea. The region occupies an area of about 20,570 square kilometres or 8.6 per cent of the total land area of Ghana (http://www.modernghana.com/GhanaHome/regions/volta.asp). The population of the Volta Region is 2,099,876 with a population growth rate of 2.4% and a

68

population   density   of   102   persons   per   square   kilometer

(http://www.ghana.gov.gh/census/phc2010.pdf).

The region has a length of about 500 kilometres, stretching from the south to the north. It encompasses most of the vegetation zones found in the country, that is, the coastal grassland and mangrove swamps, replete with sandy beaches, the guinea savannah through moist semideciduous forests in the central highland areas to the undulating sahel-savannah and the mountainous wooded savannah in the north.

As in all other parts of the country, the Volta Region has a tropical climate, characterised by moderate temperatures, 21-32° Celsius (70 - 90°F) for most of the year. The region has two rainfall regimes in the year, the first; from March to July and the second from mid- August to October. Rainfall figures, which vary greatly throughout the region, are highest in the central highland areas and in the forest zone; they are lowest in the sahel-savannah zone in the north of the region. The maximum average annual rainfall figure is 2,103mm and 1,168mm, minimum. More than half of the land area of the region falls within the Volta River Basin, with the Volta Lake draining a substantial portion of the region (http://www.modernghana.com/GhanaHome/regions/ashanti.asp). Malaria   prevalence   in   Volta   region   is   17.9% (http://www.statsghana.gov.gh/Prm.html). Patients were recruited from the Volta Regional Hospital in Ho.

Study design

This study was cross-sectional by design. Simple random sampling was employed to recruit participants into the study from regional hospitals in six of the ten regional capitals of Ghana, which are Sunyani, Kumasi, Sekondi, Ho, Cape Coast and Accra. Blood samples were collected from participants who had been clinically diagnosed as having malaria (i.e. symptomatic participants) as well as those who did not show any malaria symptoms (i.e. asymptomatic participants) but reported to the health facility with a non-malaria ailment . Sample collection was undertaken in the dry season as well as in the rainy season.

One thousand two hundred and sixty-two (1,262) participants were recruited into the study from the six regional hospitals. The sample size was calculated using the method as described by Fisher, et al. (1998) as follows;

$$n = \frac{z^2\, p\, q}{d^2} \text{ , where}$$

n = the desired sample size (when population is great than 10,000);

z = the standard normal deviation, usually set at 1.96, which corresponds to the 95% confidence interval;

P = the proportion in the target population estimated to have a particular characteristic(s);

$q = 1.0 - p;$

d = *degree of accuracy desired, usually set at 0.05 level*

Using the prevalence of malaria for each study area (http://www.statsghana.gov.gh/Prm.html), the numbers of participants sampled from each region were as follows; 158 from Greater Accra (11.6% malaria prevalence), 13 from Central Region (10.1% malaria prevalence, 232 from Western Region (18.5% malaria prevalence), 226 from Volta Region (17.9% malaria prevalence), 208 from Ashanti Region (16.1% malaria prevalence) and 301 from Brong Ahafo Region (26.7% malaria prevalence).

Inclusion Criteria

Patients were eligible for inclusion into the study if they were 6 months old or older, weighed more than 5kg, and were resident in the region where sampling is being done.

Exclusion Criteria

Patients were excluded from participating in the study if they were unconscious, hemophilic, experiencing palpitation at the time of sample collection, had transfused or had been transfused blood within the previous 48 hours.

71

Ethical clearance

Ethical clearance was obtained from the Ghana Health Service Ethics Committee before the study was conducted (GHS-ERC-16/7/09). Approval was also sought from the Medical directors and administrators of the various health facilities before sample collection. The study was explained to the prospective participants in their own language after which they were given the chance to ask questions. After ensuring that inclusion criteria were met, written informed consent of the participants or parents/guardians/representatives were sought before blood samples were collected from them. To ensure anonymity of study subjects, the samples were coded using initials of the names of the regional hospital where sampling was being done as well as order of arrival of participants for sampling. For example the code CRH001 represented the first subject who was sampled in the Central Regional Hospital.

Samples were collected and handled solely by trained laboratory technologists and stored at -20°C. Left over blood samples were autoclaved at 121°C for 15 minutes and then buried. The study posed no risk to participants except for the transient pain they felt during blood collection. Sterile techniques and disposable, single use materials were used at all times to avoid any infection.

Blood sample collection

Five milliliters (5ml) of blood sample was collected from each participant into tubes containing EDTA by trained and licensed medical laboratory technologists from the regional hospitals. All blood samples collected were stored on ice and transported to the research laboratory of the Department of Biomedical and Forensic Sciences, University of Cape Coast.

Haematology

Haemoglobin levels

The haemoglobin level of each sample collected was estimated using a Cell Dyn 1800 automated blood analyzer. A participant was considered anaemic if his/her haemoglobin level was below 8g/dl. Anaemia status of participants were scored using cut-offs published by WHO in 1968.

Sickling status

The sickling status of each participant was determined by the sickle cell slide test method as described (Cheesbrough, 2000). A drop of each sample collected was delivered on a clean white tile and an equal volume of freshly prepared 2% w/v sodium metabisulphite solution (a reducing reagent) was added. The blood and reducing reagent were thoroughly mixed and carefully covered with a cover slip without trapping air bubbles. The slides were

73

examined with 40X objective lens for sickle cells after 20 minutes. The reducing agent deoxygenates the haemoglobin in the red cells providing the conditions for cells containing HbS to sickle. Both negative and positive control tests were done, using known sickling negative and positive blood samples respectively, to check the viability of the prepared 2% w/v sodium metabisulphite solution.

Fig 6: A sickling positive slide

74

Fig 7: A sickling negative slide

ABO blood grouping

The ABO blood group of each sample collected was determined using commercial anti-A and anti-B sera (Span Diagnostics Ltd. India) following the previously described tile method (Cheesbrough, 2000). Control tests were conducted, using blood samples with known ABO blood groups, to ascertain the viability of the anti-A and anti-B grouping sera. Two drops of each blood was delivered separately onto a clean white tile. Equal volumes of anti-A and anti-B grouping sera were added to the first and second drops of the blood respectively. The mixtures were mixed with a small clean applicator stick and

75

the tile was gently tilted from side to side to look for agglutination. After 2 minutes results were recorded as follows;

Anti-A	Anti-B	ABO Blood Group
Agglutination	No agglutination	**A**
No agglutination	agglutination	**B**
Agglutination	Agglutination	**AB**
No agglutination	No agglutination	**O**

Diagnosis of falciparum malaria

Malaria was diagnosed using three techniques namely microscopy, rapid diagnostic test (RDT) and polymerase chain reaction (PCR). *P. falciparum* was considered sequestered when PCR detected falciparum malaria infection but not microscopy.

Microscopy

Thick and thin blood smears were prepared, giemsa-stained and examined microscopically for detection of *P. falciparum* parasites, following a

76

described procedure (Cheesbrough, 2005). The thin films were used to identify *P. falciparum* from other *Plasmodium* species. The thick films were used to estimate the parasite density. Parasite density was estimated per 200 white blood cells assuming 8000 WBC/μl of blood as follows;

$$\text{Parasite Density} = \frac{8000 \text{ WBC/μl X Parasites counted against 200 WBC}}{200 \text{ WBC}}$$

A minimum of 100 high power fields were examined before a thin film was declared negative. Each slide was read independently by two experienced microscopists.
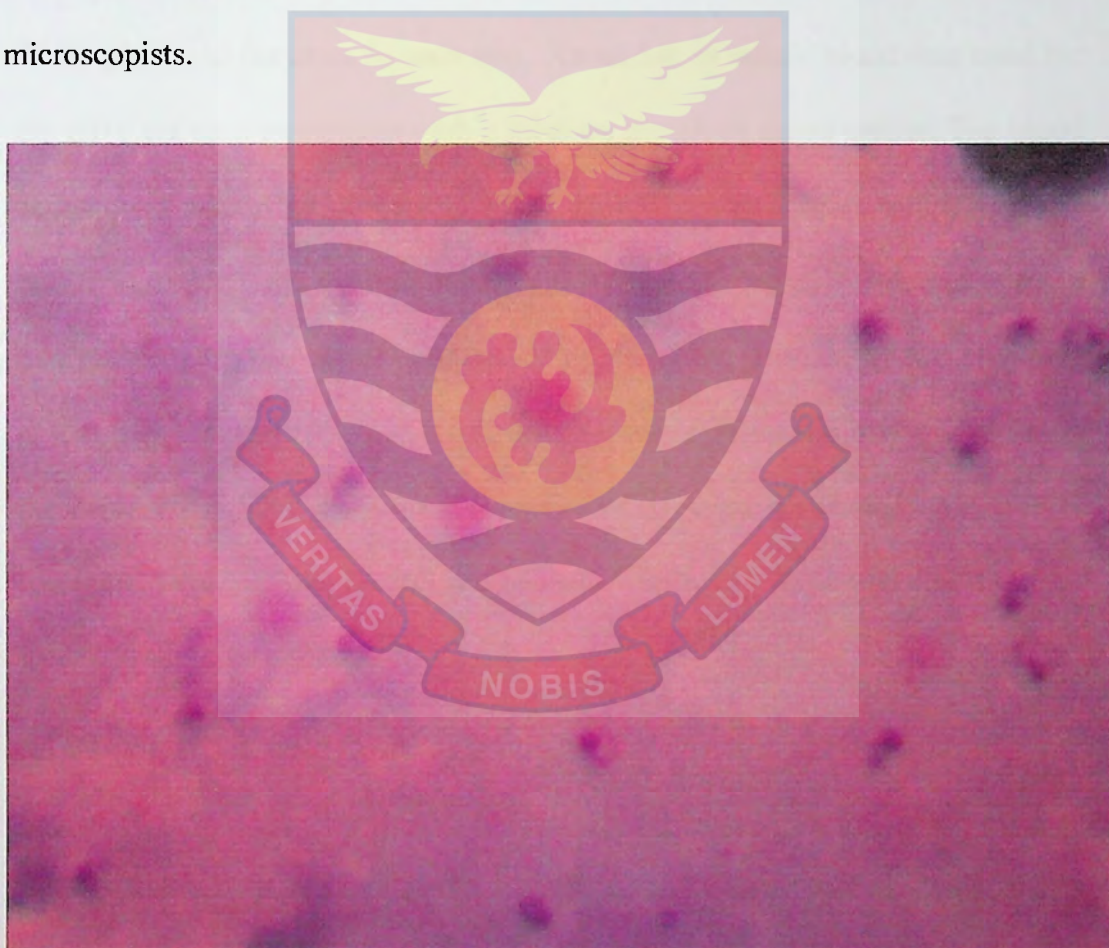
Fig 8: Malaria positive blood smear

Rapid diagnostic test (RDT)

P. falciparum identification was confirmed with first response *P. falciparum* Histidine-rich Protein-II (PfHRP-II) malaria rapid-diagnostic kits. The kit used is specific for *P. falciparum* and has a test band along its length impregnated with monoclonal antibodies which are specific for the HRP-II antigen for *P. falciparum*. The test was conducted according to the specifications provided by the manufacturer. Both positive and negative controls were set for each box of the kit that was used to be sure of the viability of the pieces of cassettes in each box. About 5μl of whole blood was used for the RDT for each participant with a label on the kit as stated earlier. The blood sample was added into the sample well after which two drops (60μl) of assay buffer was added into the buffer well and the results read in 20 minutes at room temperature. A positive reaction was identified by the presence of two rose-pink colour bands at the control (C) and test (T) labels. A visible rose-pink label at the control (C) label only was indicative of a negative reaction. Absence of rose-pink colour at both control and test labels indicated an invalid result.

A   B

Fig 9: RDT kits showing positive (A) and negative (B) results

Using microscopy as reference test, sensitivity and specificity of the RDT kit was calculated as follows

Sensitivity (%) $\quad = \dfrac{TP}{TP + FN} \times 100\%$

Specificity (%) $\quad = \dfrac{TN}{TN + FP} \times 100\%$

Where

TP (True Positives): samples which tested positive by both reference kit and test kit

TN (True Negative): samples which tested negative by both reference kit and test kit

FP (False Positive): samples which tested negative by reference kit but positive by test kit

FN (False Negative): samples which tested positive by reference kit but negative by test kit

Detection of *P. falciparum* using molecular methods

Parasite DNA extraction

About 1ml of each blood sample collected was spotted on a 3MM Whatman filter paper. The blood spot was allowed to air-dry before storage in a plastic envelope containing silica gel. The filter papers were stored at -20°C. DNA was extracted from all filter paper spots using the described chelex extraction method (Bereczky, Martensson, Gil, & Farnert, 2005) with modification. About 6 x 10mm portion of each blood spot was cut into a 1.5ml tube and 900µl of 1X Phosphate buffered saline (PBS) and 100µl of 10% saponin was added. The mixtures were incubated at room temperature for about

80

8 hours after which they were centrifuged for 2 minutes at 13000 revolutions per minute (rpm) and the supernatant discarded. The filter papers were then washed twice with 1ml 1X PBS. About 150µl of sterile double distilled water and 50µl of 20% chelex-100 was added to the filter papers and vortexed for 30 seconds. The mixture was boiled at 95°C for 10 minutes, vortexing every 2 minutes.

After the 10 minutes the content of the tubes were centrifuged for 5 minutes at 13000 rpm and about 140µl of the supernatants transferred into new 1.5ml tubes. Content of the new tubes were further centrifuged for 10 minutes at 13000 rpm and 120µl of the supernatant transferred into new 1.5ml tubes, to ensure complete removal of chelex beads. All extracted DNA were electrophoretically analyzed in horizontal plate with 2% agarose gels, containing 0.1mg/mL ethidium bromide, and analyzed under UV light (Syngene UGenius gel documentation). The remaining DNA samples were stored at -20°C.

Polymerase chain reaction (PCR) amplification

Four PCR fragments were needed to cover all required SNP locations of the *Pfcrt, Pfmdr1, and PfATPase6* genes. Amplicon sizes were 526 and 799 bp for *Pfmdr1* fragments 1 and 2, 200 bp for *Pfcrt* and 799 bp for *PfATPase6*. A nested PCR method was used to amplify target genes. PCR was carried out using previously published oligonucleotide pairs (Beck & Ley, 2008). An initial

81

primary amplification of the genes from the genomic DNA followed by a secondary (nested) amplification of the primary PCR products was carried ouy. In all the PCR reactions, final concentrations of 1X PCR buffer without $MgCl_2$, 0.2mM of each deoxynucleotide triphosphate (dNTP), 3mM $MgCl_2$, 0.2µM of each oligonucleotide, and 0.05U/µl Taq polymerase were used. PCR amplification was done using Biorad C1000 Thermal Cycler.

Amplification of *PfCRT* gene

In order to analyse the 76K mutation in the *Pfcrt* gene, a 200 bp amplification was done. The primary PCR was done using the following pair of outer primers, P10-1for (5' TTGTCGACCTTAACAGATGGCTCAC 3') and P10-1rev (5' AATTTCCCTTTTTATTTCCAAATAAGGA 3'). The primary PCR amplification for this gene was done with the following conditions 96°C for 15 minutes followed by 40 cycles (96°C for 30 seconds; 56°C for 90 seconds; 72°C for 90 seconds) 72°C for 10 minutes.

The primer pair for the nested PCR are as follows P10for (5' CTTGTCTTGGTAAATGTGCTC 3') and P10rev (5' GAACATAATCATACAAATAAAGT 3'). Amplification was done with the following conditions 96°C for 15 minutes followed by 40 cycles (96°C for 30 seconds; 50°C for 90 seconds; 72°C for 90 seconds) 72°C for 10 minutes

82

Amplification of *PfMDR1* gene first fragment

In order to analyze the 86 and 184 mutations in the *Pfmdr1* gene a 526 bp amplification of the gene was carried out. The oligonucleatide pair used to carry out the primary PCR was P1-1for (5' TTAAATGTTTACCTGCACAACATAGAAAATT 3') and P1-1rev (5' CTCCACAATAACTTGCAACAGTTCTTA 3'). The PCR conditions used for the primary PCR were 96°C for 15 minutes followed by 40 cycles (96°C for 30 seconds; 52°C for 90 seconds; 72°C for 90 seconds) 72°C for 10 minutes.

The nested PCR amplification was done with P1for (5' GAATTATTGTAAATGCAGCTTTA 3') and P1rev (5' GCAGCAAACTTACTAACACG 3') primer pair at the following conditions; 96°C for 15 minutes followed by 40 cycles (96°C for 30 seconds; 52°C for 90 seconds; 72°C for 90 seconds) 72°C for 10 minutes.

Amplification of *PfMDR1* gene second fragment

Amplification of a second fragment of the *Pfmdr1* gene was required to analyze the 1034, 1042 and 1246 mutations. The primary PCR was carried out using P3-1for (5' AATTTGATAGAAAAAGCTATTGATTATAA 3') and P3-1rev (5' TATTTGGTAATGATTCGATAAATTCATC 3') outer primer pair at PCR conditions 96°C for 15 minutes followed by 40 cycles (96°C for 30 seconds; 52°C for 90 seconds; 72°C for 90 seconds) 72°C for 10 minutes.

83

The nested amplification was done with P3for (5' GAATTATTGTAAATGCAGCTTTA 3') and P3rev (5' GCAGCAAACTTACTAACACG 3') oligonucleotide pair at the following conditions; 96°C for 15 minutes followed by 40 cycles (96°C for 30 seconds; 52°C for 90 seconds; 72°C for 90 seconds) 72°C for 10 minutes.

Amplification of *PfATPase6* gene

A fragment of the *PfATPase6* gene was amplified to analyze the G639D, S769N, and I898I mutations. The primary PCR was carried out at 96°C for 15 minutes followed by 40 cycles (96°C for 30 seconds; 56°C for 90 seconds; 72°C for 90 seconds) 72°C for 10 minutes, using P17-1for (5' AATATTGTTATTCAGAATATGATTATAA 3') and P17-1 rev (5' TGGATCAATAATACCTAATCCACCTA 3') primer pair.

The nested PCR amplification of the primary product was done at the following conditions; 96°C for 15 minutes followed by 40 cycles (96°C for 30 seconds; 56°C for 90 seconds; 72°C for 90 seconds) 72°C for 10 minutes, using P17for (5' AGCAAATATTTTCTGTAACGATAATA 3') and P17rev (5' TGTTCTAATTTATAATAATCATCTGT 3') primer pair.

Restriction fragment length polymorphism (RFLP)

To identify some of the various mutations in the *Pfmdr1*, *Pfcrt* and *PfATPase6* genes, the nested PCR products were digested with restriction

84

endonucleases. All restriction endonucleases were acquired from New England Biolabs Inc, Beverly, MA, USA. The procedure for the Restriction fragment Length Polymorphism is described elsewhere (Duraisingh et al. 2000; Veiga, Ferreira, Bjorkman & Gil, 2006). Restriction digestions for the genes were carried out at a final volume of 22µl, comprising 5µl PCR product, 1U of restriction enzyme (supplemented with 100µg/ml Bovine Serum Albumin when necessary), and 1X appropriate NEBuffer. Digestion conditions for the various mutations were done as shown in Table 2 below;

Sequencing

Three regions of the *PfATPase6* gene were amplified for sequencing as described elsewhere (Dahlstrom et al., 2008). Region 1 primary amplification was done using 5' TGGTAATAAAACTCCCGCTGATGC 3' and 5' CCGTTGTACATCCTAACGTCTCAACAC 3' oligonucleotide pair and nested amplification with 5' GTTGAACAGAGTATGTTAACAGGAGAATCCTG 3' resulting in a product of 526 bp. Region 2 primary amplification was done with 5'   GATGAAGCTGATCCATATAGT   3'   and   5' TTACGTGGTGGATCAATAA   3';   nested   amplification   with   5' AGTAGGAGTGGTGCTAAGAG   3'   and   5' ATAAGCAAAGCTAAGTGTTCT 3' resulting in a product of 897 bp.

85

Table 2: RFLP conditions for the detection of the various SNPs

Gene	Mutation	Restriction Enzyme	Recognition Site	Digestion Condition
Pfmdr 1	N86	ApoI	5'...R$^\vee$AATTY...3' 3'...YTTAA$_\wedge$R...5'	Incubate at 50°C for 6hrs Heat inactivation at 80°C for 20min
	86Y	AflIII	5'...A$^\vee$CRYGT...3' 3'...TGYRC$_\wedge$A...5'	Incubate at 37°C for 6hrs Heat inactivation at 80°C for 20min
	184F	DraI	5'...TTT$^\vee$AAA...3' 3'...AAA$_\wedge$TTT...5'	Incubate at 37°C for 6hrs Heat inactivation at 65°C for 20min
	1034S	DdeI	5'...C$^\vee$TNAG...3' 3'...GANT$_\wedge$C...5'	Incubate at 37°C for 6hrs Heat inactivation at 65°C for 20min
	1042N	AseI	5'...AT$^\vee$TAAT...3'	Incubate at 37°C for 6hrs

			3'...TAAT$_\wedge$TA...5'	Heat inactivation at 65°C for 20min
	1246D	DpnII	5'...$^\vee$GATC...3' 3'... CTAG$_\wedge$...5'	Incubate at 37°C for 6hrs
				Heat inactivation at 65°C for 20min
	1246Y	EcoRV	5'...GAT$^\vee$ATC...3' 3'...CTA$_\wedge$TAG...5'	Incubate at 37°C for 6hrs
				Heat inactivation at 80°C for 20min
Pfcrt	K76T	ApoI	5'...R$^\vee$AATTY...3' 3'...YTTAA$_\wedge$R...5'	Incubate at 50°C for 6hrs
				Heat inactivation at 80°C for 20min
PfATPase6	G639D	BspHI	5'...T$^\vee$CATGA...3' 3'...AGTAC$_\wedge$T...5'	Incubate at 37°C for 6hrs
				Heat inactivation at 65°C for 20min
	S769N	AflII	5'...C$^\vee$TTAAG...3' 3'...GAATT$_\wedge$C...5'	Incubate at 37°C for 6hrs

87

			Heat inactivation at 65°C for 20min
I898I	Tsp509I	5'...VAATT...3' 3'....TTAA∧...5'	Incubate at 37°C for 6hrs
			Heat inactivation at 65°C for 20min

Region 3 first amplification was carried out using 5' AGCATGGCACAAGTTTTGA 3', and 5' TAGCTACCTCCGTTCCATTAA 3' while nested amplification was carried out using 5' AAATAAATACCACATCAACACA 3' and 5' CTGTCATAGCAACTGTTTCTC 3' resulting in a product of 780 bp.

Sequencing was done with the following oligonucleotide pairs: region one 5' AGTTGACAAATATGCTGAAAA 3', and 5' TACCATTCTTCTTGTTCCTAAA 3'; region two 5' AGTAAATTGTAATGAAGCAAATAT 3', and 5' GGTGCACCTTTACAATACAATA 3'; region 3 5' AGTGAATGTATTTCTTCTTGGA 3', and 5' TAGCTCTGGCCGTATTAAT 3'. The sequencing reaction was performed using ABI 3500xL genetic analyzer followed by analysis using CodonCode Aligner V.4.0.2 (LI-COR, Inc). Sequencing results were included in a project in CodonCode Aligner. In the

88

CodonCode Aligner interface, a web browser page was opened for the NCBI BLAST server and sequence was selected into the search field and the BLAST search done against GenBank database. The BLAST search was repeated using different sequences.

Statistical analysis

Data were entered onto worksheets of Minitab® Statistical Software Version16. Bivariate relations were analysed using Pearson Chi-square. Parametric values were compared using Student's t-test and Analysis of Variance (ANOVA) wherever necessary. Results were presented in table and charts where appropriate. In all the analyses, a P < 0.05 was considered significant.
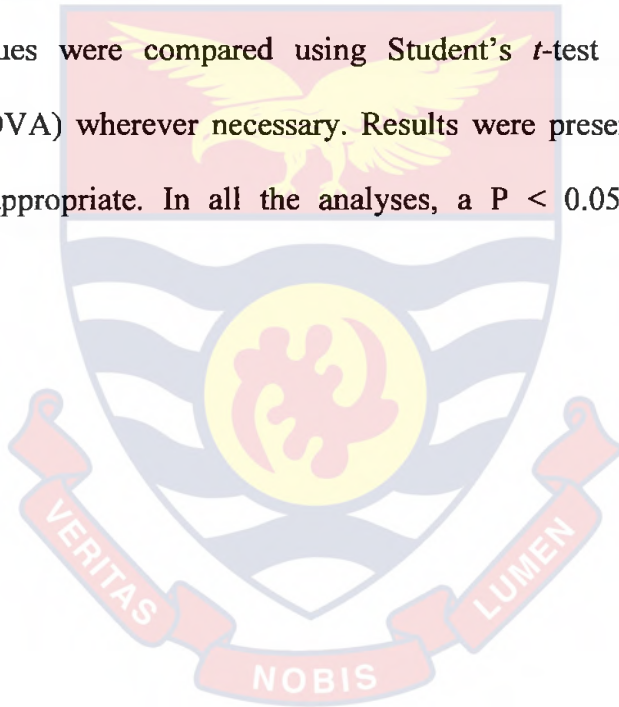
CHAPTER FOUR

RESULTS

General characteristics of study participants

A total of one thousand three hundred and eighteen (1,318) participants, with ages ranging from 6 weeks to 102 years old, were successfully recruited for this study. One hundred and sixty six of the recruited participants tested positive for malaria parasites. The mean age, haemoglobin level (Hb) and parasite density of the participants were 32.5573 years, 11.1222 g/dl of blood and 133.202 parasites/μl of blood, respectively. Eight hundred and twenty-nine (829) of the 1318 participants, representing 62.87%, were found to be anaemic while the remaining 489 (37.13%) had normal haemoglobin levels per their ages. Severe anaemia was found in 111 (13.89%) of the 829 anaemic participants. No association was observed between anaemia and sickle cell ($\chi^2 = 0.424$, DF = 1, P = 0.515)

Six hundred and thirty-five (635) participants were recruited in the rainy season whilst the remaining six hundred and eighty-three (683) were recruited in the dry season. About 16.46% of the study participants were recruited from the Greater Accra region, 16.01% from the Central region, 15.78% from the Western region, 15.63% from the Volta region, 17.83% from the Ashanti region and 18.29% from the Brong-Ahafo region. The baseline characteristics of the

90

study participants from the various regions are as summarized in Fig. 10, Fig. 11 and Table 3 below.
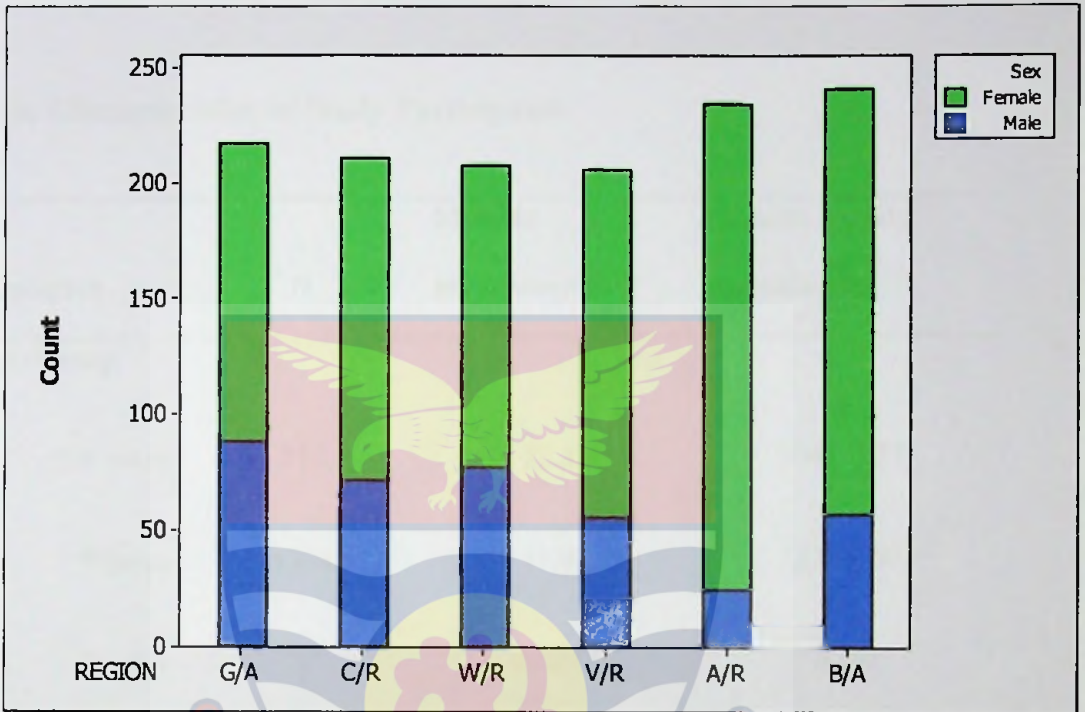
Fig 10: Distribution of study participants from the six study sites

Nine hundred and forty-three of the participants, representing 71.55%, were females while the remaining 375 (28.45%) were males. The number of female participants was found to be significantly higher than that of the males (P < 0.0001) as seen in Table 3. Of the 375 males that were recruited into the study, 55 (14.67%) were diagnosed with malaria whereas 113 (11.98%) of the 943 females were diagnosed with malaria. Parasitaemaia was not found to be significantly different in male and female participants (χ^2 = 1.737, DF = 1, P =

91

0.187). However, parasite density in male participants was significantly higher than that in the female participants (P = 0.01). The mean parasite density of the 375 male participants was 200 parasites/µl, while that of the 943 females was 106 parasites/µl. (Table 3)

Table 3: Characteristics of Study Participants

Category	N	Malaria prevalence (%)	Parasite density (parasite/µl)*
Age Group			
≤ 5 years	110	21.82	204 (±477)
> 5 years	1208	11.92	127 (±492)
P-value	-	*0.003* [‡]	*0.108*
Gender			
Male	375	14.67	200 (±653)
Female	943	11.98	106 (±407)
P-value	*< 0.0001* [‡]	*0.187* [‡]	*0.01*
Haemoglobin Level			
Anaemic	829	14.6	145 (±503)

92

Non-Anaemic	489	12.75	144 (±471)
P-value	-	*0.009* [‡]	*0.26*
Sickle cell			
Sickling positive	219	8.22	64 (±274)
Sickling negative	1099	13.65	147 (±523)
P-value	-	*0.028* [‡]	*0.001*
Blood Group			
Group O	631	13.79	156.7 (±560.1)
Group A	43	20.93	162.8 (±380.5)
Group B	587	11.41	113.2 (±436.0)
Group AB	57	8.77	56.5 (±194.8)
P-value	*< 0.0001* [‡]	*0.175* [‡]	*0.266*
Season			
Rainy Season	635	13.86	159 (±575)
Dry Season	683	11.71	109 (±397)
P-value	-	*0.243* [‡]	*0.069*

93

Region

Greater Accra	217	10.60	107.5 (±435.1)
Central Region	211	13.74	137.1 (±417.0)
Western Region	208	9.13	194.2 (±825.0)
Volta Region	206	10.19	118.9 (±420.4)
Ashanti Region	235	15.74	107.8 (±312.3)
Brong-Ahafo	241	16.18	137.2 (±405.8)
P-value	*0.418 [‡]*	*0.102 [‡]*	*0.463*

* mean (standard deviation)
[‡] chi-square
[†] ANOVA

Age of participants was stratified into two; participants who were five years old or younger and participants who were older than 5 years. One hundred and ten (110) (8.35%) of the participants were 5 years or younger, while 1,208 (91.65%) were older than 5 years. Of the 110 under-five years participants, 24 (21.82%) were diagnosed with malaria while 144 (11.92%) of the 1208 over-five-years participants were diagnosed with malaria (Table 3). The prevalence of malaria in the under-five years children was found to be significantly higher than that in the older participants ($\chi^2 = 8.880$, DF = 1, P = 0.003). There was, however, no significant difference between the parasite densities of the two

94

groups, but a significant negative correlation was observed between parasite density and age of the participants ($r = -0.083$, $P = 0.002$).

Malaria prevalence was observed to be significantly higher in the 829 anaemic participants than in the 849 non-anaemic ones but parasite densities in these two groups did not significantly differ from each other (Table 3)

Two hundred and nineteen (219) of the study participants, representing 16.62%, were found to be sickling positive where as 1,099 (83.38%) were sickling negative. A total of 8.22% of participants with the sickle cell had malaria parasitaemia whereas 13.65% of participants without the sickle cell had malaria parasitaemia. The mean parasite densities for participants with the sickle cell and those without it were 64 parasites/µl, and 147 parasites/µl respectively. Malaria prevalence and parasite density were both significantly higher in participants without the sickle cell than in those with it. A binary logistic regression analysis, however, showed no association between malaria parasitaemia and sickling status (OR (95% CI) = 1.0 (1.0 – 1.0) $P = 0.28$).

Malaria prevalence and parasite densities of samples collected from the six study sites did not significantly differ from one another (Table 3).

A majority (631/1318) of the study participants belonged to blood group O of the ABO blood group system. Blood group O was observed to be significantly more frequent than all the other groups ($\chi^2 = 951.584$, DF = 3, P < 0.0001). Of the 1,318 participants, 47.88% had blood group O, 3.26% had blood

95

group A, 44.54% had blood group B and 4.32% had blood group AB (fig 11). The frequencies of all the other blood groups put together (687) was not significantly higher than that of blood group O (631) ($\chi^2 = 2.379$, DF = 1, P = 0.123).

Of the 168 microscopy positive samples, 88 (52.4%) were collected in the rainy season and 80 (47.6%) in the dry season. The mean parasite density of positive samples collected in the rainy season was 159 parasites/μl while that for the dry season samples was 109 parasites/μl. No significant difference was found between the means of parasite densities between the two groups. The prevalence of malaria in the rainy season (13.86%) was also not significantly different from that in the dry season (11.71%) ($\chi^2 = 1.362$, DF = 1, P = 0.243).
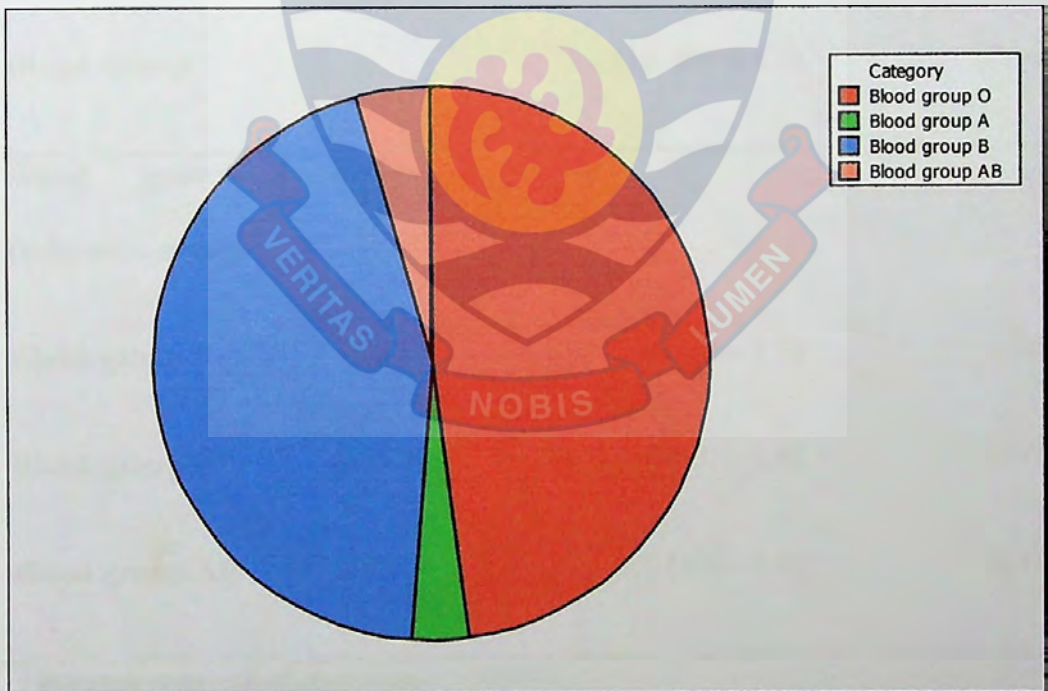
Fig 11: ABO blood group distribution of study participants

The mean parasite densities recorded for participants belonging to the various blood groups were as shown in Table 3. No significant differences were observed between the means of parasite densities of the four blood groups (P = 0.266) as shown in Table 3. Again, there was no association between the risk of contracting malaria and blood group (Table 4). All comparisons, using nominal logistic regression, generated odds ratio of 1.00.

Table 4: Nominal logistic regression analysis of malaria risk among blood groups

Blood Group	Malaria positive/malaria negative	OR (95% CI)	P-value[†]
Blood group O (reference event)	87/544	-	-
Blood group A	5/38	1.0 (1.0 – 1.0)	0.306
Blood group B	67/520	1.0 (1.0 – 1.0)	0.373
Blood group AB	5/52	1.0 (1.0 – 1.0)	0.328

[†] P-value was calculated using ANOVA

97

Generally, the 375 male participants were older than their 943 female counterparts (Table 5). Statistical analysis, however, show no significant difference between the ages of the male and female participants (P = 0.211). Haemoglobin level and parasite density were both significantly higher in the male participants than in the females (P < 0.0001 and P = 0.01 respectively) as seen in Table 5.

On the other hand, haemoglobin levels in the young children was observed to be significantly lower than that in the over-five-years group (Table 6).

Table 5: Age and Hb levels of male and female participants

Gender	Age (years)*	Hb (g/dl)*
Male	33.7(±22.9)	11.78(±2.95)
Female	32.1(±17.6)	10.86(±2.10)
P-value [†]	*0.211*	*<0.0001*

* mean (standard deviation)
[†] P-value was calculated using Student's T-test

No significant correlation was found between parasite density and haemoglobin level of participants (r = 0.013, P = 0.631).

98

Table 6: Hb level of participants of the two age groups

Age Group	n	Hb (g/dl)*
≤ 5 years	110	10.45(±2.20)
> 5 years	1208	11.18(±2.41)
P-value [†]		*0.001*

* **mean (standard deviation)**
[†] **P-value was calculated using Student's T-test**

Diagnosis of *P. falciparum*

Of the 1,318 participants recruited into the study 168 (12.75%) were diagnosed by microscopy to habour *Plasmodium falciparum*. The remaining 1,150 (87.25%) were diagnosed negative for *P. falciparum* by microscopy. Thus, the study recorded a 12.75% prevalence of *P. falciparum* in the six study sites. Polymerase Chain Reaction (PCR) and Rapid Diagnostic Test (RDT) diagnosed more *P. falciparum* positives than microscopy; 246 (18.66%) and 209 (15.86%) respectively.

In all the categories observed in Table 7 below, RDT recorded more positives than microscopy and PCR recorded more positives than RDT and microscopy except in Ashanti Region where RDT recorded 55 positives while PCR recorded 53 positives. Significant diagnostic differences in the three tools used were observed in participants older than 5 years, and also in samples taken during the rainy and dry seasons as seen in Table 7.

A total of 27.38% of the samples that tested positive for *Plasmodium falciparum* by microscopy were diagnosed negative by rapid diagnostic test (RDT), while 7.57% of the microscopy negative samples were diagnosed positive by RDT. Using microscopy as gold standard, true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) were calculated.

Table 7: Diagnostic differences between Microscopy, RDT and PCR

CATEGORIES	n	POSITIVE MALARIA DIAGNOSIS			P-value [†]
		Microscopy	RDT	PCR	
Age					
≤ 5 years	110	24	25	30	0.597
> 5 years	1208	144	184	216	< 0.0001
Season					
Rainy Season	635	88	111	134	0.003
Dry Season	683	80	98	112	0.045
Region					
G. Accra Region	217	23	19	30	0.226
Central Region	211	29	40	40	0.262
Western Region	208	19	26	34	0.086
Volta Region	206	21	21	32	0.156
Ashanti Region	235	37	55	53	0.079
B. Ahafo Region	241	39	48	57	0.122

[†] **P-value was calculated using Pearson Chi-square test**

The RDT kit recorded a sensitivity of 72.6% and specificity of 92.4%. A chi-square (χ^2) analysis of microscopy and RDT revealed a significant difference in the results obtained by the two tests ($\chi^2 = 464,933$; DF = 1; P < 0.0001).

Of the 168 samples that were found by microscopy to be positive for *P. falciparum*, 89.29% were confirmed by PCR while the remaining 10.71% were diagnosed negative by PCR. Using microscopy as reference test, sensitivity and specificity of PCR was calculated as 89.3% and 91.7% respectively. A chi-square analysis showed a significant difference in the results obtained by microscopy and PCR ($\chi^2 = 632.552$, DF = 1, P < 0.0001).

A comparison between *P. falciparum* diagnosis by PCR and RDT showed similar results as the previous two above. Out of the 209 samples diagnosed positive by RDT, PCR confirmed 178 and out of the 1109 samples that were diagnosed negative by RDT, 68 were diagnosed positive by PCR. Using PCR as the reference test, the sensitivity and specificity of RDT was calculated to be 67.4% and 97.1% respectively. Diagnosis using PCR was found to be significantly different from that using RDT ($\chi^2 = 732.621$, DF = 1, P < 0.0001).

101

Table 8: Performance of PCR, RDT and Microscopy at diagnosing malaria

	MICROSCOPY			Sensitivity	Specificity
	POSITIVE	**NEGATIVE**	**TOTAL**		
RDT				**72.6%**	**92.4%**
POSITIVE	122 (TP)	87 (FP)	**209**		
NEGATIVE	46 (FN)	1063 (TN)	**1109**		
TOTAL	168	1150	**1318**		
PCR				**89.3%**	**91.7%**
POSITIVE	150 (TP)	96 (FP)	**246**		
NEGATIVE	18 (FN)	1054 (TN)	**1072**		
TOTAL	168	1150	**1318**		

TP: True Positive
TN: True Negative
FP: False Positive
FN: False Negative

Sensitivity (%) = $\dfrac{TP}{TP + FN}$ X 100%

Specificity (%) = $\dfrac{TN}{TN + FP}$ X 100%

Table 9: Performance of PCR and RDT at diagnosing malaria

	PCR			Sensitivity	Specificity
	POSITIVE	NEGATIVE	TOTAL		
RDT				72.4%	97.1%
POSITIVE	178 (TP)	31 (FP)	209		
NEGATIVE	68 (FN)	1041 (TN)	1109		
TOTAL	246	1072	1318		

TP: True Positive
TN: True Negative
FP: False Positive
FN: False Negative

Sensitivity (%) = $\dfrac{TP}{TP + FN}$ X 100%

Specificity (%) = $\dfrac{TN}{TN + FP}$ X 100%

DNA extraction

Genomic DNA was successfully extracted from 1,209 of the 1,318 samples collected. DNA extraction was successful in all samples which were diagnosed positive by both microscopy and RDT as seen in Plate 1.

PCR amplification

P. falciparum genes in each of 246 samples were successfully amplified by PCR using Pfcrt, Pfmdr1 and PfATPase6 specific oligonucleotides.

103

1 - 13 = Genomic DNA (> 1000bp)

Plate 1: Agarose gel electrophorograph of extracted genomic DNA

PCR-RFLP of PfCRT

Nested PCR amplification of the PfCRT gene resulted in a 200 bp fragment. Out of the 246 samples successfully amplified, 144 were found to contain the 76T mutation whereas the remaining 102 had the wild-type allele K76, after digestion with ApoI. In samples with the *PfCRT* 76T mutation, ApoI digestion of the 200 bp fragment resulted in a 110 bp and a 90 bp fragments.

Out of the 144 PfCRT 76T mutations found, 15, 19, 21, 24, 28, and 37 were found in Greater Accra, Volta, Western, Central, Ashanti and Brong-Ahafo

104

regions, respectively. The 76T mutation of the PfCRT gene was not found to be associated with a particular regions ($\chi^2 = 2.757$, DF = 5, P = 0.737).
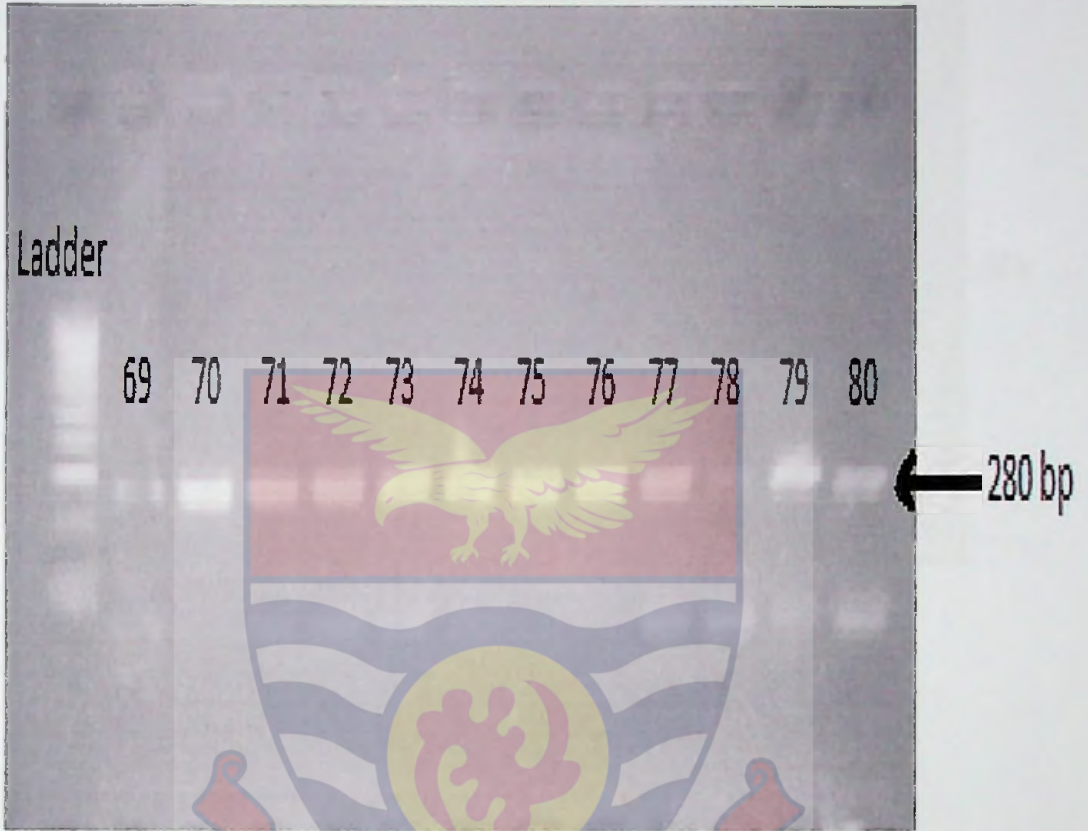
Plate 2: Primary PCR products of *Pfcrt* gene

105

200 bp

Plate 3: Nested PCR products of *Pfcrt* gene

PCR-RFLP of PfMDR1 fragment I

A 526 bp fragment of the PfMDR1 gene was amplified in a nested PCR reaction to analyze the N86Y and Y184F mutations.

Digestion of the PfMDR1 first fragment with ApoI (when the asn codon was present) and AflIII (when the tyr codon was present) revealed 57 samples with the mutant 87Y allele and 189 samples with the N86 wild-type allele.

Of the 57 samples with the mutation, 11 originated from Greater Accra, 5 each from Central and Western, 3 from Volta, 15 from Ashanti and 18 from

106

Brong-Ahafo regions. Prevalence of the PfMDR1 N86Y mutation was observed to be associated with the regions where samples were collected (χ^2 = 13.465, DF = 5, P = 0.019). Western region had the highest prevalence of 31.58%, followed by Ashanti region (26.32%), Greater Accra region (19.30%), Central and Western regions (8.77% each) and lastly Volta region (5.26%).
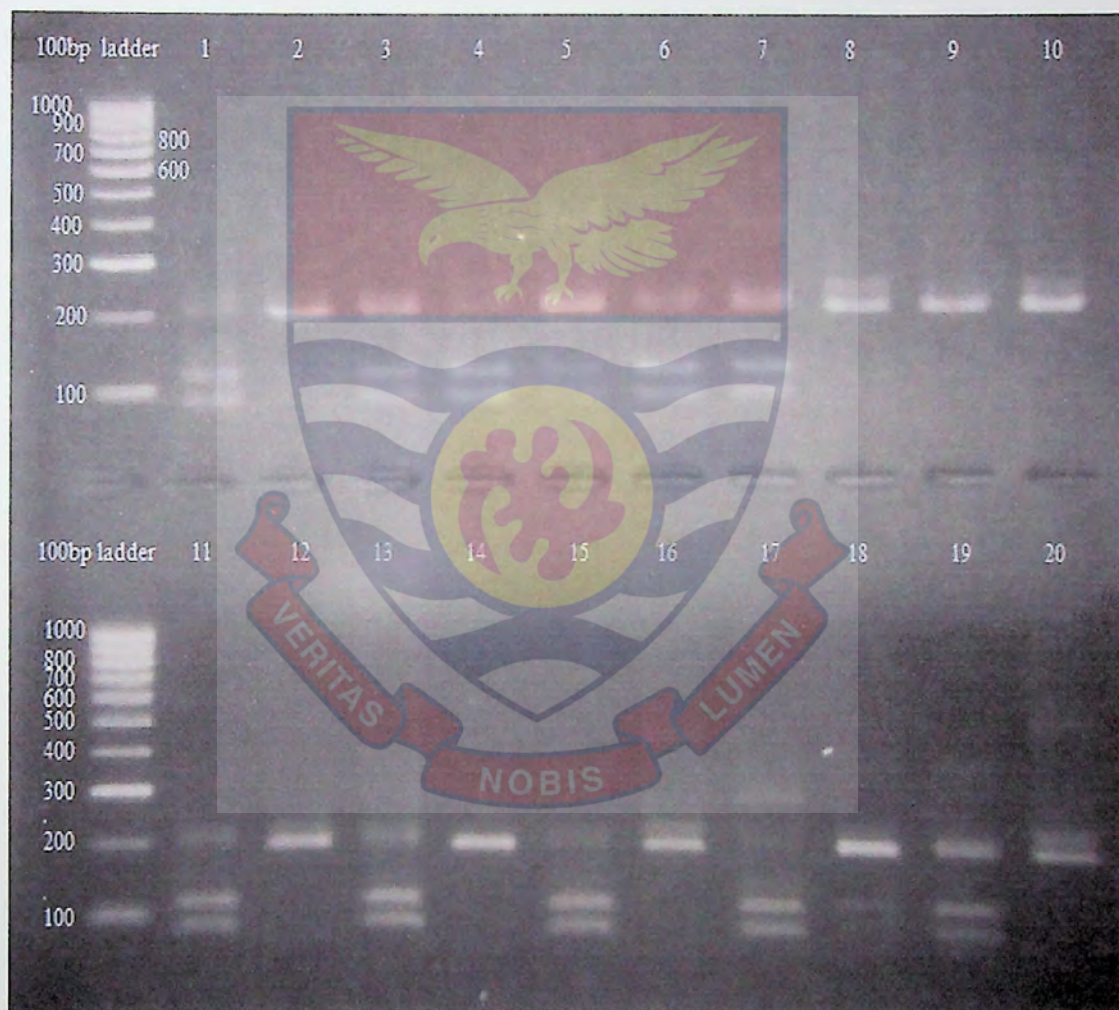
Plate 4: ApoI digestion of *Pfcrt* fragment

107

Table 10: Regional distribution of drug resistant mutants of *P. falciparum*

REGIONS	PfCRT 76T	PfMDR1 86Y	PfMDR1 184F
GREATER ACCRA	15	11	20
CENTRAL REGION	24	5	26
WESTERN REGION	21	5	26
VOLTA REGION	19	3	21
ASHANTI REGION	28	15	44
BRONG-AHAFO	37	18	44
TOTAL	144	57	181
P-value (χ^2)	0.737	0.019	0.283

DraI digestion of the MDR1 first fragment revealed 181 mutants at position 184 of the MDR1 gene. The 184F mutation of the MDR1 gene did not show any association with the regions of study ($\chi^2 = 6.251$, DF = 5, P = 0.283).
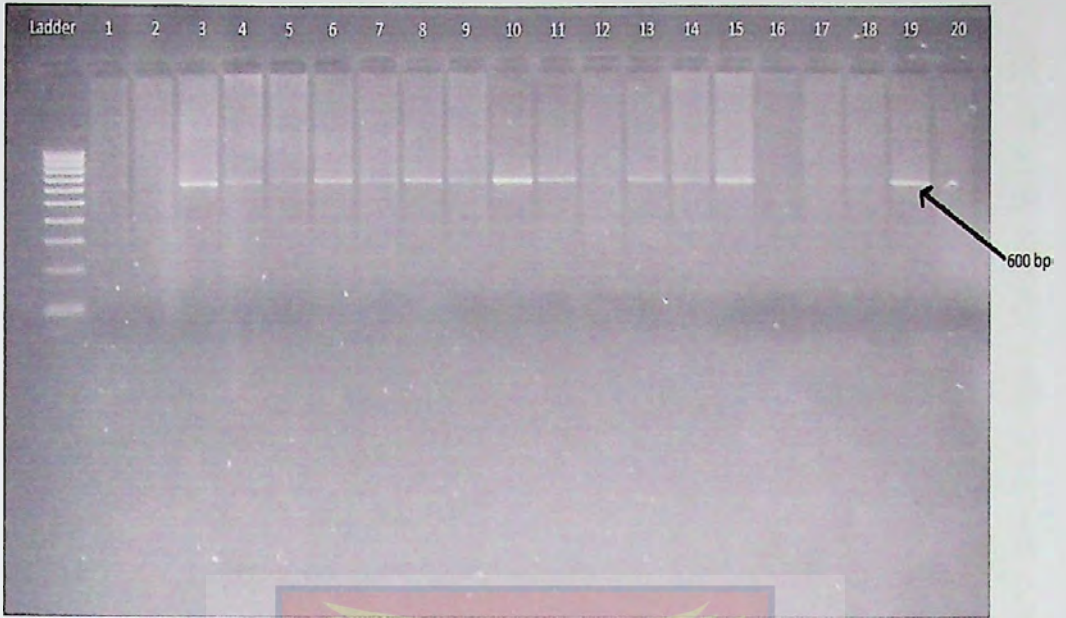
Plate 5: Primary PCR products of *Pfmdr1* 1st fragment
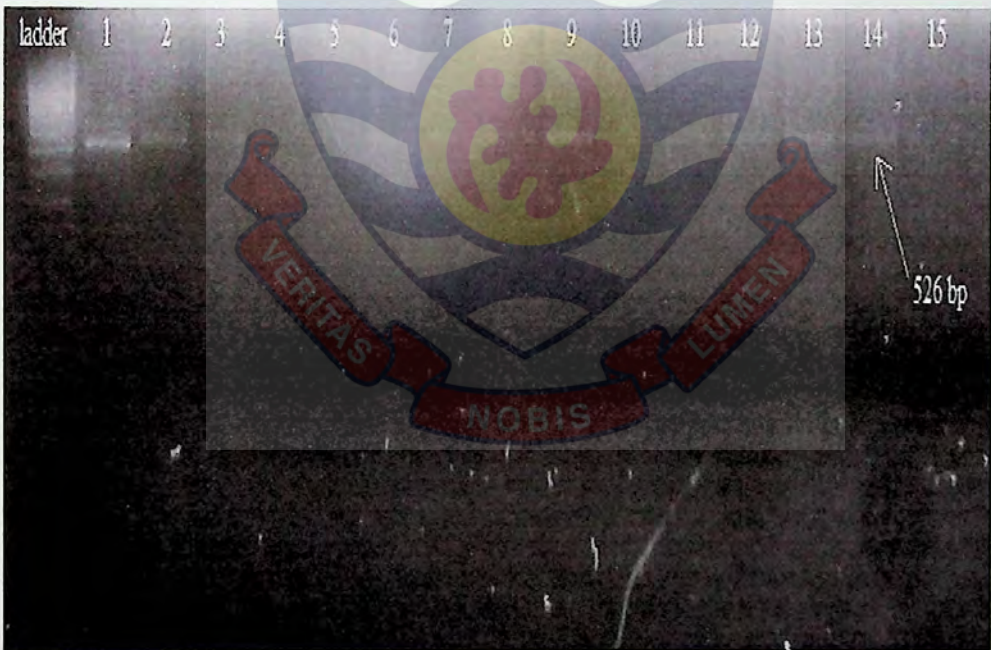
Plate 6: Nested PCR products of *Pfmdr1* first fragment

109

Plate 7: AflIII digestion of *Pfmdr1* first fragment

PCR-RFLP of PfMDR1 fragment II

A 799 bp second fragment of the MDR1 gene was also successfully amplified by a nested PCR reaction in all 264 samples. No mutation was found in all 246 samples at positions 1034, 1042, and 1246 of the MDR1 gene upon digestion with DdeI, AseI and DpnII/EcoRV.

110

Plate 8: ApoI digestion of *Pfmdr1* first fragment

Plate 9: Primary PCR products of *Pfmdr1* second fragment

111
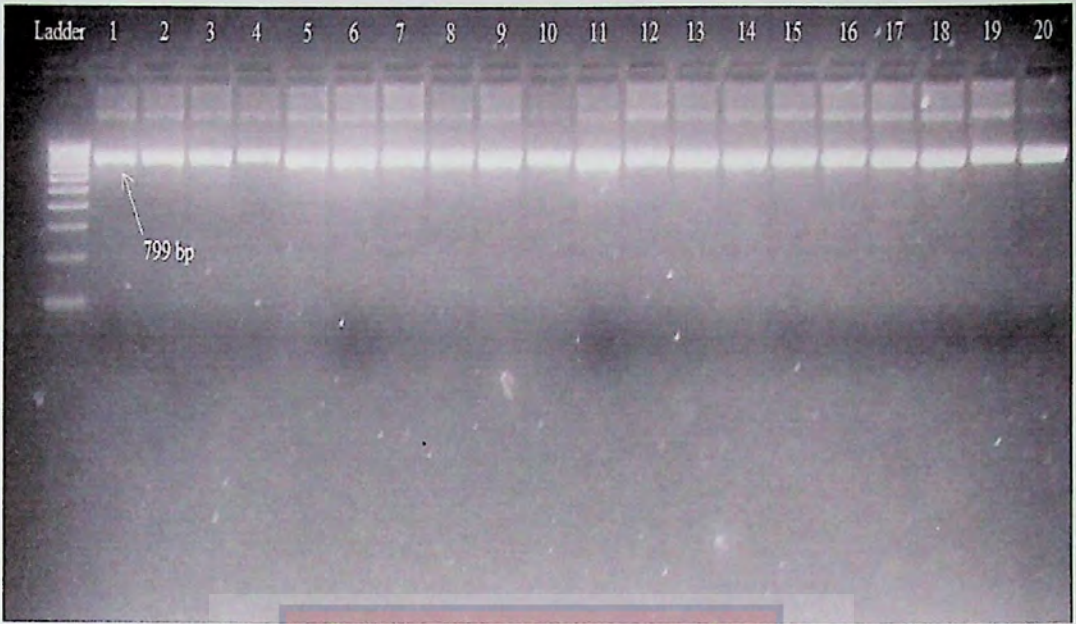
Plate 10: Nested PCR products of *Pfmdr1* second fragment
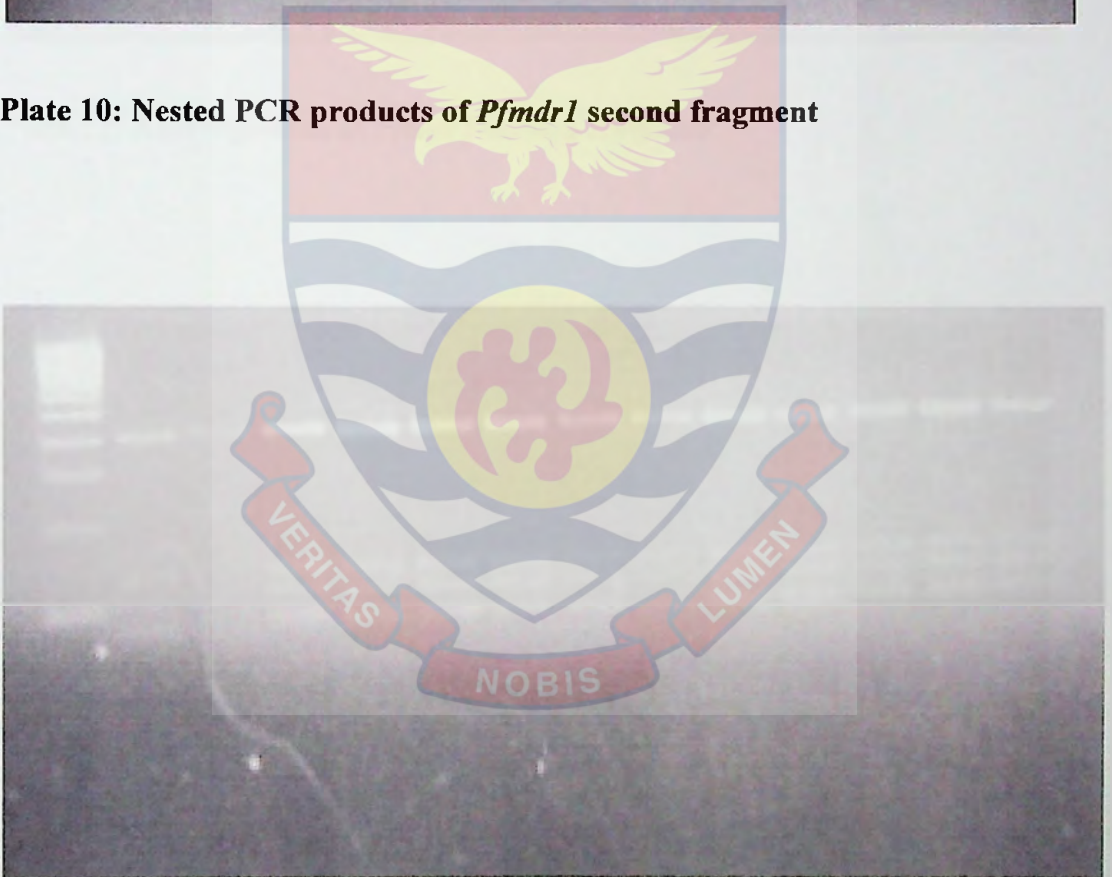
Plate 11: DdeI digestion of *Pfmdr1* second fragment

112

Plate 12: AseI digestion of *Pfmdr1* second fragment
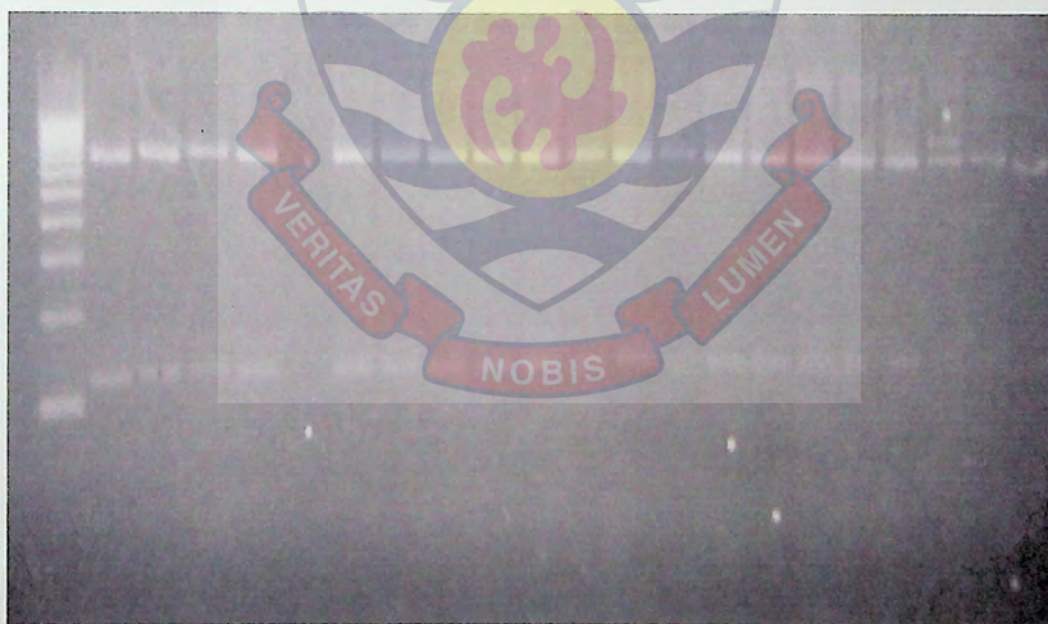
Plate 13: DpnII digestion of *Pfmdr1* second fragment

PCR-RFLP of ATPase6

Amplification of the PfATPase6 gene was successful in all 246 samples in a nested PCR reaction. The amplification resulted in a 799 bp fragment. No mutation was observed at positions 639, 769 and 898 upon digestion with BspHI, AflII and Tsp509I.

Plate 14: Primary PCR products of *PfATPase6*
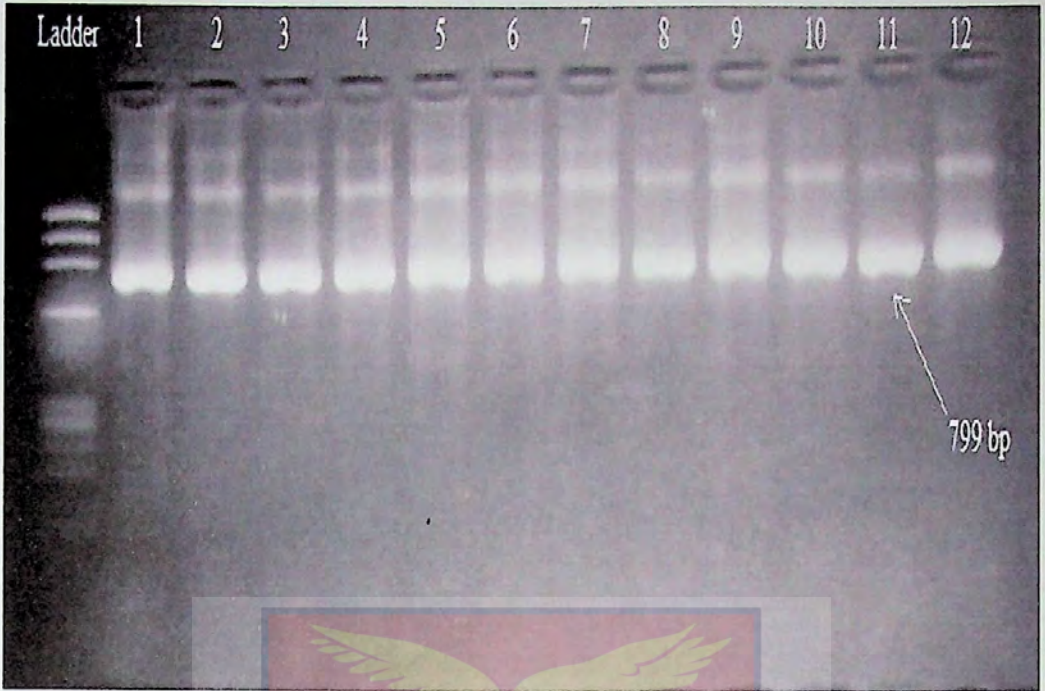
Plate 15: Nested PCR products of *PfATPase6*
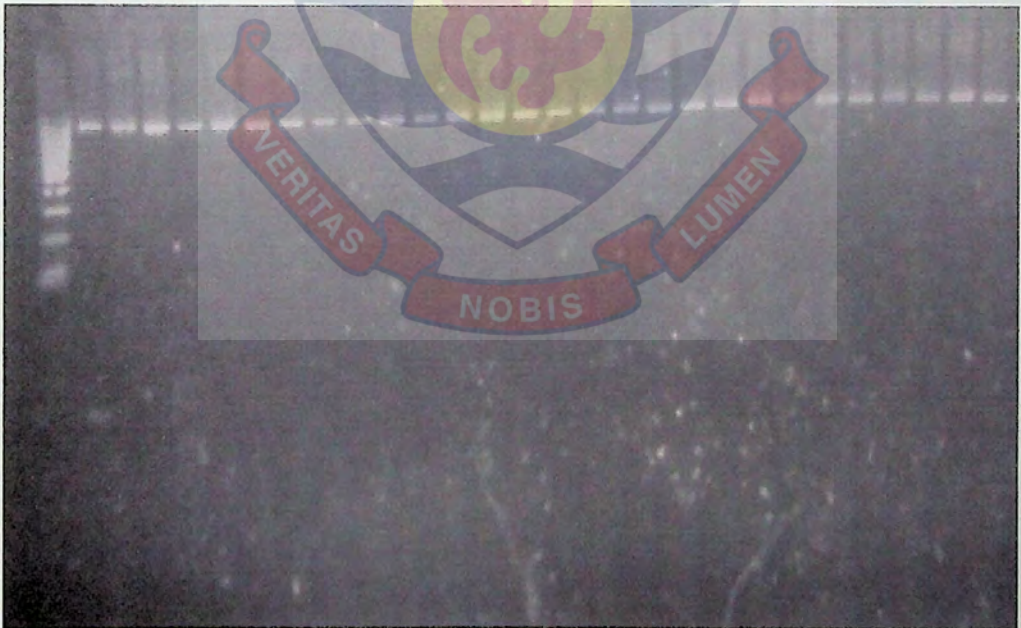
Plate 16: BspHI digestion of *PfATPase6* gene

115

Plate 17: AflII digestion of *PfATPase6* gene
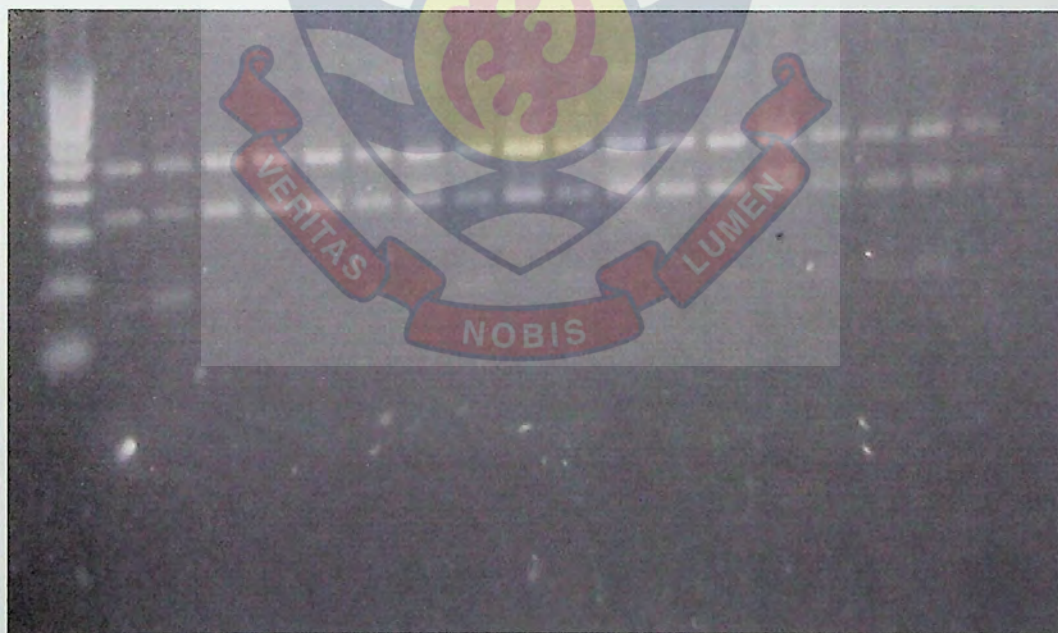
Plate 18: Tsp509I Digestion of *PfATpase6* gene

Sequencing of PfATPase6 fragments

All 246 samples that were successfully amplified by PCR were sequenced as described in section 3.7.4. Sequencing of regions 1and 3 of the PfATPase6 gene was successful in 144 of the 246 samples. Sequencing of region 2 of the PfATPase6 gene was not successful in any of the 246 samples. Sequencing of the first and third regions were used to analyze SNPs at the following described codon positions of the PfATPase6; 229, 243, 263, 683, 723, 747, 756, 758, 769, 771, 776, 783, 801, 809 and 898 (Afoakwah et al., 2011).
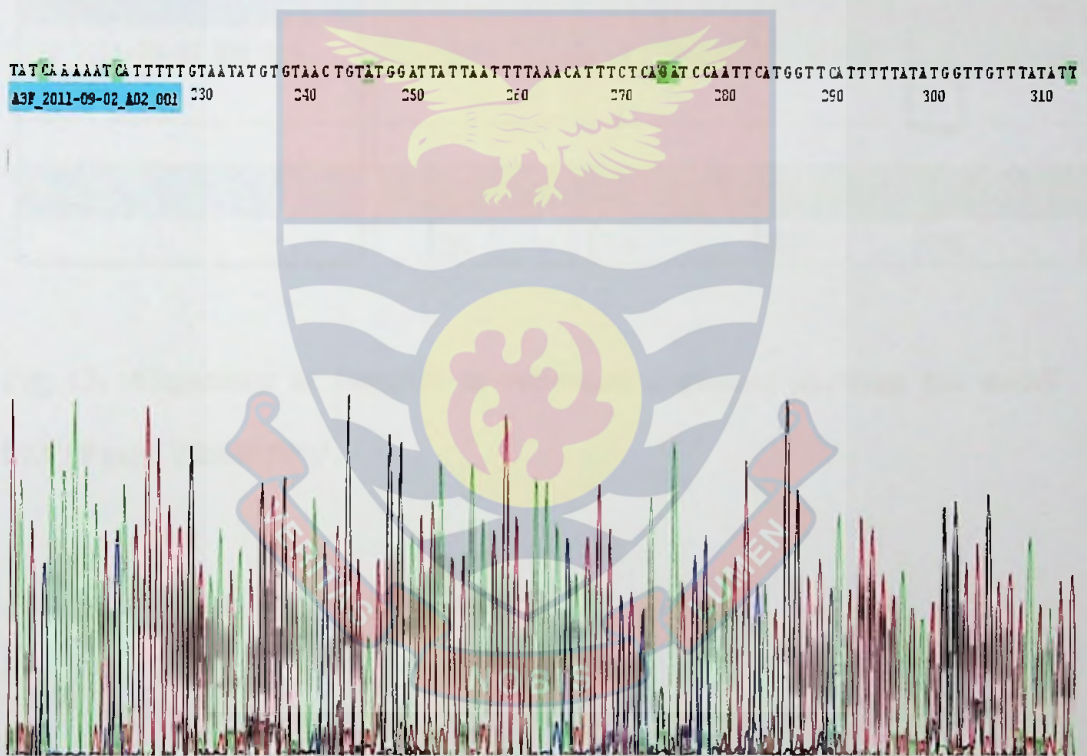
Fig 12: Nucleotide sequence of a section of PfATPase6 region 1

Nucleotide sequence of samples were compared with a reference PfATPase 6 sequence with GenBank ID AB576313.1

117

No mutations were found at any of the described codon positions. However, two novel SNPs were found. The first, Y264F, was found in only one sample and the second, D289N, was found in six samples. All samples containing these novel SNPs were collected from Brong-Ahafo region of Ghana.

Fig 13: Alignment of samples to reference sequence showing the novel PfATPase6 Y264F SNP

Anaemia in samples with mutations

Of the 246 samples that were diagnosed *P. falciparum* positive by PCR, 24 were found to be anaemic (Hb < 8.0 g/dl) and the remaining 222 with Hb levels higher than 8.0 g/dl. Upon analaysis, anaemia was observed in 11 of the 144 samples with PfCRT 76T mutation. No significant association was found between the PfCRT mutation and anaemia in falciparum malaria ($\chi^2 = 1.768$, DF = 1, P = 0.184) (Table 11)

118
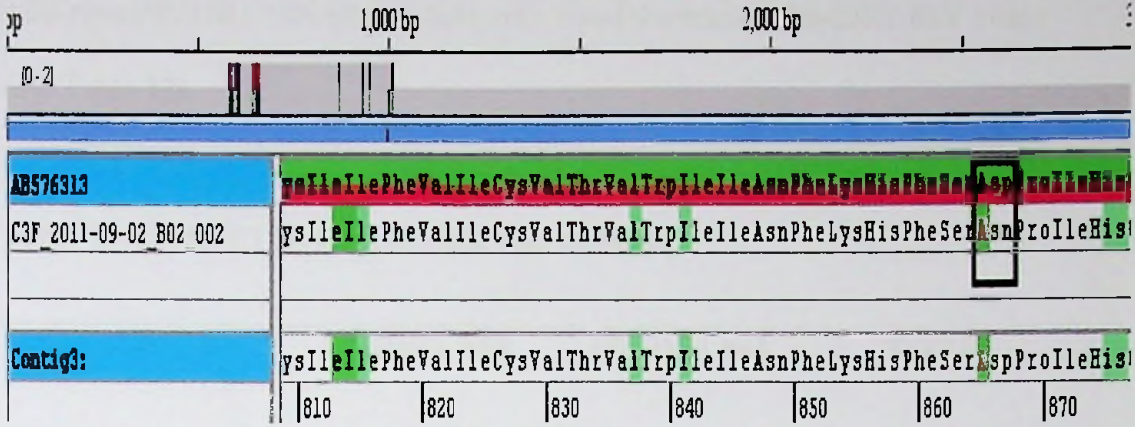
Fig 14: Alignment of samples to reference sequence showing the novel PfATPase6 D289N SNP

Table 11:   PfCRT K76T mutation and anaemia

	PfCRT 76T	PfCRT K76	TOTAL
ANAEMIA	11	13	24
NORMAL Hb	133	89	222
TOTAL	144	102	246

The wild-type allele N86 of PfMDR1 gene was significantly associated with anaemia (χ^2 = 5.395, DF = 1, P = 0.02) but not the mutant allele 86Y. Of the 24 anaemic samples that were successfully amplified by PCR, 23 had the

119

wild-type PfMDR1 N86 allele while only 1 had the mutant PfMDR1 86Y allele

(see Table 12).

Table 12: PfMDR1 N86Y mutation and anaemia

	PfMDR1 N86	PfMDR1 86Y	TOTAL
ANAEMIA	23	1	24
NORMAL Hb	166	56	222
TOTAL	189	57	246

The wild-type allele Y184 of PfMDR1 gene was also significantly associated with anaemia ($\chi^2 = 13.930$, DF = 1, P < 0.0001) but not the mutant PfMDR1 184F allele. Of the 24 anaemic samples that were successfully amplified by PCR were found with the Y184 wild-type allele whereas 10 were found with the 184F mutant allele of the PfMDR1 gene (see Table 13)

Though there were differences in the observed counts of SNPs in the rainy and dry seasons, statistical analysis revealed no significant seasonal variation in the observed SNPs (see Table 14).

120

Table 13: PfMDR1 Y184F mutation and anaemia

	Y184	184F	TOTAL
ANAEMIA	14	10	24
NORMAL Hb	51	171	222
TOTAL	65	181	246

Table 14: Chi-square analysis of seasonal variation in observed SNPs

Locus/SNP	N	Rainy Season	Dry Season	P-Value (χ^2)
PfCRT K76T				
K76	102	62	40	
76T	144	72	72	0.094
PfMDR1 N86Y				
N86	189	102	87	
86Y	57	32	25	0.773

PfMDR1 Y184F

Y184	65	32	33	
184F	181	102	79	*0.323*

122

CHAPTER FIVE

DISCUSSION

General characteristics of participants

Malaria continues to be a leading cause of morbidity and mortality in Ghana, and most parts of sub-saharan Africa. About 7.2 million malaria cases are recorded in Ghana annually (WHO, 2008). The prevalence of malaria in this study was found to be 12.75% using microscopy as diagnostic tool. PCR, a more sensitive diagnostic tool, found a higher prevalence of 18.66%. These prevalence rates suggest a high endemicity of malaria in the study sites. There has not been any evidence of reduction in malaria cases in Ghana since 2001 (WHO, 2008; WHO, 2010a), notwithstanding the increase in malaria control strategies. Insecticide treated nets (ITNs) have been the centre stage of malaria control in Ghana, with pregnant women and children under five being the most targeted group (WHO, 2008). ITNs in Ghana is subsidized by government leading to a 100% of households owing at least one ITN (WHO, 2010a). Since 2004 Artesunate + Amodiaquine and Artemether + Lumefantrine have been used as first line treatment for confirmed and uncomfirmed *P. falciparum* malaria. Intermittent preventive treatment with sulphadoxine-pyrimethamine has been used to prevent malaria in pregnancy and indoor-residual spraying has been the primary vector control intervention since 2005 (WHO, 2008).

123

In the face of the numerous interventions, the lack of corresponding decrease in malaria cases could partly be attributed to treatment-seeking behavior of patients. Research has revealed that most patients try to manage febrile cases at their own level at home (Malik et al., 2006; Sumba et al., 2008; Boampong, Acquah, & Achiamaa, 2009; Yadav, 2010; Getahun, Deribe, & Deribew, 2010; Xu, Xu, liu, & Zeng, 2012) and seek treatment from health professionals only when the home-based management fails. In most of these cases, children under 5 year are the worst hit. These children lack immunity against malaria. Treating them with counterfeit or under dose drugs makes recovery from malaria quite difficult. In this present study the percentage of under five children suffering from malaria was found to be significantly higher than that of the older participants. Most of these children might have received prior home-based treatments.

Home-based management of malaria in itself is not a bad practice. It is accepted by WHO as a means to provide prompt and effective treatment of malaria episodes for individuals who cannot readily access hospitals and clinics. The practice is, however, heavily challenged by counterfeit or sub-standard antimalarials, non-compliance to full treatment regimen and incorrect dosing of antimalarials. The implementation of home-based management of malaria should rely on sound evidence of public health benefit (Hopkins, Talisuna, Whitty, & Staedke, 2007). Most studies have found no obvious public health

124

benefit of this intervention (Spencer et al., 1987a; Spencer et al., 1987b; Boampong et al., 2009) while a few others have found decrease in morbidity (Delacollete, van der Stuyft, & Molima, 1996) and mortality (Kidane and Morrow, 2000).

Gender plays a role in the success of many public health programmes. Biological differences between males and females can affect susceptibility to certain infectious diseases, while gender norms, cultural practices, and behaviours can strongly influence disease prevention and care-seeking, as well as access to treatment. Given equal exposure, adult men and women are equally vulnerable to malaria infection, except for pregnant women, particularly those in their first pregnancy, who are at greater risk of severe malaria in most endemic areas.

Gender differences, however, often play a role in access to prevention and treatment. In many cultures, men tend to endure discomfort, leading to delays in seeking medical care and subsequent reporting, while women may delay seeking health care due to the lack of control of financial resources, as well as household duties which result in less time available to travel to a clinic. In many malarious areas, certain gender-specific occupations may increase exposure to malaria vectors. For example, in areas where forest-dwelling malaria vectors are common, men entering the forest for logging or gem mining may place themselves at greater risk.

125

In this study, 55 (14.67%) of the 375 male participants were diagnosed with malaria whereas 113 (11.98%) of the 943 female participants had malaria. On one hand, no significant difference was observed in the prevalence of malaria among the male and their female counterparts, suggesting that exposure to infective bites may be equal in male and female Ghanaians. On the other hand, parasite density among the male participants was significantly higher than the female participants. This result seem to propose that Ghanaian males tend to endure uncomplicated malaria symptoms, seeking health care only when symptoms seem not to subside, by which time they may be hyperparsitaemic. This assertion is supported by the fact that male participants formed a minority (28.45%) of the study subjects as well as malaria positive participants (32.74%) yet they had a higher mean parasite density than their female counterparts.

Anaemia is a common cause of morbidity and mortality in sub-Saharan Africa (deMaeyer & Adiels-Tegman, 1985; Desai et al., 2005). The aetiology of anaemia in this region involves interactions between nutritional deficiencies, haemoglobinopathies, malaria, helminthiasis, bacterial infections and HIV (Bouyou-Akotet et al., 2009). Malaria is suspected to be the major cause of anaemia in patients suffering from the disease. The pathogenesis of anaemia in malaria is multifactorial involving rupture of infected RBCs to release merozoites, opsonization and clearance of infected RBCs, clearance of uninfected RBCs coated with parasite-derived antigens, sequestration of infected RBCs, rostting of infected and uninfected RBCs and failure of the bone

126

marrow to increase RBC production to compensate for the losses (Laminkara et al., 2007).

Anaemia is an important factor to malaria-attributable deaths in hospitals. Severe anaemia alone accounts for between 17% and 54% of malaria-attributed deaths in under 5 children (Slutsker, Taylor, Wirima, & Steketee, 1994; Marsh et al., 1995; Biemba, Dolmans, Thuma, Weiss, & Gordeuk, 2000). Both severe and more moderate or mild anaemia may act as risk factors that predispose children to fatal outcomes because of other conditions (McDermott et al., 1996; Brabin, Premji, & Verhoeff, 2001; Brabin, Prinsen-Geerligs, Verhoeff, & Kazembe, 2003).

A significant majority of participants (62.89%) were anaemic. This could be a worrying observation considering the fact that sampling was carried out in very urban parts of the country where the standards of living are relatively high. However, this observation is expected since participants of this study were selected from patients visiting regional hospitals that serve as referral points in the region. Thus, respondent were necessarily not enjoying good health. The observed anaemia may therefore be largely due to infections. Malaria was found to be a significant contributor to the anaemia, accounting for 9.18% of all the observed cases of anaemia. Helminth infections and nutritional deficiencies might have also been significant contributors to the observed anaemia. It is also worthy of noting that about as many anaemic participants that were sickle-cell positive were sickle cell negative. This implies that participants

127

with sickle cell consciously took measures to curtail sickle-cell related anaemia, thus, the aetiology of the anaemia found in such participants is most likely infections like malaria.

Like most studies conducted in the sub-Saharan Africa region, this current study recorded a significant association between malaria and anaemia. A total of 72.02% of participants with malaria suffered from anaemia, confirming the suggestion that malaria is a major cause of anaemia in malaria patients. The anaemia in these patients may be exacerbated by co-morbidities and nutritional deficiencies. Unexpectedly, the observed parasite densities had no correlation with haemoglobin levels. This finding is, however, not absolutely surprising. Most patients reporting to health facilities might have commenced malaria treatment resulting in the reduction of parasite density. Hence the actual mass of parasites responsible for the degree of anaemia observed at the time of hospital attendance may be missed. A large majority (93.45%) of malaria positive participants, however, did not have severe anaemia. Age of the participants might have played a significant role in this observation. Most of the participants were older than 5 years and, thus, might have developed a level of premunition against malaria and are therefore not expected to suffer from the consequences of severe disease including severe malaria.

Innate immunity to complicated and uncomplicated malaria, due to erythrocyte abnormalities in haemoglobin and enzyme as well as presence or absence of membrane proteins, has been found in persons living in endemic

128

regions. The high prevalence of HbAS (May et al., 2007) and blood group O (Cserti and Dzik, 2007; Martin et al., 1979) is considered to have resulted from a malaria-influenced selection over years. Sixteen percent (16.61%) of participants were found to have sickle-cell (HbSS or HbAS). Considering that the estimated prevalence of the sickle-cell in some malaria non-endemic European countries are so very low (3% in Albania, 0.6% in France, 0.57% in Portugal, 0.53% in Greece. 0.47% in the Netherlands, 0.47% in England and Wales, and 0.44% in Turkey) (Modell et al., 2007), the observed prevalence in this study is very much on the high side. This observation is in tune with others from other malaria endemic countries (Leikin et al., 1989; Platt et al., 1994; Kato et al., 2006).

Expectedly, blood group O was the most frequent (47.88%) blood group among the study participants. Again, similar observations have been made among persons living in malaria endemic countries (Fischer & Boone, 1998; Loscertales & Brabin, 2006; Rowe et al., 2007; Pathirana et al., 2005; Tekeste & Petros, 2010). These observations confirm malaria as a strong force in the evolutionary history of the human genome (Kwiatkowski, 2005).

The sickling trait protects against severe and uncomplicated malaria (Hill et al., 1991; Aidoo et al., 2002) while the protection conferred by blood group O is only against severe malaria (Carlson & Walgren, 1992; Udomsangpetch, et al., 1993; Rowe, Obeiro, Newbold & Marsh, 1995; Chotivanich et al., 1998). Results of this study fit the above description. All the

recorded malaria cases in this study were uncomplicated cases and it was observed that parasite density was significantly higher in sickle-cell negative participants than in sickle-cell positive participants (P = 0.001) (Table 3) but parasite density in the four blood groups didn't significantly differ from one another (P = 0.266; Table 3). Severe cases of malaria were excluded from the study due to ethical issues. The risk of getting malaria, however, was equal in sickling positive and negative participants as well as in all the four blood groups (OR = 1.00, 95%C.I = +1.00, -1.00).

Falciparum malaria diagnosis

The technical capability to perform a correct diagnosis of malaria is of the utmost importance in preventing the progression of uncomplicated malaria to a complicated one, as well as preventing the development of drug resistance. The diagnostic accuracy of microscopy, the standard method for diagnosing malaria, has been questioned by several studies (Kain et al., 1998; Milne et al., 1994; Thomson et al., 2000; Houwen, 2002; Mckenzie et al., 2003; Trampuz et al., 2003; Johnston et al., 2006; Maguire et al., 2006; Wongsrichanalai et al., 2007). This study also found similar concerns as those raised by previous ones. Consistently, microscopy underdiagnosed falciparum malaria in all observed categories as seen in Table 7. Generally, parasite densities recorded in the study were low. This, coupled with the fact that *P. falciparum* is capable of sequestration and hence disappearance from peripheral blood, makes the underdiagnosis by microscopy not surprising. RDT and PCR both performed

130

better than microscopy in diagnosing falciparum malaria. In all the categories observed in Table 7, PCR was the best diagnostic tool except in Ashanti and Central Regions. In total, significant differences were observed in the diagnosis by the three tools employed. No significant differences were, however, seen in the diagnoses by microscopy, RDT and PCR in the individual study sites, and in the participants younger than 6 years old. This phenomenon could have been as a result of the small group sizes of these categories, since when categories with larger sizes (such as rainy season, dry season and older participants) were analyzed, significant differences were found.

RDT recorded sensitivity and specificity of 72.6% and 92.4% respecitively while PCR recorded 89.3% and 91.7% sensitivity and specificity respectively. The sensitivities of these two diagnostic tools would have been better if the reference diagnostic tool (i.e. microscopy) had itself not underdiagnose.

Considering that PCR is the most sensitive and most specific of the three frequently used tools (Bronzan et al., 2008), its use as the reference tool in the determination of sensitivity and specificity should be considered among researchers in the bid to achieve malaria eradication. When microscopy and RDT were each compared against PCR, microscopy recorded a sensitivity of 60.97% while RDT recorded 72.4%. This obviously flaws microscopy and RDT as efficient diagnostic tools, failing to diagnose, among others, cases of very low parasite densities. These cases may remain asymptomatic and, possibly,

131

untreated resulting in the reservation of a pool of the parasites in endemic areas
to sustain transmission.

Seasonality of malaria

The different ecological zones of sub-saharan Africa, and other
malarious regions, support a wide range of malaria transmission conditions
(MacDonald, 1957). The complex interactions between malaria parasites,
human host, *Anopheles* vector and environmental conditions make transmission
pattern of malaria vary from one geographic region to another. Understanding
the disease transmission pattern within a particular area is fundamental for the
description of disease risk and control (Bruce-Chwatt, 1980; Molineaux, 1988).
Malaria is hyperendemic in all parts of Ghana, although transmission rates are
lower in urban areas. Transmission is said to occur all year round with seasonal
variations during the rainy season (Dery et al., 2010). However, no convincing
empirical relationship has been observed between seasonality in environmental
factors and seasonality in malariometric indices that could be used to ascertain
malaria seasonality in endemic regions (Mabaso, Craig, Ross, & Smith, 2007).

In this present study seasonality of malaria was not observed. The
prevalence of malaria in the rainy season didn't significantly differ from that in
the dry season, suggesting no seasonality in malaria prevalence in Ghana.
Parasite densities recorded in the rainy and dry seasons were also not
significantly different from each other. Seasonality in malaria is generally

132

attributed to climatic factors, in particular rainfall, which affect other climatic factors and the malaria vector population dynamics. The tropical and sub-tropical regions of the world are warm enough to support the continuous breeding of the malaria vector all year round. Rainfall, which provides the breeding habitats for the malaria vector and sustains the aquatic immature stages of the vector, is on the other hand not available all year round. Rainfall impinges on mosquito population dynamics in a rather complex manner. A large amount of rain within a short period of time may wash away aquatic stages as well as adults, while continuous, low-volume rain may not be optimal for colonizing mosquito species that require temporary breeding sites. Long term moderate to heavy rainfall synchronizes mosquito population activity by increasing near-surface humidity, which enhances mosquito flight activity and host seeking behaviours, as well as altering the abundance and type of aquatic habitats available to the mosquito for oviposition and subsequent development of the immature stages (Sharman and Day, 2007). Though different *Anopheles sp* vary greatly in their preference for particular breeding sites, clear, clean and shallow seem a common characteristic of most breeding sites of *Anopheles sp*. Due to the rapid increase in urbanization and industrilasation, numerous breeding sites which are characteristically clear, clean and shallow are created for the malaria vector. Peridomestic and industrial water collections thus serve as important breeding sites for the malaria vector all year round. Seasonal variation in malaria transmission due to variation in rainfall patterns might therefore be defeated since the vector might not need rainfall for breeding. This

133

could have accounted for the lack of seasonality in malaria transmission observed in this study. These results support the assertion that at very high transmission levels malaria prevalence is not seasonal (Smith et al., 1993).

Contribution of candidate genes to artemisinin resistance

Resistance to antimalarial drugs is the single most important threat to global malaria control (Imwong et al., 2010). The deployment of ACTs, together with other control measures, has resulted in significant decrease in malaria morbidity and mortality in many endemic countries (WHO, 2008; WHO, 2010a). This success is threatened by the recent confirmation of reduced artemisinin sensitivity in *P. falciparum* in Western Cambodia (Dondorp et al., 2009). The molecular markers responsible for this reduced sensitivity to artemisinin have not been clearly elucidated. So far five candidate genes have been suggested to confer artemisinin resistance: *Pfmdr1, Pfcrt, PfATPase6,* UBP-1 and the 6kb mitochondrial genome (Imwong et al., 2010). In this study, three of the candidate genes, namely *Pfmdr1, Pfcrt* and *PfATPase6* were analyzed. Mutations at codon positions 76 of the *Pfcrt* gene, 86 and 184 of the *Pfmdr1* gene and 264 and 289 of the *PfATPase6* gene were found.

Laboratory induced artemisinin resistance in the *P. chabaudi* model has been demonstrated in chloroquine resistant strain, suggesting that chloroquine resistance may be a prerequisite for the subsequent development of artemisinin resistance (Imwong et al., 2010). The *Pfcrt* 76T mutation was found in 144

134

(58.54%) samples and the wild-type K76 allele was found in 102 samples. *Pfcrt* 76T mutation has been reported as the single most important SNP for chloroquine resistance (Su et al., 1997; Fidock et al., 2000; Djimde et al., 2001b; Durand et al., 2001; Sidhu et al., 2002; Lakshmanan et al., 2005). Reduction in the use of chloroquine in a "chloroquine resistant" region could result in the reemergence of chloroquine sensitivity (Laufer et al., 2006). Thus, the *Pfcrt* K76 wild-type allele re-emerges at the expense of the 76T mutant allele. Studies in Africa and Asia have confirmed the reemergence of chloroquine sensitivity after the use of chloroquine was reduced (Schwenke et al., 2001; Liu et al., 1995; Nguyen et al., 2003). Prior to the abolishment of chloroquine in Ghana, a study reported about 64.53% prevalence of the *Pfcrt* 76T mutation in five health centres in Ghana (Duah et al., 2006). In this study the 58.54% prevalence of the *Pfcrt* 76T mutation is considered very high after eight years of the abolishment of chloroquine usage in Ghana. This is in sharp contrast to findings from other endemic areas where chloroquine use was reduced. Chloroquine use in Ghana might not have ceased after all or another antimalarial drug with similar mechanism of action may be causing the sustenance of the *Pfcrt* 76T mutation in the population.

Artesunate+amodiaquine was the first recommended ACT for the treatment of uncomplicated malaria in Ghana. Cross-resistance between chloroquine and amodiaquine is documented (Ochong, van den Broek, Keus & Nzila, 2003; Dokomajilar et al., 2006; Holmgren et al., 2006). The *Pfcrt* 76T mutation is a common SNP shared by both chloroquine resistance and

135

amodiaquine resistant strains of *P. falciparum*. The high prevalence of the *Pfcrt* 76T mutation could, therefore, be as a result of the high use of amodiaquine in Artesunate+amodiaquine in Ghana. This high prevalence of *Pfcrt* 76T mutation threatens ACT use in Ghana in two plausible ways. First, if the suggestion that chloroquine resistance may be a pre-requisite for the subsequent development of artemisinin is accurate, then the ground is adequately prepared for the development of artemisinin resistance in Ghana. Secondly, if the high prevalence of the *Pfcrt* 76T mutation is due to amodiaquine resistance, then the artesunate+amodiaquine combination therapy is seriously threatened and hence parasites are being exposed to artesunate drug pressure, a condition necessary for the development of resistance against artesunate.

Point mutations in the *Pfmdr1* gene have also been implicated in treatment failures with artemisinin, artesunate and dihydroartemisinin (Duraisingh et al., 2000; Reed et al., 2000; Sidhu et al.S, 2002; Anderson et al., 2005). In this study, 76.83%, 23.17%, 26.42% and 73.58% of all samples that were successfully analyzed by PCR-RFLP contained the wild-type N86, mutant 86Y, wild-type Y184 and mutant 184F alleles of the *Pfmdr1* gene respectively. The N86Y and Y184F mutations of the *Pfmdr1* gene, together with other point mutations of the same gene, have been associated with treatment failures of artemether+lumefantrine and artesunate+amodiaquine. *Pfmdr1* N86, 184F and D1246 which have been associated with artemether+lumefantrine treatment failure (Sisowath et al., 2005; Dokomajilar et al., 2006; Sisowath et al., 2007; Happi et al., 2009) was recorded in 76.83%, 73.58% and 100% respectively of

136

the samples that were successfully analyzed by PCR-RFLP. The 86Y, Y184 and 1246Y alleles which have been associated with artesunate+amodiaquine treatment failure were seen in 23.17%, 26.42% and 0% of the samples. Considering the percentage prevalence of the above SNPs, artemether+lumefantrine treatment failure seems more ripe in Ghana than artesunate+amaodiaquine treatment failure. Artemther+lumefantrine usage may be more frequent than that of artesunate+amodiaquine due to the frequent associated side-effects of amodiaquine (Asante et al., 2009) resulting in a high drug pressure. The high drug pressure, together with a probable non-adherence to the ACT (WHO, 2001) might have caused the selection of the SNPs associated with artemethe+lumefantrine treatment falilure. It is, therefore, probably only a matter of time for the discovery of high treatment failure of this ACT in Ghana.

The significance of the *PfATPase6* gene in artemisinin resistance is gradually fading. Stable resistance to artemisinin has been found without the corresponding presence of the *PfATPase6* S769N and L263E mutations which have been proposed as candidate SNPs for artemisinin resistance (Afonso et al., 2006; Afoakwah et al., 2011). In this study, like most others, all SNPs of the *PfATPase6* gene that have been proposed to be involved in artemisinin resistance were not found. Two novel SNPs, Y264F and D289N, were, however, found. These two add to the many other SNPs that have been found in this gene (Afoakwah et al., 2011) to confirm the highly diverse nature of the gene.

137

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

Conclusion

Single Nucleotide polymorphisms (SNPs) of the *Pfmdr1* and *Pfcrt* genes that have been described to be associated with treatment failure with Artemisinin-based combination therapies (ACTs) are highly prevalent in Ghana. The SNPs of *PfATPase6* gene that are said to confer resistance to artemisinins, however, were not found. With the current drug pressure vis-a-vis the observed SNPs, it is only a matter of time for a stable drug resistance to be recorded in Ghana. Finding of this study and previous ones show that the *PfATPase6* gene is gradually becoming insignificant in artemisinin resistance.

Recommendations

Considering the high prevalence of the SNPs associated with treatment failures of the ACTs, a national programme to monitor the development of resistance to artemisinin is crucially needed.

Surveillance of treatment failure with ACT should also be considered as soon as possible to curtail the development of stable resistance agains the ACT.

138

Adherence to full treatment regime should be encouraged. If possible, supervision of patients under treatment should be enforced to avoid noncompliance to full treatment dosage. A surveillance on counterfeit drugs with subtherapeutic doses of active ingredients, should be rolled out.

Amodiaquine resistance in Ghana should be ascertained for a review of the Artesunate+amodiaquine treatment policy. If high levels of amodiaquine resisistance is found, then the Artesunate+amodiaquine combination therapy should be banned to prevent the development of resistance to artesunate.

Chloroquine availability and usage in the country should be a matter of concern, in view of the reported high levels of *Pfcrt* K76T mutation. The ban on chloroquine should be enforced to ensure nonavailability of the drug.
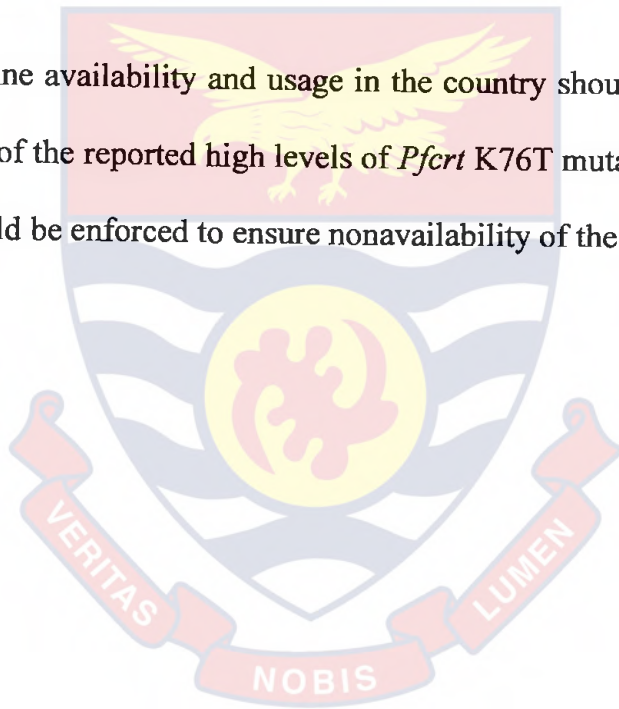
References

Acheampong, D. O., Appiah, M. G., Boamponsem, L. K., Boampong, J. N., & Afoakwah, R. (2011). The efficacy of rapid diagnostic test (rdt) in diagnosing *Plasmodium falciparum* malaria in some selected health facilities in the Cape Coast metropolis of Ghana. *Advances in Applied Science Research, 2*(4), 348-356.

Afoakwah, R., Boampong, J. N., Acheampong, O. D., & Nwaefuna, E. K. (2011). Polymorphisms in *Plasmodium falciparum* Adenosine Triphosphatase 6 (PfATPase6) gene and their significance in finding the genetic marker for Artemisinin resistance. *European Journal of Experimental Biology, 1*(3), 7 – 13.

Afoakwah, R., Boampong, J. N., Aubyn, E., & Acquah, S. Relative Susceptibility of ABO blood groups to *Plasmodium falciparum* malaria in Korle-Bu Teaching Hospital, Ghana. Unpublished data.

Afonso, A., Hunt, P., Cheesman, S., Alves, A. C., Cunha, C.V., do Rosario, V., & Cravo, P. (2006). Malaria Parasites Can Develop Stable Resistance to Artemisinin but Lack Mutations in Candidate Genes atp6 (Encoding the Sarcoplasmic and Endoplasmic

140

Reticulum Ca^{2+} ATPase), tctp, mdr1, and cg10. *Antimicrobial Agents and Chemotherapy, 50,* 480-489.

Aidoo, M., Terlouw, D. J., Kolczak, M. S., McElroy, P. D., ter Kuile, F. O. , Karuiki, S., Nahlen, B. L., Lal, A. A., & Udhayakumar, V. (2002) Protective effects of the sickle cell gene against malaria morbidity and mortality. *Lancet* 359: 1311–1312.

Aikawa, M. (1977). Variations in structure and function during the life cycle of malaria parasites. *Bulletins of WHO, 55,* 139-156.

Aikawa, M., & Sterling, C. (1974). Intracellular parasitic protozoa. New York, USA: Academic Press Inc.

Alker, A. P., Lim, P., Sem, R., Shah, N. K., Yi, P., Bouth, D. M., Tsuyuoka, R., Maguire, J. D., Fandeur, T., Ariey, F., Wongsrichanalai, C., & Meshnick, S. R. (2007). *Pfmdr1* and *in vivo* resistance to artesunate–mefloquine in falciparum malaria on the Cambodian–Thai border. *American Journal of Tropical Medicine and Hygiene, 76,* 641–647.

Allen, S. J., O'Donnell, A., Alexander, N. D., Mgone, C. S., Peto, T. E., Clegg, J. B., Alpers, M. P., & Weatherall, D. J. (1999). Prevetion of cerebral malaria in children in Papua New Guinea by Southeast Asian ovalocytosis band 3. *American Journal of Tropical Medicine and Hygiene, 60*(6),1056-1060.

Alonso, P. L., Lindsay, S. W., Armstrong, J. R. M., de Francisco, A., Shenton, F. C., Greenwood, B. M., Conteh, M., Cham, K., Hill, A. G., David, P. H., Fegan, G., & Hall, A. J. (1991) The effect of insecticide-treated bed nets on mortality of Gambian children. *Lancet, 337*(8756), 1499-1502

Anderson, T. J., Nair, S., Qin, H., Singlam, S., Brockman, A., Paiphun, L., & Nosten, F. (2005). Are transporter genes other than the chloroquine resistance locus (pfcrt) and multidrug resistance gene (pfmdr) associated with antimalarial drug resistance? *Antimicrobial Agents and Chemotherapy, 49*, 2180 – 2188.

Asante, K. P., Owusu, R., Dosoo, D., Awini, E., Adjei, G., Amenga, S. E., Chandramohan, D., & Owusu-Agyei, S. (2009). Clinical Study: Adherence to Artesunate-Amodiaquine Therapy for Uncomplicated Malaria in Rural Ghana: A Randomised Trial of Supervised versus Unsupervised Drug Administration. *Journal of Tropical Medicine, 2009*. doi:10.1155/2009/529583.

Ashton, M., Sy, N. D., Huong, N. V., Gordi, T., Hai, T. N., Huong, D. X., Nièu, N. T., & Công, L. D. (1998). Artemisinin kinetics and dynamics during oral and rectal treatment of uncomplicated malaria. *Clinical Pharmacology and Therapeutics, 63*, 482–493.

Barnes, D. A., Foote, S. J., Galatis, D., Kemp, D. J., & Cowman, A. F. (1992). Selection for high-level chloroquine resistance results in deamplification of the *pfmdr1* gene and increased sensitivityto mefloquine in *Plasmodium falciparum. EMBO* J., *11*, 3067–3075.

Barnish, G., Bates, I., & Iboro, J. (2004). Newer drug combinations for malaria. *BMJ 328*: 1511–1512.

Barragan, A., Kremser, P. G., Wahlgren, M., & Carlson, J. (2000). Blood group antigens is a receptor in Plasmodium falciparum rosetting. *Infection and Immunity. 68*(5), 2971-2975.

Baruch, D. I., Pasloske, B. L., Singh, H. B., Bi, X., Ma, X. C., Feldman, M., Taraschi, T. F., & Howard, R. J. (1995). Cloning the P. falciparum gene encoding PfEMP1, a malarial variant antigen and adherence receptor on the surface of parasitized human erythrocytes. *Cell, 82*(1): 77 – 87.

Bate, C. A. W., Taverne, J., & Playfair, J. H. (1988). Malaria Parasites induce TNF production by macrophages. *Immunology, 64*, 227-231.

Bate, C. A. W., Taverne, J., Davé, A., & Playfair, J. H. (1990). Malaria exoantigens induce T-independent antibody that blocks their ability to induce TNF. *Immunology, 70*, 315-320.

Beales, P. F., Orlov, V. S., & Kouynetsov, R. L. (eds). (1989). Malaria and planning for its control in Tropical Africa. Moscow, WHO/UNDP.

Beck, H., & Ley, S. C. (2008). Monitoring of malaria drug resistance associated SNPs in Plasmodium falciparum on microarray. In Mell, K., Ljungstrom, I., Perlmann, H., Scherf, A., & Wahlgren, M. (ed), Methods in Malaria Research (248 -257). Manassas, Virginia: MR4/ATCC.

Beg, M. A., Khan, R., Baig, S. M., Gulzar, Z., Hussain, R., & Smego, R. A. Jr. (2002). Cerebral involvement in benign tertian malaria. *American Journal of Tropical Medicine and Hygiene, 67*, 230-232.

Bell, D., Go, R., Miguel, C., Walker, J., Cacal, L., & Saul, A. (2001). Diagnosis of malaria in a remote area of the Philippines: comparison of techniques and their acceptance by health workers and the community. *Bull World Health Organ 79:* 933–941.

Bereczky, S., Mårtensson, A., Gil, J. P., & Färnert A. (2005). Short report: rapid dna extraction from archive blood spots on filter paper for genotyping of *Plasmodium falciparum. American Journal of Tropical Medicine and Hygiene., 72*(3), 249-251.

144

Biemba, G., Dolmans, D., Thuma, P. E., Weiss, G., & Gordeuk, V. R. (2000). Severe anaemia in Zambian children with *Plasmodium falciparum* malaria. *Tropical Medicine and International Health, 5,* 9–16.

Biggs, B. A., & Brown, G. V. (2001). Malaria. In Gillespie, S. & Pearson D. R. (Eds). Principles and practice of clinical Parasitology. John Wiley and Sons Ltd. New York.

Binka, F. N., Indome, F., & Smith, T. (1998). Impact of Spatial distribution of permithrin-impregnated bed nets on children mortality in rural northern Ghana. *American Journal of Tropical Medicine and Hygiene, 59,* 80-85.

Blumberg, L., Lee, R. P., Lipman, J., & Beards, S. (1996). Predictors of mortality in severe malaria: a two year experience in a non-endemic area. *Anaesthesia and Intensive Care, 24,* 217-223.

Boampong, J. N., Acquah, S., & Achiamaa A. (2009). A cross-sectional study of home-based management of malaria in Bakaano, a suburb of Cape Coast, Ghana: recognition of signs, symptoms and treatment options. *Journal of Ghana Science Association, 11*(2): 50-57

Boampong, J.N., Manno S., Koshino I., & Takakuwa Y. (2007). Erythrocyte Shape Change Prevents *Plasmodium falciparum* Invasion. *Membrane*. *32*(2) 95-103.

Bojang, K. A., Schneider, G., Forck, S., Obaro, S. K., Jaffar, S., Pinder, M., Rowley, J., & Greenwood, B. M. (1998). A trial of FansidarTM plus chloroquine or Fansidar' alone for the treatment of uncomplicated malaria in Gambian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *92*, 73-76.

Bouyou-Akotet, M. K., Dzeing-Ella, a., Kendjo, E., Etoughe, D., Ngoungou, E. B., Planche, T., Koko, J., & Kombila, M. (2009). Impact of *Plasmodium falciparum* infection on the frequency of moderate to severe anaemia in children below 10 years of age in Gabon. *Malaria Journal*, *8*:166 doi:10.1186/1475-2875-8-166

Brabin, B. J., Premji, Z., & Verhoeff, F. (2001). An analysis of anemia and child mortality. *Journal of Nutrition, 131*, 636S–648S.

Brabin, B., Prinsen-Geerligs, P., Verhoeff, F., & Kazembe, P. (2003). Anaemia prevention for reduction of mortality in mothers and children. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 97*, 36–38.

Braun-Breton, C., Pereira da Silva, H. (1993). Malaria proteases and red cell invasion. *Parasitology Taday, 9*, 92-96.

Brockman, A., Price, R. N., van Vugt, M., Heppner, D. G., Walsh, D., Sookto, P., Wimonwattrawatee, T., Looareesuwan, S., White, N. J., & Nosten, F. (2000). *Plasmodium falciparum* antimalarial drug susceptibility on the northwestern border of Thailand during five years of extensive use of artesunate-mefloquine. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 94,* 537-544.

Bronzan, R. N., McMorrow, M. L., & Kachur, S. P. (2008). Diagnosis of malaria: challenges for clincians in endemic and non-endemic regions. *Molecular Diagnosis Therapy, 12*(15), 299–306.

Brown, A. E., Webster, H. K., Teja-Isavadharm, P., & Keeratithakul, D. (1990) Macrophage activation in falciparum malaria as measured by neopterin and interferon-gamma. *Clinical and Experimental immunology, 82*:97-101.

Bruce-Chwatt, L. J. (1980). *Essential Malariology.* London: Heineman Medical Books Ltd.

Bruce-Chwatt, L. J. (1984). DNA probes for malaria diagnosis. *Lancet, 1*: 795.

Bruneel, F., Hocqueloux, L., Alberti, C., Wolff, M., Chevret, S., Bedos, J. P., Durand, R., Le Bras, J., Regnier, B., & Vachon, F. (2003). The clinical spectrum of severe imported falciparum malaria in the

ICU: Report of 188 cases in adults. *American Journal of Respiratory and Critical Care Medicine, 167*, 684-689.

Buchachart, K., Krudsood, S., Nacher, M., Chindanond, D., Rungmatcha, P., Kano, S., & Looareesuwan, S., (2004). Evaluation of the KAT-Quick malaria rapid test for rapid diagnosis of falciparum malaria in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health, 35*, 35–37.

Carlson, J., & Wahlgren, M. (1992). *Plasmodium falciparum* erythrocyte rosetting is mediated by promiscuous lectin-like interactions. *Journal of Experimental Medicine, 176*, 1311-1317.

Carruthers, V. B., & Sibley, L. D. (1999). Mobilization of intracellular calcium stimulates microneme discharge in *Toxoplasma gondii*. *Molecular Microbiology, 31*, 421-428.

Castelli, F., & Carosi, G. (1997). Diagnosis of malaria infection. In Carosi, G., Castelli, F. (eds). *The Handbook of Malaria Infection in the Tropics*. Italy. AIFO.

Cattani, J. A., Gibson, F. D., Alpers, M. P., & Crane, G. G. (1987). Hereditary ovalocytosis and reduced susceptibility to malaria in Papua New Guinea. . *Transactions of the Royal Society of Tropical Medicine and Hygiene, 81*,705-709.

148

Cerami, C., Frevert, U., Sinnis, P., Takacs, B., Clavijo, P., Santos, M. J., & Nussenzweig, V. (1992). The basolateral domain of the hepatocyte plasma membrane bears receptors for the circumsporozoite protein of *Plasmodium falciparum* sporozoite. *Cell, 70,* 1021.

Chaijaroenkul, W., Bangchang, K. N., Mungthin, M., & Ward, S. A. (2005) *In vitro* antimalarial drug susceptibility in Thai border areas from 1998 – 2003. *Malaria Journal, 4,* 37.

Chandramohan, D., Jaffar, S., & Greenwood, B. (2002). Use of clinical algorithms for diagnosing malaria. *Tropical Medicine and International Health, 7:* 45–52.

Chandramohan, D., Owusu-Agyei, S., Carneiro, I, Awine, T., Amponsa-Achiano, K., Mensah, N., Jaffar, S., Baiden, R., Hodgson, A., Binka, F., & Greenwood, B. (2005). Cluster randomised trial of intermittent preventive treatment for malaria in infants in area of high, seasonal transmission in Ghana. *BMJ, 331:*727–33.

Chavchich, M., Gerena, L., Peters, J., Chen, N., Cheng, Q., & Kyle, D. E (2010). Role of *pfmdr1* amplification and expression in induction of resistance to artemisinin derivatives in *Plasmodium falciparum. Antimicrobial Agents and Chemotherapy, 54,* 2455–2464.

Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries. Part II. Cambridge: Cambridge University Press.

Cheesbrough, M. (2005). District Laboratory Practice in Tropical Countries. Part I. (2nd edition). Cambridge: Cambridge University Press.

Chen, Q., Schlichtherle, M., & Wahlgren, M. (2000). Molecular aspects of severe malaria. *Clinical Microbiology Reviews, 13*: 236.

Chotivanich, K. T., Udomsangpetch, R., Pipitaporn, B., Angus, B., Suputtamongkol, Y., Pukrittayakamee, S., & White, N. J. (1998). Rosetting characteristics of uninfected erythrocytes from healthy individuals and malaria patients. *American Journal of Tropical Medicine and Parasitology, 92*, 45–56.

Chou, A. C., Chevli, R. & Fitch, C. D. (1980). Ferriprotoporphyrin IX fulfills the criteria for identification as the chloroquine receptor of malaria parasites. *Biochemistry, 19*, 1543-1549.

Clark, I. A., Virelizier, J. L., Carswell E. A. & Wood, P. R. (1981). Possible importance of macrophage-derived mediators in acute malaria. *Infection and Immunity, 32*, 1058-1066.

Cockburn, I. A., Mackinnon, M. J., O'Donnell, A., Allen, S. J., Moulds, J. M., Baisor, M., Bockarie, M., Reeder, J. C., & Rowe, J. A. (2004). A

human complement *falciparum* rosetting confers protection against severe malaria. *PNAS, 101*:272-277.

Coetzee, M. (2004). Distribution of the African malaria vectors of the *Anopheles gambiae* complex. *American Journal of Tropical Medicine and Hygiene, 70*(2), 103-104.

Cojean, S., Hubert, V., Le Bras, J. & Durand, R. (2006). Resistance to dihydroartemisinin. *Emerging Infectious Diseases, 12*, 1798-1799.

Cooper, R. A., Ferdig, M. T., Su, X., Ursos, L. M. B., Mu, J., Nomura, T., Fujioka, H., Fidock, D. A.,Roepe, P. D. & Wellems, T. E. (2002). Alternative mutations at position 76 of the vacuolar transmembrane protein PfCRT are associated with chloroq/uine resistance and unique stereospecific quinine and quinidine responses in *Plasmodium falciparum. Molecular Pharmacology, 61*(1),35–42.

Cox-Singh, J., Davis, T. M., Lee, K. S., Shamsul, S. S., Matusop, A., Ratnam, S., Rahman, H. A., Conway, D. J., & Singh, B. (2008). Plasmodium knowlesi malaria in humans is widely distributed and potentially life threatening. *Clinical Infectious Diseases, 46*(2), 165-171.

Cserti, C. M., & Dzik, W. H. (2007). The ABO blood group system and Plasmodium falciparum malaria. *Blood, 110*:2250–2258.

Dahlström, S., Veiga, M. I., Ferreira, P., Mårtensson, A., Kaneko, A., Andersson, B., Björkman, A. & Gil, J. P. (2008). Diversity of sarco/endoplasmic reticulum Ca^{2+} -ATPase orthologue of *Plasmodium falciparum. Infection Genetics and Evolution, 8*, 340-345.

Daneshvar, C., Davis, T. M., Cox-Singh, J., Rafa'ee, M. Z., Zakaria, S. K., Divis, P. C. S., & Singh, B. (2010). Clinical and parasitological response to oral chloroquine and primaquine in uncomplicated human *Plasmodium knowlesi* infections. *Malaria Journal, 9*, 238.

Darlow, B., Vrbova, H., Gibney, S., Jolley, D., Stace, J., & Alpers, M. (1982). Sulfadoxine-pyrimethamine for treatment of acute malaria in children in Papua New Guinea. *American Journal of Tropical Medicine and Hygiene, 31,* 1-9.

De Zuleta, J., Kafuko, G. W., McCrae, A. W. R., Cullen, J. R., Pedrsen, C. K., & Wasswa, D. F. (1964). A malaria eradication experiment in the highlands of Kigezi (Uganda). *East African Medical Journal, 41*, 102–120.

Delacollette, C., van der Stuyft, P., & Molima, K. (1996). Using community health workers for malaria control: experience in Zaire. *Bull World Health Organ*, 74:423-430.

deMaeyer, E, & Adiels-Tegman, M. (1985). The prevalence of anaemia in the world. *World Health Stat Q, 38*:302-316.

Denis, M. B., Tsuyuoka, R., Poravuth, Yi., Narann, T. S., Seila, S., Lim, C., Incardona, S., Lim, P., Sem, R., Socheat, D., Christophel, E. M., & Ringwald, P. (2006). Surveillance of the efficacy of artesunate and mefloquine combination for the treatment of uncomplicated falciparum malaria in Cambodia. *Tropical Medicine and International Health, 11*, 1360–1366.

Dery, D. B., Brown, C., Asante, K. P., Adams, M., Dosoo, D., Amenga-Etego, S., Wilson, M., Chandramohan, D., Greenwood, B., & Owusu-Agyei, S. (2010). Patterns and seasonality of malaria transmission in the forest-savannah transitional zones of Ghana. *Malaria Journal, 9*(314).

Desai, M. R., Terlouw, D. J., Kwena, A. M., Phillips-Howard, P. A., Kariuki, S. K., Wannemuehler, K. A., Odhacha, A., Hawley, W. A., Shi, Y. P., Nahlen, B. L., & Ter Kuile, F. O. (2005). Factors associated with hemoglobin concentrations in pre-school children in

Western Kenya: cross-sectional studies. *American Journal of Tropical Medicine and Hygiene, 72,* 47-59.

Devine, D. V. (1991). The regulation of complement on cell surfaces. *Transfusion Medicine Reviews, 5,* 123-131.

Dicko, A., Mantel, C., Kouriba, B., Sagara, I., Thera, M. A., Doumbia, S., Diallo, M., Poudiougou, B., Diakite, M., & Doumbo, O. K. (2005). Season, fever prevalence and pyrogenic threshold for malaria disease definition in an endemic area of Mali. *Tropical Medicine and International Health, 10,* 550–556.

Dinis, D. V., & Schapira, A. (1990). Comparative study of the efficacy and side-effects of two therapeutic regimens against chloroquine-resistant falciparum malaria in Maputo, Mozambique. *Bulletin de la Société de Pathologie Exotique, 83,* 521-528.

Djimdé, A., Doumbo, O. K., Cortese, J. F., Kayentao, K., Doumbo, S., Diourté, Y., Coulibaly, D., Dicko, A., Su, X., Nomura, T., Fidock, D. A., Wellems, T. E. & Plowe, C. V. (2001b). A molecular marker for chloroquine-resistant falciparum malaria. *New England Journal of Medicine, 344,* 257-263.

Djimde, A., Doumbo, O. K., Steketee, R.W., & Plowe, C.V. (2001a) Application of a molecular marker for surveillance of chloroquine-resistant falciparum malaria. *Lancet, 358,* 890–891.

154

Dokomajilar, C., Lankoade, M. Z., Dorsey G., Zongo, I., Ouedraogo, J. B., & Rosenthal, P. J. (2006). Roles of Specific *Plasmodium falciparum* mutations in resistance to amodiaquine and Sulfadoxine-pyrimethamine in Burkina Faso. *American Journal of Tropical Medicine and Hygiene, 75,* 162 – 165.

Dokomajilar, C., Nsobya, S. L., Greenhouse, B., Rosenthal, P. J., & Dorsey, G. (2006). Selection of *Plasmodium falciparum* pfmr1 alleles following therapy with artemether-lumefantrine in an area of Uganda where malaria is highly endemic. *Antimicrobial Agents and Chemotherapy, 50,* 1893 – 1895.

Dolan, S. A., Miller, L. H., & Wellems, T. E. (1990). Evidence for a switching mechanism in the invasion of erythrocytes by *Plasmodium falciparum. Journal of Clinical Investigation, 86,* 618–624.

Dondorp, A. M., Angus, B. J., Hardeman, M. R., Chotivanich, K. T., Silamut, K., Ruangveerayuth, R., Kager, P. A., White, N. J., & Vreeken, J. (1997). Prognostic significance of reduced red blood cell deformability in severe falciparum malaria. *American Journal of Tropical Medicine and Hygiene, 57,* 507-511.

Dondorp, A. M., Nosten, F., Yi, P., Das, D., Phyo, A. P., Tarning, J., Lwin, K. M., Ariey, F., Hanpithakpong, W., Lee, S. J., Ringwald, P., Silamut, K., Imwong, M., Chotivanich, K., Lim, P., Herdman, T.,

155

An, S.S., Yeung, S., Singhasivanon, P., Day, N. P. J., Lindegardh, N., Socheat, D. & White, N. J. (2009). Artemisinin resistance in *Plasmodium falciparum* malaria. *New England Journal of Medicine, 361,* 455–467.

Dondorp, A. M., Nyanoti, M., Kager, P. A., Mithwani, S., Vreeken, J., & Marsh, K. (2002). The role of reduced red cell deformability in the pathogenesis of severe falciparum malaria and its restoration by blood transfusion. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 96,* 282-286.

Dondorp, A. M., Pongponratn, E., & White, N. J. (2004). Reduced microcirculatory flow in severe falciparum malaria: pathophysiology and electron-microscopic pathology. *Acta Tropica, 89,* 309–317

Dondorp, A., Nosten, F., Stepniewska, K., Day, N., White, N., & South East Asian Quinine Artesunate Malaria Trial (SEAQUAMAT) group. (2005). Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet. 366*(9487): 717-25.

Dorsey, G., Staedke, S., Clark, T. D., Njama-Meya, D., Nzarubara, B., Maiteki-Sebuguzi, C., Dokomajilar, C, Kamya, M. R. & Rosenthal, P. J. (2007). Combination therapy for uncomplicated falciparum

malaria in Ugandan children: a randomized trial. *JAMA, 297,* 2210-2219.

Drakeley, C. J., Jawara, M., Targett, G. A., Walraven, G., Obisike, U., Coleman, R., Pinder, M., & Sutherland, C. J. (2004). Addition of artesunate to chloroquine for treatment of *Plasmodium falciparum* malaria in Gambian children causes a significant but short-lived reduction in infectiousness for mosquitoes. *Tropical Medicine and International Health, 9*(1), 53-61.

Duah, N. O., Wilson, M. D., Ghansah, A., Abuaku, B., Edoh, D., Quashie, N. B., & Koram, K. A. (2006). Mutations in *Plasmodium falciparum* chloroquine resistance transporter and multidrug resistance genes, and treatment outcomes in Ghanaian children with uncomplicated malaria. *Journal of Tropical Pediatrics, 53*(1), 27-31.

Duraisingh, M. T., Jones, P., Sambou, I., Von Seidlein, L., Pinder, M. & Warhurst, D. C. (2000). The tyrosine-86 allele of the *pfmdr1* gene of *Plasmodium falciparum* is associated with increased sensitivity to the anti-malarials mefloquine and artemisinin. *Molecular Biochemistry and Parasitology, 108,* 13–23.

Duraisingh, M. T., Roper, C., Walliker, D., & Warhurst, D. C. (2000). Increased sensitivity to the antimalarials mefloquine and artemisinin is

conferred by mutations in the *pfmdr1* gene of *Plasmodium falciparum. Molecular Microbiology, 36,* 955–961.

Durand, R., Jafari, S., Vauzelle, J., Delabre,J., Jesic, Z. & Le Bras, J. (2001) Analysis of pfcrt point mutations and chloroquine susceptibility in isolates of Plasmodium falciparum. *Molecular Biochemistry and Parasitology, 114*(1), 95-102.

Eckstein-Ludwig, U., Webb, R. J., van Goethem, D. A., East, J. M., Lee, A. G., Kimura, M., O'Neil, P. M., Bray, P. G., Ward, S. A., & Krishna, S. (2003). Artemisinins target the SERCA of *Plasmodium falciparum. Nature, 424,* 957-961.

Encyclopedia Britannica (2009). "Ghana." Ultimate Reference Suite. Chicago.

Esposito, F., & Habluetzel, A. (1997). The Anopheles vector. IN: Carosi, G., Castelli, F. (eds). The Handbook of Malaria Infection in the Tropics. Italy. AIFO.

Face, C. A. (1983). Merozoites of *P. falciparum* require glycophorins for invasion into red blood cells. *Bulletin de la Societe de pathologie exotique et de ses filiales, 76,* 463-469.

Facer, C. A. (1980). Direct Coombs antiglobulin reactions in Gambian children with Plasmodium falciparum malaria. II. Specificity of

erythrocyte-bound IgG. *Clinical and Experimental Immunology,*
39, 279-288.

Facer, C. A., Bray, R. S., & Brown, J. (1979). Direct Coombs antiglobulin
reactions in Gambian children with Plasmodium falciparum
malaria. I. Incidence and class specificity. *Clinical and
Experimental Immunology, 35,* 119-127.

Falade, C., Makanga, M., Premji, Z., Ortmann, C. E., Stockmeyer, M. & De
Palacios, P. I. (2005). Efficacy and safety of artemether-
lumefantrine (Coartem®) tablets (six-dose regimen) in African
infants and children with acute, uncomplicated falciparum
malaria. *Transactions of the Royal Society of Tropical Medicine
and Hygiene, 99,* 459-467.

Farcas, G. A., Zhong, K. J., Lovegrove, F. E., Graham, C. M., & Kain, K. C.
(2003). Evaluation of the Binax NOW ICT test versus
polymerase chain reaction and microscopy for the detection of
malaria in returned travelers. *American Journal of Tropical
Medicine and Hygiene, 69,* 589–592.

Farvacque-Vitkovic, C., Raghunath, M., Eghoff, C., & Boakye, C. (2008)
African Region Working Paper Series Number 110.
Development of the Cities of Ghana – Challenges, Priotities and
Tools. The World Bank.

Fernando, S. D., Karunaweera, N. D., & Fernando, W. P. (2004). Evaluation of a rapid whole blood immunochromatographic assay for the diagnosis of *Plasmodium falciparum* and *Plasmodium vivax* malaria. *Ceylon Medical Journal, 49,* 7–11.

Ferreira, I. D., Lopes, D., Martinelli, A., Ferreira, C., do Rosário, V. E., & Cravo, P. (2007). In vitro assessment of artesunate, artemether and amodiaquine susceptibility and molecular analysis of putative resistance-associated mutations of *Plasmodium falciparum* from São Tomé and Príncipe. *Tropical Medical International Health, 12,* 353-362.

Fidock, D. A., Eastman, R. T., Ward, S. A., & Meshnick, S. R. (2008). Recent highlights in antimalarial drug resistance and chemotherapy research. *Trends in Parasitology, 24*(12), 537-544.

Fidock, D. A., Nomura, T., Talley, A. K., Cooper, R. A., Dzekunov, S. M., Ferdig, M. T., Ursos, L. M. B., Sidhu, A. B. S., Naudé, B., Deitsch, K. W., Su, X.,Wootton, J. C., Roepe, P. D. & Wellems, T. E. (2000). Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. *Molecular Cell, 6*(4), 861-871.

Fischer, P. R., & Boone, P. (1998). Short report: severe malaria associated with blood group. *American Journal of Tropical. Medicine and Hygiene, 58,* 122-123.

Flint, J., Harding, R. M., Boyce, A. J., & Clegg, J. B. The population genetics of the haemoglobinopathies. *Baillière's Clinical Haematology, 11*:1-51.

Foo, L. C., Rekhraj, V., Chiang, G. L., & Mak, J. W. (1992). Ovalocytosis protects against severe malaria parasitemia in the Malayan aborigines. *American Journal of Tropical Medicine and Hygiene, 47,* 271-275.

Foote, S. A., & Cowman, A. F. (1994). The mode of action and the mechanism of resistance to antimalarial drugs. *Acta Tropica, 56,* 157-171.

Foote, S. J., Kyle, D. E., Martin, R. K., Oduola, A. M., Forsyth, K., Kemp, D. J. & Cowman, A. F. (1990). Several alleles of the multidrug-resistance gene are closely linked to chloroquine resistance in *Plasmodium falciparum. Nature, 345,* 255–258.

Foote, S. J., Thompson, J. K., Cowman, A. F. & Kemp, D. J. (1989). Amplification of the multidrug resistance gene in some chloroquine resistant isolates of *Plasmodium falciparum. Cell, 57,* 921–930.

161

Forney, J. R., Wongsrichanalai, C., Magill, A. J., Craig, L. G., Sirichaisinthop, J., Bautista, C. T., Miller, R. S., Ockenhouse, C. F., Kester, K. E., Aronson, N. E., Andersen, E. M., Quino-Ascurra, H. A., Vidal, C., Moran, K. A., Murray, C. K., DeWitt, C. C., Heppner, D. G., Kain, K. C., Ballou, W. R., & Gasser, R. A. Jr, (2003). Devices for rapid diagnosis of malaria: evaluation of prototype assays that detect *Plasmodium falciparum* histidine-rich protein 2 and a *Plasmodium vivax*-specific antigen. *Journal of Clinical Microbiology, 41,* 2358–2366.

Garrett-Jones, C. (1964) Prognosis for interruption of malaria transmission through assessment of the mosquito's vectorial capacity. *Nature, 204,* 1173–1174.

Genton, B., Al-Yaman, F., Mgone, C. S., Alexander, N., Paniu, M. M., Alpers, M. P., Mokela, D. (1995). Ovalocytosis and cerebral malaria. *Nature, 378,* 564-565.

Getahun, A., Deribe, K., & Deribew, A. (2010). Determinants of delay in malaria treatment seeking behavior for under-five children in south-west Ethiopia: a case control study. *Malaria Journal, 9*(320). Doi: 10.1186/1475-2875-9-320.

Gillies, M. T. (1988). Anopheline mosquitoes: vector behavior and bionomics. In Wernsdorfer, W. H., McGregoor, I. (Eds). Malaria: Principles

162

and Practice of Malariology (pp. 453 – 485). Edinburgh, Churchill Livengstone.

Greenwood, B. (2006). Review: Intermittent preventive treatment – a new approach to the prevention of malaria in children in areas with seasonal malaria transmission. *Tropical Medicine and International Health, 11*(17), 983 – 991. doi:10.1111/j.1365-3156.2006.01657.x

Grobusch, M. P., Alpermann, U., Schwenke, S., Jelinek, T., & Warhurst, D. C. (1999). False-positive rapid tests for malaria in patients with rheumatoid factor. *Lancet 353:* 297.

Grobusch, M. P., Hanscheid, T., Gobels, K., Slevogt, H., Zoller, T., Rogler, G., & Teichmann, D. (2003). Comparison of three antigen detection tests for diagnosis and follow-up of falciparum malaria in travellers returning to Berlin, Germany. *Parasitology Research, 89,* 354–357.

Hadley, T. J., Klotz, F. W., Pasvol, G., Haynes, J. D., McGinniss, M. H., Okubo, Y., & Miller, L. H. (1987). Falciparum malaria parasites invade erythrocytes that lack glycophorin A and B (MkMk): strain differences indicate receptor heterogeneity and two pathways for invasion. *Journal of Clinical Investigation, 80,* 1190–1193.

Hansford, C. F. (1972). Recent trends in the control and treatment of malaria. *South African Medical Journal, 46,* 635–637.

Happi, C. T., Gbotosho, G. O., Folarin, O. A., Sowunmi, A., Hudson, T., O'Neil, M., Milhous, W., Wirth, D. F., & Oduola, A. M. J. (2009). Selection of *Plasmodium falciparum* multi-drug resistance gene 1 alleles in asexual stages and gametocytes by artemether-lumefantrine in Nigerian children with uncomplicated *falciparum* malaria. *Antimicrobial Agents and Chemotherapy, 53,* 888-895.

Hawley, A. W., Philips-Howard, P. A., ter Kuile, F. O., Terlouw, D. J., Vulule, J. M., Ombok, M., Nahlen, B. I., Gimnig, J. E., Kariuki, S. K., Kolczak, M. S., & Hightower, A. W. (2003). Community-wide effects of permethrin treated bed nets on child mortality and malaria morbidity in Western Kenya. *American Journal of Tropical Medicine and Hygiene, 68*(Suppl 4), 121-127.

Hay, S. I., Guerra, C. A., Tatem, A. J., Noor, A. M., & Snow, R. W. (2004). The global distribution and population at risk of malaria: past, present, and future. *Lancet Infectious Diseases, 4,* 327-336.

Hein, T. T., & White, N. J. (1993). Qinghaosu. *Lancet, 341,* 603-608.

Hien, T. T.,Davis, T. M. E., Chuong, L. V., Ilett, K. F., Sinh, D. X. T.,Phu, N. H., Agus, C., Chiswell, G. M., White, N. J., & Farrar, J. (2004).

164

Comparative pharmacokinetics of intramuscular artesunate and artemether in patients with severe falciparum malaria. *Antimicrobial Agents and Chemotherapy, 48*(11), 4234–4239.

Hill, A. V. S. (1992). Malaria resistance genes: a natural selection. *Transactions of the Royal Society for Tropical Medicine and Hygiene, 86*, 225-226.

Hill, A. V., Allsopp, C. E., Kwiatkowski, D., Anstey, N. M., Twumasi, P., Rowe, P. A., Bennett, S., Brewster, D., McMichael, J. A., & Greenwood, B. M. (1991) Common west African HLA antigens are associated with protection from severe malaria. *Nature, 352*, 595–600.

Holmgren, G., Gil, J. P., Ferreira, P. M., Veiga, M. I., Obonyo, C. O., & Bjorkman, A. (2006). Amodiaquine resistant *Plasmodium falciparum* malaria *in vivo* is associated with selection of pfcrt 76T and fmdr1 86Y. *Infection Genetics and Evolution, 6*, 309 – 314.

Holmgren, G., Harmin, J., Svard, J., Martensson, A., Gil, J. P., Bjorkman, A. (2007). Selection of pfmdr1 mutations after amodiaquine monotherapy and amodiaquine plus artemisinin combination therapy in East Africa. *Infection Genetics and Evolution, 7*, 562 – 569.

165

Hopkins, H., Talisuna, A., Whitty, C. J. M., & Staedke, S. G. (2007). Impact of home-based management of malaria on health outcomes in Africa: a systematic review of the evidence. *Malaria Journal*, *6*, 134

Houwen, B. (2002). Blood film preparation and staining procedures. *Clinics in Laboratory Medicine*, *22*, 1–14.

http://en.wikipedia.org/wiki/Greater_Accra_Region 18th February, 2012 last visited.

http://ghanadistricts.com/region/ 18th February, 2012 last visited.

http://www.ghana.gov.gh/census/phc2010.pdf

http://www.statsghana.gov.gh/ 18th February, 2012 last visited.

http://www.statsghana.gov.gh/Prm.html 20th February, 2012 last visited.

Humphries, D., Nguyena, S., Boakye, D., Wilson, M., & Cappelloa, M. (2012). The promise and pitfalls of mass drug administration to control intestinal helminth infections. *Current Opinion in Infectious Diseases*, *25*, 584–589. DOI:10.1097/QCO.0b013e328357e4cf

Hunt, R. H., Coetzee, M. & Fettene, M. (1998). The Anopheles gambiae complex: a new species from Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *92*, 231–235.

Imwong, M., Dondorp, A. M., Nosten, F, Yi, P., Mungthin, M., Hanchana,S., Das, D., Phyo, A. P., Lwin, K.M., Pukrittayakamee, S., Lee, S. J., Saisung, S., Koecharoen, K., Nguon, C., Day, N. P. J., Socheat, D. & White, N. J.   (2010). Exploring the contribution of candidate genes to artemisinin resistance in *Plasmodium falciparum. Antimicrobial Agents and Chemotherapy, 54*, 2886–2892

Iqbal, J., Muneer, A., Khalid, N. & Ahmed, M. A. (2003). Performance of the OptiMAL test for malaria diagnosis among suspected malaria patients at the rural health centers. *American Journal of Tropical Medicine and Hygiene, 68*, 624–628.

Ittarat, W., Pickard, A.L., Rattanasinganchan, P., Wilairatana, P., Looareesuwan, S., Emery, K., Low, J., Udomsangpetch, R. & Meshnick, S. R. (2003). Recrudescence in Artesunate-Treated Patients with Falciparum Malaria is Dependent on Parasite Burden not on Parasite Factors. *American Journal of Tropical Medicine and Hygiene, 68*(2), 147-152.

Jackson, Y., Chappuis, F., Loutan, L. & Taylor, W. (2006). Malaria Treatment Failures after Artemisinin-Based Therapy in three Expatriates: Could Improved Manufacturer Information Help to Decrease the Risk of Treatment Failure? *Malaria Journal, 5*, 81.

167

Jakeman, G, N., Saul, A., Hogarth, W. L. & Collins, W. E. (1999). Anaemia of acute malaria infections in non-immune patients primarily results from destruction of uninfected erythrocytes. *Parasitology, 119,* 127-133.

Jambou, R., Legrand, E., Niang, M., Khim, N. & Mercereau-Puijalon, O. (2005). Resistance of *Plasmodium falciparum* field isolates to *in vitro* artemether and point mutation of *SERCA-type PfATPase6. Lancet, 366,* 1960-1963.

Jarolim, P., Palek, J., Amato, D., Hassan, K., Sapak, P., Nurse, G. T., Rubin, H. L., Zhai, S., Shar, K. E. & Liu, S. C. (1991). Deletion in the erythrocyte band 3 gene in malaria-resistant Southeast Asian ovalocytosis. *Proceedings of the National Academy of Science, 88,* 11022-11026.

Jenkins, N. E., Chakravorty, S. J., Urban, B. C., Kai, O. K., Marsh, K. & Craig, A. G. (2006). The effect of *Plasmodium falciparum* infection on expression of monocyte surface molecules. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 100,* 1007-1012.

Johnson, D. J., Fidock, D. A., Mungthin, M., Lakshmanan, V., Sidhu, A. B. S., Bray, P. G. & Ward, S. A. (2004). Evidence for a central role for

PfCRT in conferring *Plasmodium falciparum* resistance to diverse antimalarial agents. *Molecular Cell, 15(6)*, 867–877.

Johnston, S. P., Pieniazek, N. J., Xayavong, M. V., Slemenda, S. B., Wilkins, P. P. & da Silva, A. J. (2006). PCR as a confirmatory technique for laboratory diagnosis of malaria. *Journal of Clinical Microbiology, 44*, 1087–1089.

Juliano, R. L. & Ling, V. (1976). A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochimica et Biophysica Acta, 455*, 152–162.

Kai, O. K. & Roberts, D. J. (2008). The pathophysiology of malarial anaemia: Where have all the red cells gone? *BMC Medicine*, 6, 24. doi:10.1186/1741-7015-6-24.

Kain, K. C. & Keystone, J. S. (1998). Malaria in travelers. Epidemiology, disease, and prevention. *Infectious Disease Clinics of North America, 12*, 267-284.

Kato, G. J., McGowan, V., Machado, R. F., Little, J. A., Taylor, J. 6th, Morris, C. R., Nichols, J. S., Wang, X., Poljakovic, M., Morris, S. M. Jr. & Gladwin, M. T. (2006). Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension, and death in patients with sickle cell disease. *Blood, 107(6)*, 2279–85.

Kayser, F. H., Bienz, K. A., Eckert, J. & Zinkernagel, R. M. (2005). Medical Microbiology. New York, NY: Thieme.

Kidane, G. & Morrow, R. H. (2000). Teaching mothers to provide home treatment of malaria in Tigray, Ethiopia: a randomised trial. *Lancet*, 356, 550-555.

Kitchen, S. F. (1949). Symptomatology: general considerations and falciparum malaria. In Boyd M.F. (Ed). Malariology, Vol. 2 (pp 996-1017). Philadelphia: WB Saunders.

Kouznetsov, R. L. (1977). Malaria control by application of indoor spraying of residual insecticides in Tropical Africa and its impact on population health. *Tropical Doctor*, 7, 81–91.

Krogstad, D. J., Gluzman, I. Y., Kyle, D. E., Oduola, A. M. & Martin, S. K. (1987). Efflux of chloroquine from *Plasmodium falciparum*: mechanism of chloroquine resistance. *Science*, 238, 1283–1285.

Krogstad, D. & Schlesinger, P. H. (1987). Acid vesicle function, intracellular pathogens and the action of chloroquine against *Plasmodium falciparum*. *New England Journal of Medicine*, 317, 542-549.

Kwiatkowski, D. P. (2005). How malaria has affected the human genome and what human genetics can teach us about malaria. *American Journal of Epidemiology*, 77, 171-192.

170

Kwiatkowski, D., Cannon, J. G., Manogue, K. R., Dinarello C. A. & Greenwood, B. M. (1989). Tumour necrosis factor production in Falciparum malaria and its association with schizont rupture. *Clinical and Experimental Immunology, 77,* 361-366.

Ladhani, S., Lowe, B., Cole, A. O., Kowuondo, K. & Newton, C. R. (2002). Changes in white blood cells and platelets in children with falciparum malaria: relationship to disease outcome. *British Journal of Haematology,* 119, 839-847.

Laferl, H., Kandel, K., Pichler, H. (1997). False positive dipstick test for malaria. *New England Journal of Medicine, 337,* 1635–1636.

Lakshmanan, V., Bray, P. G., Verdier-Pinard, D., Johnson, D. J., Horrocks, P., Muhle, R. A., Alakpa, G. E., Hughes, R. H.,Ward, S. A. Krogstad, D. J., Sidhu, A. B. & Fidock, D. A. (2005). A critical role for PfCRT K76T in *Plasmodium falciparum* verapamil-reversible chloroquine resistance. *EMBO Journal, 24,* 2294–2305.

Lamikanra, A. A., Brown, D., Potocnik, A., Casals-Pascual, C., Langhorne, J., Roberts, D. J. (2007). Malarial anemia: of mice and men. *Blood, 110,* 18-28.

Laufer, M. K., Thesing, P. C., Eddington, N. D., Masonga, R., Dzinjalamala, F. K., Takala, S. L., Taylor, T. E., & Plowe, C.V. (2006). Return of

chloroquine antimalarial efficacy in Malawi. *New England Journal of Medicine*, *355*, 1959-1966.

Le Hesran, J.Y., Cot, M., Personne, P., Fievet, N., Doubois, B., Beyeme, M., Boudin, C. & Deloron, P. (1997) Maternal placental infection with Plasmodium falciparum and malaria morbidity during the first 2 years of life. *American Journal of Epidemiology, 146*, 826-831.

Leikin, S. L., Gallagher, D., Kinney, T. R., Sloane, D., Klug, P., Rida, W. (1989). Mortality in children and adolescents with sickle cell disease. Cooperative Study of Sickle Cell Disease. *Pediatrics*, *84*(3), 500–508.

Lim, P., Alker, A. P., Khim, N., Shah, N. K., Incardona, S., Doung, S., Yi, P., Bouth, D. M., Bouchier, C., Puijalon, O. M., Meshnick, S. R. Wongsrichanalai, C., Fandeur, T., Le Bras, J., Ringwald, P. & Ariey, F. (2009). Pfmdr1 copy number and artemisinin derivatives combination therapy failure in falciparum malaria in Cambodia. *Malaria Journal, 8,* 11.

Lindsay, S. W. & Gibson, M. E. (1988). Bed-nets revisited- old idea, new angle. *Parasitology Today*, *4*, 270-272.

Liu, D. Q., Liu, R. J., Ren, D. X., Gao, D. Q., Zhang, C. Y., Qui, C. P., Cai, X. Z., Ling, C. F., Sang, A. H., & Tang, X. (1995). Changes in the

172

resistance of Plasmodium falciparum to chloroquine in Haina, China. *Bulletins of World Health Organization, 73*(4), 483-486.

Lividas, G., Mouchet, J., Gariou, J. & Chastang, R. (1958). Peut-on envisager l'éradication du paludisme dans la région forestière du Sud Cameroun? *Rivistade Malariologia, 37,* 229–256.

Loscertales, M. P. & Brabin, B. J. (2006). ABO phenotypes and malaria related outcomes in mothers and babies in The Gambia: a role for histo-blood groups in placental malaria. *Malaria Journal, 5,* 1-6.

Luse, S. A. & Miller, L. H. (1971). *Plasmodium falciparum* malaria: ultrastructure of parasitized erythrocytes in cardiac vessels. *American Journal of Tropical Medecine and Hygiene, 20,* 655–660.

Luxemburger, C., Brockman, A., Silamut, K., Nosten, F., van Vugt, M., Gimenez, F., Chongsuphajaisiddhi, T., & White, N. J. (1998). Two patients with falciparum malaria and poor in vivo responses to artesunate. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 92,* 668–669.

Mabaso, M. L., Craig, M., & Smith, T. (2007). Environmental predictors of the seasonality of malaria transmission in Africa: the challenge. *American Journal of Tropical Medicine and Hygiene, 76,* 33-38.

173

MacDonald, G. (1957). The Epidemiology and Control of Malaria. London: Oxford University Press.

Macete, E., Aide, P., Aponte, J. J., Sanz, S., Mandomando, I., Espasa, M., Sagauque, B., Dobano, C., Mabunda, S., Dgedge, M., Alonso, P. & Menendez, C. (2006). Intermittent preventive treatment for malaria control administered, at the time of routine vaccinations in Mozambican infants: a randomized, placebo-controlled trial. *Journal of Infectious Disease, 194,* 276–85.

Maguire, J. D., Lederman, E. R., Barcus, M. J., O'Meara, W. A., Jordon, R. G., Duong, S., Muth, S., Sismadi, P., Bangs, M. J., Prescott, W. R., Baird, J. K. & Wongsrichanalai, C. (2006). Production and validation of durable, high quality standardized malaria microscopy slides for teaching, testing and quality assurance during an era of declining diagnostic proficiency. *Malaria Journal, 5,* 92.

Makkar, R. P., Mukhopadhyay, S., Monga, A., Monga, A., & Gupta, A. K. (2002). *Plasmodium vivax* malaria presenting with severe thrombocytopenia. *Brazilian Journal of Infectious Diseases, 6,* 263-265.

Malaria Research & Development Alliance (2005). Malaria Research and Development: An Assessment of Global Investment. www.MalariaAlliance.org.

Malik, E. M., Hanafi, K., Ali, S. H., Ahmed, E. S., & Mohammed, K. A. (2006). Treatment seeking behavior for malaria in children under five years of age: implication for home management in rural areas with high seasonal transmission in Sudan. *Malaria Journal, 5*(60), doi:10.1186/1475-2875-5-60.

Marsh, K., Forster, D., Waruiru, C., Mwangi, I., Winstanley, M., Marsh, V., Newton, C., Winstanley, P., Warn, P., Peshu, N., Pasvol, G. & Snow, R. (1995) Indicators of lifethreatening malaria in African children. *New England Journal of Medicine, 332,* 1399–1404.

Martin, S. K., Miller, L. H., Hicks, C. U., David-West, A., Ugbode, C. & Deane, M. (1979). Frequency of blood group antigens in Nigerian children with falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 73,* 216–218.

Massaga, J. J., Kitua, A. Y., Lemnge, M. M., Akida, J. A., Malle, L. N., Ronn, A. M., Theander, T. G. & Bygbjerg, I. C. (2003). Effect of intermittent treatment with amodiaquine on anaemia and malarial fevers in infants in Tanzania: a randomised placebo-controlled trial. *Lancet, 361,* 1853–60

May, J., Evans, J. A., Timman, C., Ehmen, C., Busch, W., Thye, T., Agbenyaga, T., & Horstmann, R. D. (2007). Haemoglobin variants and disease manifestations in severe falciparum Malaria. *JAMA, 297*, 222002226.

Mboera, L. E., Fanello, C. I., Malima, R. C., Talbert, A., Fogliati, P., Bobbio, F. & Molteni, F. (2006). Comparison of the Paracheck-Pf test with microscopy, for the confirmation of *Plasmodium falciparum* malaria in Tanzania. *Annals of Tropical Medicine and Parasitology, 100,* 115–122.

McAdam, A. J. & Sharpe, A. H. (2005). Infectious Disease. In V. Kumar, A. K. Abbas, N. Fausto (Eds.), Pathologic basis of disease (pp. 343-414). Elsevier Saunders. Philadelphia.

McDermott, J. M., Slutsker, L., Steketee, R. W., Wirima, J. J., Breman, J. G. & Heymann, D. L. (1996). Prospective assessment of mortality among a cohort of pregnant women in rural Malawi. *American Journal of Tropical Medicine and Hygiene, 55,* 66–70.

McGregor, I. A. (1984). Epidemiology, malaria and pregnancy. *American Journal of Tropical Medicine and Hygiene, 33,* 517-525.

McKenzie, F. E, Sirichaisinthop, J., Miller, R. S., Gasser, R. A. Jr. & Wongsrichanalai, C. (2003). Dependence of malaria detection and species diagnosis by microscopy on parasite density.

American Journal of Tropical Medicine and Hygiene, 69, 372–376.

Mehta, K. S., Halankar, A. R., Makwana, P. D., Torane, P. P., Satija, P. S., & Shah, V. B. (2001). Severe acute renal failure in malaria. *Journal of Postgraduate Medicine, 47:*24-26.

Mendis, K., Sina, B. J., Marchesini, P., & Carter, R. (2001). The neglected burden of *Plasmodium vivax* malaria. *American Journal of Tropical Medicine and Hygiene, 64,* 97-106.

Meshnick, S. R., Taylor, T. E. & Koachonwongspaisan, S. (1996). Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. *Microbiological Reviews, 60,* 301 – 315.

Miller, L. H., Baruch, D. I., Marsh, K. & Doumbo, O. K. (2002). The pathogenic basis of malaria. *Nature, 415,* 673-679.

Miller, L. H., Manson, S. J., Clyde, D. F., & McGiniss, M. H. (1976). The resistance factor of *P. vivax* in Blacks: the Duffy-blood group genotype FyFy. *New England Journal of Medicine, 295,* 302-304.

Miller, R. S., McDaniel, P. & Wongsrichanalai, C. (2001). Following the course of malaria treatment by detecting parasite lactate dehydrogenase enzyme. *British Journal of Haematology, 113,* 558–559.

177

Milne, L. M., Kyi, M. S., Chiodini, P. L. & Warhurst, D. C. (1994). Accuracy of routine laboratory diagnosis of malaria in the United Kingdom. *Journal of Clinical Pathology, 47,* 740–742.

Mishra, B., Samantaray, J. C., Kumar, A. & Mirdha, B. R. (1999). Study of false positivity of two rapid antigen detection tests for diagnosis of *Plasmodium falciparum* malaria. *Journal of Clinical Microbiology, 37,* 1233.

Mitchell, G. H., Thomas, A. W., Margos, G., Dluzewski, A. R., & Bannister, L. H. (2004). Apical membrane antigen 1, a major malaria vaccine candidate, mediates the close attachment of invasive merozoites to host red blood cells. *Infection and Immunity, 72,*154-158.

Modell, B., Darlison, M., Birgens, H., Cario, H., Faustino, P., Giordano, P. C., Gulbis, B., Hopmeier, P., Lena-Russo, D., Romao, L. & Theodorsson, E. (2007) Epidemiology of haemoglobin disorders in Europe: an overview. *Scandinavian Journal of Clinical & Laboratory Investigation, 67,* 39–70.

Mohan, K., Dubey, M. L., Ganguly, N. K. & Mahajan, R. C. (1995). *Plasmodium falciparum*: role of activated blood monocytes in erythrocyte membrane damage and red cell loss during malaria. *Experimental Parasitology, 80,* 54-63.

Mohapatra, M. K., Padhiary, K. N., Mishra, D. P., & Sethy, G. (2002). Atypical manifestations of *Plasmodium vivax* malaria. *Indian Journal of Malariology, 39,* 18-25.

Molineaux, L. (1988). The epidemiology of human malaria as an explanation of its distribution, including some implications for its control. In W. H. Wernsdorfer & McGregor, I (Eds.), *Malaria: Principles and Practice of Malariology.* Volume 2 (pp. 913-998). London: Churchill Livingstone.

Moody, A. H. & Chiodini, P. L. (2000). Methods for the detection of blood parasites. *Clinical Laboratory of Haematology, 22,* 189-201.

Moody, A. H. & Chiodini, P. L. (2002). Non-microscopic method for malaria diagnosis using OptiMAL IT, a second-generation dipstick for malaria pLDH antigen detection. *British Journal of Biomedical Science, 59,*228–231.

Moody, A., Hunt-Cooke, A., Gabbett, E. & Chiodini, P. (2000). Performance of the OptiMAL malaria antigen capture dipstick for malaria diagnosis and treatment monitoring at the Hospital for Tropical Diseases, London. *British Journal of Haematology, 109,* 891–894.

Moreno, S. N. & Docampo, R. (2003). Calcium regulation in protozoan parasites. *Current Opinion Microbiology, 6*(4), 359 – 364.

Mulenga, M., VangGeertruyden, J. P., Mwananyanda, L., Chalwe, V., Moerman, F., Chilengi, R., Van Overmeir, C., Dujardin, J. C. & D'Alessandro, U. (2006). Safety and efficacy of lumefantrine-artemether (Coartem®) for the treatment of uncomplicated *Plasmodium falciparum* malaria in Zambian adults. *Malaria Journal, 5,* 73.

Mutabingwa, T. K., Anthony, D., Heller, A., Hallett, A. J., Drakeley, C, Greenwood, B. M. & Whitty, C. J. M. (2005). Amodiaquine alone, amodiaquine – sulfadoxine – pyrimethamine, amodiaquine – artesunate, and artemether-lumefantrine for outpatient treatment of malaria in Tanzanian children: a four-arm randomised effectiveness trial. *Lancet, 365,* 1474-1480.

Mwangi, T. W., Mohammed, M., Dayo, H., Snow, R. W. & Marsh, K. (2005). Clinical algorithms for malaria diagnosis lack utility among people of different age groups. *Tropical Medicine & International Health, 10,* 530–536.

Nagamune, K., Moreno, S. N., Chini, E. N. & Sibley, L. D. (2008). Calcium regulation and signaling in apicomplexan parasites. *Subcellular Biochemistry, 47,* 70 – 81.

Naqvi, R., Ahmad, E., Akhtar, F., Naqvi, A., & Rizvi, A. (2003). Outcome in severe acute renal failure associated with malaria. *Nephrology, Dialysis, Transplantation, 18,* 1820-1823.

Nester, E., Anderson, D., Roberts, E. J., Pearsall, N. & Nester, M. (2004). Microbiology: A Human Perspective (4th ed.). New York, NY: McGraw Hill Companies Inc.

Newton, P. N., Van Vugt, M., Teja-Isavadharm, P., Siriyanonda, D., Rasameesoroj, M., Teerapong, P., Ruangveerayuth, R., Slight, T., Nosten, F., Suputtamongkol, Y., Looareesuwan, S. & White, N. J. (2002). Comparison of oral artesunate and dihydroartemisinin antimalarial bioavailabilities in acute falciparum malaria. *Antimicrobial Agents and Chemotherapy, 46*(4), 1125–1127.

Nguyen, M. H., Davis, T. M., Cox-Singh, J., Hewitt, S., Tran, Q. T., Tran, B. K., Nguyen, T. H., Vo, N. P., Doan, H. N., & Le, D. C. (2003). Treatment of uncomplicated falciparum malaria in southern Vietnam: can chloroquine or sulfadoxine-pyrimethamine be reintroduced with artesuante? *Clinical Infectious Diseases, 37*(11), 1461-1466.

181

Nocht, B. & Werner, H. (1910). Beobachtungen uber relative Chininresistenz bei Malaria aus Brasilien. *Deutsche Medizinische Wochenschrift, 36,* 1557-1560.

Noedl, H. (2005). Artemisinin Resistance: How Can We Find It? *Trends in Parasitology, 21,* 9.

Noedl, H., Se, Y., Schaecher, K., Smith, B. L., Socheat, D. & Fukuda, M. M. (2008). Evidence of artemisinin-resistant malaria in western Cambodia. *New England Journal of Medicine, 359,* 2619–2620.

Nosten, F., ter Kuile, F., Chongsuphajaisiddhi, T., Luxemburger, C., Webster, H. K., Edstein, M., Phaipun, L., Thew, K. L. & White, N. J. (1991). Mefloquine-resistant falciparum malaria on the Thai-Burmese border. *Lancet, 337,* 1140-1143.

Ochong, E. O., van den Broek, I. V. F., Keus, K. & Nzila, A. (2003). Short report: Association between chloroquine and amodiaquine resistance and allelic variation in the *plasmodium falciparum* multiple drug resistance 1 gene and the chloroquine resistance transporter gene in isolates from the upper Nile in southern Sudan. *American Journal of Tropical Medicine and Hygiene, 69*(2), 184–187

Ockenhouse, C. F., Barbosa, A., Blackall, D. P., Murphy, C. I., Kashala, O., Dutta, S., Lanar, D. E. & Daugherty J. R. (2001). Sialic acid-

dependent binding of baculovirus-expressed recombinant antigens from Plasmodium falciparum EBA-175 to Glycophorin A. *Molecular and Biochemical Parasitology, 113*(1), 9-21.

Oh, M. D., Shin, H., Shin, D., Kim, U., Lee, S., Kim, N., Choi, M. H., Chai, J. Y., & Choe, K. (2001). Clinical features of vivax malaria. *American Journal of Tropical Medicine and Hygiene, 65*, 143-146.

Okoyeh, J. N., Pillai, C. R. & Chitnis, C. E. (1999). *Plasmodium falciparum* field isolates commonly use erythrocyte invasion pathways that are independent of sialic acid residues of glycophorin A. *Infection and Immunity, 67*, 5784–5791.

Olliaro. P. L., Haynes, R. K., Meunier, B., & Yuthavong, Y. (2001). Possible modes of action of the artemisinin-type compounds. *Trends in Parasitology, 17*(3), 122-126.

Olotu, A., Fegan, G., Williams, T. N., Sasi, P., Ogada, E., Bauni, E., Wambua, J., Marsh, K., Borrmann, S. & Bejon, P. (2010) Defining Clinical Malaria: The Specificity and Incidence of Endpoints from Active and Passive Surveillance of Children in Rural Kenya. *PLoS ONE, 5*(12), e15569. doi:10.1371/journal.pone.0015569

Othnigue, N., Wyss, K., Tanner, M. & Genton, B. (2006). Urban malaria in the Sahel: prevalence and seasonality of presumptive malaria and

183

parasitaemia at primary care level in Chad. *Tropical Medicine & International Health, 11,* 204–210.

Palmer, C. J., Bonilla, J. A., Bruckner, D. A., Barnett, E. D., Miller, N. S., Haseeb, M. A., Masci, J. R. & Stauffer, W. M. (2003). Multicenter study to evaluate the OptiMAL test for rapid diagnosis of malaria in U.S. hospitals. *Journal of Clinical Microbiology, 41,* 5178–5182.

Pasvol, G., Weatherall, D. J., & Wilson, R. J. M. (1977). Effects of fetal hemoglobin on susceptibility of red cells to Plasmodium falciparum. *Nature, 270,* 171-173.

Pathirana, S. L., Alles, H. K., Bandara, S., Phone-Kyaw, M., Perera, M. K., Wickremasinghe, A. R., Mendis, K. N. & Handunnetti, S. M. (2005). ABO-blood-group types and protection against severe *Plasmodium falciparum* malaria. *Annals of Tropical Medicine and Parasitology, 99,* 119-124.

Pattanasin, S., Proux, S., Chompasuk, D., Luwiradaj, K., Jacquier, P., Looareesuwan, S., & Nosten F. (2003). Evaluation of a new Plasmodium lactate dehydrogenase assay (OptiMAL-IT) for the detection of malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 97,* 672–674.

Payne, D. (1988). Use and limitations of light microscopy for diagnosing malaria at the primary health care level. *Bull World Health Organ, 66,* 621–626.

Pearce, R. J., Drakeley, C., Chandramohan, D., Mosha, F., & Roper, C. (2003). Molecular Determination of Point Mutation Haplotypes in the Dihydrofolate Reductase and Dihydropteroate Synthase of *Plasmodium falciparum* in Three Districts of Northern Tanzania. *Antimicrobial Agents and Chemotherapy, 47*(4), 1347 – 1354.

Peel, S. A., Bright, P., Yount, B., Handy, J. & Baric, R. S. (1994). A strong association between mefoloquine and holofantrine resistance and amplification, overexpression, and mutation in the P-glycoprotein gene homolog (pfmer) of *Plasmodium falciparum in vitro. American Journal of Tropical Medicine and Hygiene, 51,* 648 – 658.

Phyo, A. P., Nkhoma, S., Stepniewska, K., Ashley, E. A., Nair, S., McGready, R., ler Moo, C., Al-Saai, S., Dondorp, A. M., Lwin, K. M., Singhasivanon, P., Day, N. P. J., White, N. J., Anderson, T. J. C. & Nosten, F. (2012). Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. *The Lancet, 379* (9830), 1960 – 1966.

Piola, P., Fogg, C., Bajunirwe, F., Biraro, S., Grandesso, F., Ruzagira, E., Babigumira, J., Kigozi, I., Kiguli, J., Kyomuhendo, J., Ferradini, L., Taylor, W., Checchi, F. & Guthmann, J. P. (2005). Supervised versus unsupervised intake of six-dose artemether-lumefantrine for treatment of acute, uncomplicated *Plasmodium falciparum* malaria in Mbarara, Uganda: a randomised trial. *Lancet, 365*, 1467-1473.

Platt, O. S., Brambilla, D. J., Rosse, W. F., Milner, P. F., Castro, O., Steinberg, M. H. & Klug, P. P. (1994). Mortality in sickle cell disease. Life expectancy and risk factors for early death. *New England Journal of Medicine, 330*(23), 1639–44.

Ponnudurai, T., Lensen, A. H., van Gemert, G. J., Bolmer, M. G. & Meuwissen, J. H. (1991). Feeding behaviour and sporozoite ejection by infected Anopheles stephensi. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 85*(2), 175-180.

Praise, M. E., Ayisi, J. G., Nahlen, B. L., Schultz, L. J., Roberts, J. M., Misore A, Muga, R, Oloo, A. J. & Steketee, R. W. (1998). Efficacy of sulfadoxinepyrimethamine for prevention of placental malaria in an area of Kenya with a high prevalence of malaria and human immunodeficiency virus infection. *American Journal of Tropical Medicine and Hygiene, 59*(5), 813-822.

Prakash, J., Singh, A. K., Kumar, N. S., & Saxena, R. K. (2003). Acute renal failure in *Plasmodium vivax* malaria. *Journal of the Association of Physicians of India,* 2003, 51:265–267.

Price, R. N., Cassar, C., Brockman, A., Duraisingh, M., Van vugt, M., White, N. J., Nosten, F. & Krishna, S. (1999). The pfmdr1 gene is associated with a multidrug resistant phenotype in *Plasmodium falciparum* from the western border of Thailand. *Antimicrobial Agents and Chemotherapy, 43,* 2943 – 2949.

Price, R. N., Uhlemann, A., Brockman, A., McGready, R., Ashley, E., Phaipun, L., Patel, R., Laing, K., Looareesuwan, S., White, N. J., Nosten, F. & Krishna. S. (2004). Mefloquine resistance in *Plasmodium falciparum* and increased *pfmdr1* gene copy number. *Lancet, 364,* 438–447.

Prommano, O., Chaisri, U., Turner, G. D., Wilairatana, P., Ferguson, D. I., Viriyavejakul, P., White, N. J., & Pongponratn, E., (2005). A quantitative ultrastructural study of the liver and the spleen in fatal falciparum malaria. *South-east Asian Journal of Tropical Medicine and Public Health, 36,* 1359-1370.

Pukrittayakamee, S., Chotivanich, K., Chantra, A., Clemens, R., Looareesuwan, S., & White, N. J. (2004). Activities of artesunate and primaquine against asexual- and sexual-stage parasites in

187

falciparum malaria. *Antimicrobial Agents and Chemotherapy*, *48*(4), 1329-1334.

Qilin, H., Weichuan, O., Jiexian, Z., Zhu, W., Kunyan, Z., Jiankang, H., Xianzheng, C., Xuejian, P., Shigang, F., Xiangfeng, W., & Jian, L. (1988). Effectiveness of amodiaquine, sulfadoxine-pyrimethamine, and combinations of these drugs for treating chloroquine-resistant falciparum malaria in Hainan Island, China. *Bulletin of the World Health Organization*, *66*(3), 353-358.

Reed, M. B., Saliba, K. J., Caruana, S. R., Kirk, K. & Cowman, A. F. (2000). Pgh1 modulates sensitivity and resistance to multiple antimalarials in *Plasmodium falciparum*. *Nature*, *403*, 906–909.

Reyburn, H., Ruanda, J., Mwerinde, O. & Drakeley, C. (2006). The contribution of microscopy to targeting antimalarial treatment in a low transmission area of Tanzania. *Malaria Journal, 5*, 4.

Richardson, D. C., Ciach, M., Zhong, K. J., Crandall, I. & Kain, K. C. (2002). Evaluation of the Makromed dipstick assay versus PCR for diagnosis of *Plasmodium falciparum* malaria in returned travelers. *Journal of Clinical Microbiology, 40*, 4528–4530.

Rogers, W. O., Sem, R., Tero, T., Chim, P., Lim, P., Muth, S., Socheat, D., Ariey, F. & Wongsrichanalai, C. (2009). Failure of artesunate-

mefloquine combination therapy for uncomplicated *Plasmodium falciparum* malaria in southern Cambodia. *Malaria Journal, 8,* 10.

Rohrbach, P., Sanchez, P. C., Hayton, K., Friedrich, O., Patel, J., Sidhu A. B., Ferdig, M. T., Fidock, D. A. & Lanzer, M. (2006). Genetic linkage of pfmdr1 with food vacuolar solute import in *Plasmodium falciparum. EMBO Journal, 25,* 3000 – 3011.

Rosenburg, R. & Wirtz, R. A. (1990). An estimation of the number of sporozoites ejected by a feeding mosquito. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 84,* 209-212.

Rowe, A., Obeiro, J., Newbold, C. I. & Marsh, K. (1995). *Plasmodium falciparum* rosetting is associated with malaria severity in Kenya. *Infection and Immunity, 63,* 2323-2326.

Rowe, J. A., Handel, I. G., Thera, M. A., Deans, A. M., Lyke, K. E., Kone, A., Diallo, D. A., Raza, A., Kai, O., Marsh, K., Plowe, C. V., Doumbo, O. K. & Moulds, J. M. (2007). Blood group 'O' protects against severe *Plasmodium falciparum* malaria through the mechanism of reduced rosetting. *Proceedings of the National Academy of Science, 104,* 17471-17476.

Rowe, J. A., Moulds, J. M., Newbold, C. I. & Miller, L. H. (1997). *P. falciparum* rosetting mediated by a parasite-variant erythrocyte

189

membrane protein and complement-receptor 1. *Nature, 388*, 292-295.

Russell, P. F. (1955). Man's Mastery of Malaria. London: Oxford University Press.

Ruwende, C., Khoo, S. C., Snow, R. W., Yates, S. N. R., Kwiatkowski, D., Gupta, S., Warn, P., Allsopp, C. E. M., Gilbert, S. C., Peschu, N., Newbold, C. I., Greenwood, B. M., Marsh, K. & Hill, A. V. S. (1995). Natural selection of hemi- and heterozygotes for G6P D deficiency in Africa by resistance to severe malaria. *Nature, 376*, 246-249.

Sachs, J., & Malany, P. (2002). The economic and social burden of malaria. *Nature, 415*, 680 – 615.

Sahr, F., Willoughby, V. R., Gbakima, A. A. & Bockarie, M. J. (2001). Apparent drug failure following artesunate treatment of *Plasmodium falciparum* malaria in Freetown, Sierra Leone: four case reports. *Annals of Tropical Medicine Parasitology, 95*, 445–449.

SAMC. (2000). Malaria methods: towards better informed malaria control in Southern Africa. Southern African Malaria Control (SAMC)/WHO, Zimbabwe.

Sanchez, C. P., Rotmann, A., Stein, W. D., & Lanzer, M. (2008). Polymorphisms within PfMDR1 alter the substrate specificity for antimalarial drugs in Plasmodium falciparum. *Molecular Microbiology, 70,* 786-798.

Schapira, A. & Schwalbach, J. F. L. (1988). Evaluation of four therapeutic regimens for falciparum malaria in Mozambique. *Bulletin of the World Health Organization, 66,* 219-226.

Schellenberg, D., Menendez, C., Aponte, J. J., Kahigwa, E., Tanner, M., Mshinda, H. & Alonso, P. (2005). Intermittent preventive antimalarial treatmentfor Tanzanian infants: follow-up to age 2 years of a randomised, placebo-controlled trial. *Lancet, 365,* 1481–3.

Schellenberg, D., Menendez, C., Kahigwa, E., Aponte, J., Vidal, J., Tanner, M., Mshinda, H. & Alonso, P. (2001). Intermittent treatment for malaria and anaemia control at time of routine vaccinations in Tanzanian infants: a randomised, placebo-controlled trial. *Lancet, 357,* 1471–7.

Schultz, L. J., Steketee, R. W., Macheso, A., Kazembe, P., Chitsulo, L. & Wirima, J. J. (1994). The efficacy of antimalarial regimens containing sulfadoxine-pyrimethamine and/or chloroquine in preventing peripheral and placental Plasmodium falciparum

191

infection among pregnant women in Malawi. *American Journal of Tropical Medicine and Hygiene, 51*(5), 515-522.

Schwartz, E., Sadetzki, S., Murad, H. & Raveh, D. (2001). Age as a risk factor for severe *Plasmodium falciparum* malaria in nonimmune patients. *Clinical Infectious Diseases, 33*, 1774-1777.

Schwenke, A., Brandts, C., Philips, J., Winkler, S., Wernsdorfer, W. H., & Kremsner, P. G. (2001). Declining chloroquine resistance of Plasmodium falciparum in Lambarene, Gabon from 1992 to 1998. *Wien Win Wochenschr, 113*, 63-64.

Sharman, J. & Day, J. F. (2007). Reproductive phase locking of mosquito population in response to rainfall frequency. *PLOS ONE, 2*(3), e331.

Sharp, B. L., Le Sueur, D. & Becker, P. (1990). Effect of DDT on survival and blood feeding success of Anopheles arabiensis in northern KwaZulu-Natal, South Africa. *Journal of the American Mosquito Control Association, 6*, 197–202.

Shiff, C. J., Minjas, J. & Premji, Z. (1994). The ParaSight-F test: a simple rapid manual dipstick test to detect *Plasmodium falciparum* infection. *Parasitology Today, 10*, 494–495.

Shulman, C. E., Dorman, E. K., Cutts, F., Kawuondo, K., Bulmer, J. N., Peshu, N. & Marsh, K. (1999). Intermittent sulphadoxine-pyrimethamine to prevent severe anaemia secondary to malaria in pregnancy: a randomised placebo-controlled trial. *Lancet, 353,* 632-636.

Sidhu, A. B., Verdier-Pinard, D. & Fidock, D. A. (2002). Chloroquine resistance in *Plasmodium falciparum* malaria parasites conferred by pfcrt mutations. *Science, 298,* 210 – 213.

Sim, B. K., Chitnis, C. E., Wasniowska, K., Hadley, T. J., & Miller, L. H. (1994). Receptor and ligand domains for invasion of erythrocytes by *Plasmodium falciparum. Science 264,* 1941–1944.

Simon, F., Le Bras, J., Gaudebout, C. & Girard, P. M. (1988). Reduced sensitivity of *Plasmodium falciparum* to mefloquine in West Africa. *Lancet, 331*(8583), 467-468.

Sisowath, C., Ferreira, P. E., Bustamante, L. Y., Dahlström, S., Mårtensson, A., Björkman, A., Krishna, S. & Gil, J. P. (2007). The role of *pfmdr1* in *Plasmodium falciparum* tolerance to artemether-lumefantrine in Africa. *Tropical Medicine and International Health, 12*(6), 736–742.

Sisowath, C., Petersen, I., Veiga, M. I., Martensson, A., Premji, Z., Bjorkman, A., Fidock, D. A. & Gil, J. P. (2009). *In vivo* selection of

Plasmodium falciparum parasites carrying the chloroquine-susceptibility pfcrt K76 allele after treatment with artemether-lumefantrine in Africa. *Journal of Infectious Diseases, 199,* 750 - 757.

Sisowath, C., Strömberg, J., Mårtensson, A., Msellem, M., Obondo, C., Björkman, A & Gil, J. P. (2005). In vivo selection of *Plasmodium falciparum pfmdr1* 86N coding alleles by artemether-lumefantrine (Coartem). *Journal of Infectious Diseases, 191,* 1014–1017.

Slutsker, L., Taylor, T. E., Wirima, J. J. & Steketee, R. W. (1994). In-hospital morbidity and mortality due to malaria-associated severe anaemia in two areas of Malawi with different patterns of malaria infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 88,* 548–551.

Smith, A. T. (2007). Measures of clinical malaria in field trials of interventions against *Plasmodium falciparum. Malaria Journal, 6,* 53. doi:10.1186/1475-2875-6-53

Smith, T., Charlwood, J. D., Kihonda, J., Mwankusye, S., Billingsley, P., Meuwissen, J., Lymo, J., Takken, W., Teusch, T. & Tanner, M. (1993). Absence of seasonal variation in malaria parasitaemia in

194

an area of intense seasonal transmission. *Acta Tropica, 54,* 55–72.

Snounou, G., Viriyakosol, S., Jarra, W., Thaithong, S. & Brown, K. N. (1993). Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. *Molecular and Biochemical Parasitology, 58,* 283–292.

Snow, R. W., Omumbo, J. A., Lowe, B., Molyneux, C. S., Obiero, J. O., Palmer, A., Weber, M. W., Pinder, M., Nahlen, B., Obonyo, C., Newbold, C., Gupta, S. & Marsh, K. (1997). Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. *Lancet, 349,* 1650-1654.

Spencer, H. C., Collins, W. E., Chin, W. & Skinner, J. C. (1979). The enzyme-linked immunosorbent assay (ELISA) for malaria. I. The use of *in vitro*-cultured *Plasmodium falciparum* as antigen. *American Journal of Tropical Medicine and Hygiene, 28,* 927–932.

Spencer, H. C., Kaseje, D. C., Collins, W. E., Shehata, M. G., Turner, A., Stanfill, P. S., Huong, A. Y., Roberts, J. M., Villinski, M. & Koech, D. K. (1987a). Community-based malaria control in Saradidi, Kenya: description of the programme and impact on

parasitaemia rates and antimalarial antibodies. *Annals of Tropical Medicine and Parasitology*, *81*(1), 13-23.

Spencer, H. C., Kaseje, D. C., Mosley, W. H., Sempebwa, E. K., Huong, A. Y. & Roberts, J. M. (1987b). Impact on mortality and fertility of a community-based malaria control programme in Saradidi, Kenya. *Annals of Tropical Medicine and Parasitology*, *81*(1), 36-45.

Stephens, J. K., Phanart, K., Rooney, W. & Barnish, G. (1999). A comparison of three malaria diagnostic tests, under field conditions in North-west Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health, 30,* 625–630.

Stephens, M. A. (1974). "EDF Statistics for Goodness of Fit and Some Comparisons". *Journal of the American Statistical Association, 69,* 730–737

Su, X. Z., Heatwole, V. M., Wertheimer, S. P., Guinet, F., Herrfeldt, J. A., Peterson, D. S., Ravetch, J. A. & Wellems, T. E. (1995). The large diverse gene family *var* encodes proteins involved in cytoadherence and antigenic variation of *plasmodium falciparum*-infected erythrocytes. *Cell, 82*(1), 89 – 100.

Su, X., Kirkman, L. A., Fujioka, H. & Wellems, T. W. (1997). Complex polymorphisms in an approximately 330 kDa protein are linked

to chloroquine resistant *Plasmodium falciparum* in Southeast Asia and Africa. *Cell, 91*(5), 593 – 603.

Sulzer, A. J., Wilson, M. & Hall, E. C. (1969). Indirect fluorescentantibody tests for parasitic diseases. V. An evaluation of a thick-smear antigen in the IFA test for malaria antibodies. *American Journal of Tropical Medicine and Hygiene, 18,* 199–205.

Sumba, P. O., Wong, S. L., Kanzaria, H. K., Johnson, K. A., & John, C. C. (2008). Malaria treatment seeking behavior and recovery from malaria in a highland area of Kenya. *Malaria Journal, 7*(245). doi10.1186/1475-28757-254.

Svenson, J. E., MacLean, J. D., Gyorkos, T. W. & Keystone, J. (1995). Imported malaria. Clinical presentation and examination of symptomatic travelers. *Archives of International Medicine, 155,* 861–868. doi: 10.1001/archinte.155.8.861.

Tahar, R., Ringwald, P., & Basco, L. K. (2009). Molecular epidemiology of malaria in Cameroon. XXVIII. In vitro activity of dihydroartemisinin against clinical isolates of *Plasmodium falciparum* and sequence analysis of the *P. falciparum* ATPase 6 gene. *American Journal of Tropical Medicine and Hygiene, 81,* 13–18.

Tanios, M. A., Kogelman, L., McGovern, B., & Hassoun, P. M. (2001). Acute respiratory distress syndrome complicating *Plasmodium vivax* malaria. *Critical Care Medicine, 29,* 665-667.

Targett, G., Drakeley, C., Jawara, M., von Seidlein, L., Coleman, R., Deen, J., Pinder, M., Doherty. T., Sutherland, C., Walraven, G., & Milligan, P. (2001). Artesunate reduces but does not prevent posttreatment transmission of *Plasmodium falciparum* to Anopheles gambiae. *Journal of Infectious Diseases, 183*(8), 1254-1259.

Tekeste, Z. & Petros, B. (2010). The ABO blood group and *Plasmodium falciparum* malaria in Awash, Metehara and Ziway areas, Ethiopia. *Malaria Journal, 9,* 280.

ter Kuile, F., White, N. J., Hollaway, P. H., Pasvol, G., & Krishner, S. (1993) *Plasmodium falciparum* in vitro studies of the pharmacodynamic properties of drugs used for the treatment of severe malaria. *Experimental Parasitology, 76,* 85-95.

Thomson, S., Lohmann, R. C., Crawford, L., Dubash, R. & Richardson, H. (2000). External quality assessment in the examination of blood films for malarial parasite within Ontario, Canada. *Archives of Pathology and Laboratory Medicine, 124,* 57–60.

198

Trampuz, A., Jareb, M., Muzlovic, I. & Probhu, R. M. (2003). Clinical Review: Severe malaria. *Critical care, 7*, 315 – 323. doi: 10.1188/cc2183

Trape, J. F. (2001). The public health impact of chloroquine resistance in Africa. *American Journal of Tropical Medicine and Hygiene, 64*(1-2), 12-17.

Udomsangpetch, R., Todd, J., Carlson, J. & Greenwood, B. M. (1993). The effects of hemoglobin genotype and ABO blood group on the formation of rosettes by *Plasmodium falciparum*–infected red blood cells. *American Journal of Tropical Medicine and Hygiene, 48*, 149–153.

Udomsangpetch, R., Wahlin, B., Carlson, J., Berzins, K., Torii, M., Aikawa, M., Perlman, P. & Wahlgren, M. (1989). *Plasmodium falciparum*-infected erythrocytes form spontaneous erythrocyte rosettes. *Journal of Experimental Medicine, 169*, 1835-1840.

Uhlemann, A. C., Yuthavong, Y. & Fidock, D. A. (2005). Mechanisms of antimalarial drug action and resistance. In: Sherman, I. W (Eds), Molecular Approaches to Malaria (p. 429). ASM Press.

Valderramos, S. G., Scaanfeld, D., Uhlemann, A., Fidock, D. A. & Krishna, S. (2010). Investigations into the role of the *Plasmodium falciparum* SERCA (PfATP6) L263E mutation in artemisinin

action and resistance. *Antimicrobial Agents and Chemotherapy,* *54*(8), 3842–3852.

Valderramos, S. G. & Fidock, D. A. (2006). Transporters involved in resistance to antimalarial drugs. *Trends in Pharmacological Science, 27,* 594-601.

van der Hoek, W., Premasiri, D. A. R. & Wickremasinghe, A. R. (1997). Early diagnosis and treatment of malaria in a refugee population in Sri Lanka. *Southeast Asian Journal of Tropical Medicine and Public Health, 28,* 12–17.

Veiga, M. I., Ferreira, P. E., Bjorkman, A., & Gil, J. P. (2006). Multiplex PCR–RFLP methods for pfcrt, pfmdr1 and pfdhfr mutations in Plasmodium falciparum. *Molecular and Cellular Probes 20,* 100–104

Verdrager, J. (1986). Epidemiology of emergence and spread of drug-resistant falciparum malaria in South-east Asia. *Southeast Asian Journal of Tropical Medicine and Public Health, 17,*111-118.

Verhoeff, F. H., Brabin, B. J., Chimsuku, L., Kazembe, P., Russel, W. B. & Broadhead, R. L. (1998). An evaluation of intermittent sulfadoxine-pyrimethamine treatment in pregnancy on parasite clearance and risk of low birth weight in rural Malawi. *Annals of Tropical Medicine and Parasitology, 92,* 141-150.

200

Waitumbi, J. N., Donvito, B., Kisserli, A., Cohen, J. H. & Stoute, J. A. (2004). Agerelated changes in red blood cell complement regulatory proteins and susceptibility to severe malaria. *Journal of Infectious Diseases, 190,* 1183-1191.

Waitumbi, J. N., Opollo, M. O., Muga, R. O., Misore, A. O. & Stoute, J. A.(2000). Red cell surface changes and erythrophagocytosis in children with severe *Plasmodium falciparum* anemia. *Blood, 95,* 1481-1486.

Wang, P., Lee, C. S., Bayoumi, R., Djimde, A., Doumbo, O., Swedberg, G., Das, L. D., Mshinda, H., Tanner, M., Watkins, W. M., Sims, P. F. G., & Hyde, J. E. (1997). Resistance to antifolate in *Plasmodium falciparum* monitored by sequence analysis of dihydropteroate synthetase and dihydrofolate reductase alleles in a large number of field samples of diverse origin. *Molecular and Biochemical Parasitology, 89,* 161–177.

Wang, Z., Parker, D., Meng, H., Wu, L., Li, J., Zhao, Z., Zhang, R., Fan, Q., Wang, H., Cui, L. & Yang Z. (2012). *In Vitro* Sensitivity of *Plasmodium falciparum* from China-Myanmar Border Area to Major ACT Drugs and Polymorphisms in Potential Target Genes. *PLoS ONE,* *7*(5), e30927. doi:10.1371/journal.pone.0030927

Warrell, D. A. & Gilles, H. M. (2002). Essential malariology (4th ed). London: Arnold.

Weatherall, D. J. (1997). Thalassaemia and malaria, revisited. *Annals of Tropical Medicine and Parasitology, 91*, 885-890

White, N. J. & Ho, M. (1992). The pathophysiology of malaria. *Advances in Parasitology, 31*, 34–173.

White, N. J. (1992). Antimalarial drug resistance: the pace quickens. *Journal of Antimicrobial Chemotherapy, 30*, 571-585.

White, N. J. (1997a). Assessment of the pharmocodynamic properties of antimalarial drugs *in vivo. Antimicrobial Agents Chemotherapy, 4*(7), 1413 – 1422.

White, N. J. (1997b). The treatment of malaria. In Carosi, G., Castelli, F (Eds), The handbook of malaria infection in the tropics. AIFO. Italy.

White, N. J. (1999). Antimalarial drug resistance and combination chemotherapy. *Philosophical Transactions of the Royal Society B., 354*, 739-749.

White, N. J. (2008). *Plasmodium knowlesi*: the fifth human malaria parasite. *Clinical Infectious Diseases, 46*, 172 – 173.

White, N. J. (2008). Qinghaosu (artemisinin): the price of success. *Science, 320*, 330-334.

202

White, N. J. (2009). Malaria. In G. C. Cook and A. I. Zumla (Eds), Manson's tropical disease (pp 1201-1300). Saunder Elsevier Ltd.

Whitty, C. J. M., Chandler, C., Ansah, E., Leslie, T. & Staedke, S. G. (2008). Deployment of ACT antimalarials for treatment of malaria: challenges and opportunities. *Malaria Journal, 7*, S7. doi:10.1186/1475-2875-7-S1-S7

WHO. (1991). *Basic Malaria Microscopy.* Geneva: World Health Organization.

WHO. (2000a). Severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 94*(1), S1-S90.

WHO. (2000b). Malaria Diagnosis: New Perspectives. Report of a Joint WHO/USAID Informal Consultation. Geneva: World Health Organization.

WHO. (2001). Antimalarial Drug Combination Therapy. Report of a WHO Technical Consultation, WHO/CDS/RBM/2001.35, 2001.

WHO. (2003). Malaria rapid diagnosis: making it work. Informal consultation on field trials and quality assurance on malaria rapid diagnostic test. Geneva, World Health Organization.

WHO. (2005). Susceptibility of *Plasmodium falciparum* to antimalarial drugs. Report on global monitoring 1996-2004. Geneva.

WHO. (2005). The Roll Back Malaria strategy for improving access to treatment trough home management of malaria. World Health Organization, Geneva. *WHO/HTM/MAL/20051101*

WHO. (2006a). Indoor residual spraying: use of indoor residual spraying for scaling up global malaria control and elimination. Geneva, World Health Organization/Global Malaria Programme.

WHO. (2006b). Guidelines for malaria treatment. Geneva. World Health Organization.

WHO. (2008). World Malaria Report 2008. Geneva. World Health Organization.

WHO. (2010b). Global Report on Antimalarial Drug Efficacy and Drug Resistance: 2000-2010. Geneva. World Health Organization.

WHO. (2010a). World Malaria Report 2010. Geneva. World Health Organization.

WHO. (2011). World Malaria Report 2011. Geneva. World Health Organization.

WHO/Global Malaria Programme. Insecticide-treated mosquito nets: A World Health   Organization   Position   Statement http://www.un.org/millenniumgoals

Wilson, C. M., Volkmana, S. K., Thaithongb, S., Martinc, R. K., Kylec, D. E., Milhousc, W. K. & Wirth, D. F. (1993). Amplification of *pfmdr*1 associated with mefloquine and halofantrine resistance in *Plasmodium falciparum* from Thailand. *Molecular and Biochemical Parasitology, 57,* 151–60.

Wiser, M. F. (1999). Cellular and molecular biology of plasmodium. Retrieved August 22, 2011, from http://www.tulane.edu/~wiser/malaria/cmb.html

Wongsrichanalai, C., M. J. Barcus, S. Muth, Sutamihardja, A., & Wernsdorfer, W. H. (2007). A Review of Malaria Diagnostic Tools: Microscopy and Rapid Diagnostic Test (RDT). *American Society of Tropical Medicine and Hygiene, 77(6),* 119-127.

Woodrow, C. J., & Krishna, S., (2006). Antimalarial drugs: recent advances in molecular determinants of resistance and their clinical significance. *Cell. Mol. Life Sci., 63,* 1586–1596.

Wootton, J. C., Feng, X., Ferdig, M. T., Cooper, R. A., Mu, J., Baruch, D. I., Magill, A. J. & Su, X. (2002). Genetic diversity and chloroquine selective sweeps in *Plasmodium falciparum. Nature, 418,* 320–323.

Xiang, L., Rundles, J. R., Hamilton, D. R., & Wilson, J. G. (1999). Quantitative alleles of CR1: coding sequence analysis and comparison of

haplotypes in two ethnic groups. *Journal of Immunology, 163,* 4939-4945.

Xu, J. W., Xu, Q., Liu, H., & Zeng, Y. (2012). Malaria treatment-seeking behavior and related factors of Wa ethnic minority in Myanmar: a cross-sectional study. *Malaria Journal, 11*(46). Dio: 10.1186/1475-2875-11-417.

Yadav, S. P. (2010). A study of treatment seeking behavior for malaria and its management in febrile children in rural part of desert, Rajasthan. *India Journal of Vector Borne Diseases, 47,* 235-242.

Zhang, G., Guan, Y., Zheng, B., Wu, S. & Tang, L. (2008). No PfATPase6 S769N mutation found in *Plasmodium falciparum* isolates from China. *Malaria Journal, 8,* 122.

206
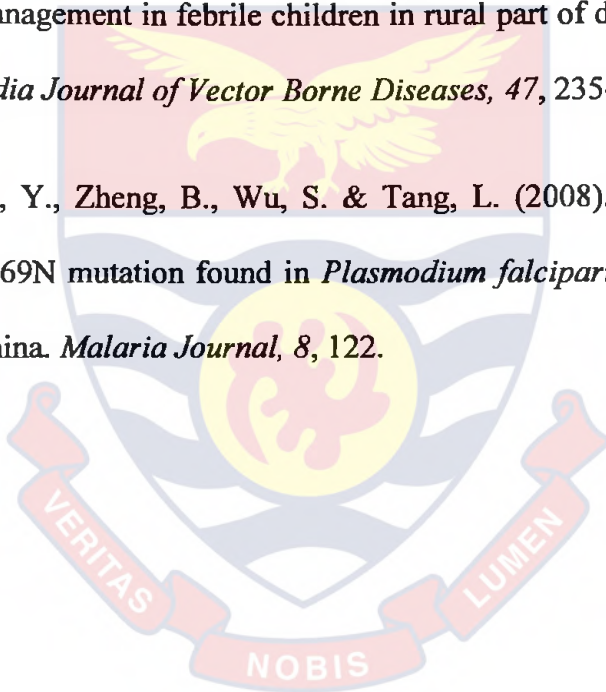
APPENDICES

APEENDIX I

ETHICAL APPROVAL OF STUDY

GHANA HEALTH SERVICE ETHICAL REVIEW COMMITTEE

In case of reply the
number and date of this
Letter should be quoted.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra

My Ref. GHS-ERC/ 3
Your Ref. No.

26th November 2009

Tel: - 233-21-681109
Fax - 233-21-226739
Email: Hannah.Frimpong a hru-ghs.org

<u>MR. AFOAKWAH RICHMOND,   PRINCIPAL INVESTIGATOR</u>

<u>ETHICAL CLEARANCE</u>

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol titled:

"MOLECULAR MONITORING OF PLASMDIUM FALCIPARUM RESISTANCE TO ARTEMISININS IN GHANA" - ID NO: GHS-ERC-16/7/09

This approval requires that you submit periodic review of the protocol to the Committee and a final full review to the Ethical Review Committee (ERC) on completion of the study. The ERC may observe or cause to be observed procedures and records of the study during and after implementation.
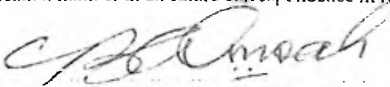
Please note that any modification of the project must be submitted to the ERC for review and approval before its implementation.

You are also required to report all serious adverse events related to this study to the ERC within seven days verbally and fourteen days in writing.

You are requested to submit a final report on the study to assure the ERC that the project was implemented as per approved protocol. You are also to inform the ERC and your mother organization before any publication of the research findings.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

SIGNED

PROFESSOR ALBERT GEORGE BAIDOE AMOAH
(GHS-ERC CHAIRMAN)

Cc   The Director, Research & Development Division, Ghana Health Service, Accra

APPENDIX II
RAW DATA OF STUDY

Sample ID	REGION	SEASON	Gender	Ages	Hb	Anaemia	Severe Anemia	Group	Sickling	Microscopy	Par Den	RDT	PCR
16227	Ashanti	Rainy	Female	37	12.6	No	No	B +	Neg	Neg	0	Neg	Neg
16225	Ashanti	Rainy	Female	19	11.1	Yes	No	A +	Neg	Neg	0	Neg	Neg
16224	Ashanti	Rainy	Female	62	11.4	Yes	No	O +	Neg	Neg	0	Neg	Neg
16222	Ashanti	Rainy	Female	27	10	Yes	No	O +	Neg	Neg	0	Neg	Neg
16223	Ashanti	Rainy	Female	28	12.7	No	No	A +	Neg	Neg	0	Neg	Neg
16229	Ashanti	Rainy	Female	38	10.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
16220	Ashanti	Rainy	Female	18	11.2	Yes	No	A +	Pos	Neg	0	Pos	Pos
16217	Ashanti	Rainy	Female	35	10.1	Yes	No	O +	Pos	Neg	0	Neg	Neg
16208	Ashanti	Rainy	Female	20	12	No	No	A +	Neg	Neg	0	Neg	Neg
16211	Ashanti	Rainy	Female	33	8	Yes	No	O +	Neg	Neg	0	Pos	Pos
16214	Ashanti	Rainy	Female	5	11.2	Yes	No	A +	Neg	(+)	970	Pos	Pos
16207	Ashanti	Rainy	Female	30	12	No	No	O +	Neg	(+)	480	Neg	Neg
16249	Ashanti	Rainy	Female	42	12.2	No	No	B -	Neg	Neg	0	Neg	Neg
16244	Ashanti	Rainy	Female	30	11	Yes	No	O +	Neg	Neg	0	Neg	Neg
16239	Ashanti	Rainy	Female	20	7.4	Yes	Yes	O +	Neg	(+)	900	Pos	Pos
16288	Ashanti	Rainy	Male	26	11.1	Yes	No	O +	Neg	Neg	0	Neg	Neg
16246	Ashanti	Rainy	Female	50	10.5	Yes	No	B +	Neg	Neg	0	Neg	Pos
16245	Ashanti	Rainy	Female	34	10.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
16247	Ashanti	Rainy	Female	14	11	Yes	No	A +	Neg	Neg	0	Neg	Neg
16131	Ashanti	Rainy	Female	46	10.6	Yes	No	O +	Neg	(+)	290	Pos	Pos
16231	Ashanti	Rainy	Female	39	8.8	Yes	No	O +	Neg	Neg	0	Neg	Neg
16230	Ashanti	Rainy	Female	30	9.1	Yes	No	O +	Neg	(++)	2130	Pos	Pos
16229	Ashanti	Rainy	Female	28	10.2	Yes	No	A +	Neg	Neg	0	Neg	Neg
16238	Ashanti	Rainy	Female	76	8.9	Yes	No	A +	Neg	Neg	0	Neg	Neg
16251	Ashanti	Rainy	Female	40	9.8	Yes	No	O +	Neg	Neg	0	Neg	Neg

208

ID	Region	Season	Sex	Age	Hb			Blood Group					
16252	Ashanti	Rainy	Female	29	10.6	Yes	No	O+	Neg	Neg	0	Neg	Neg
16254	Ashanti	Rainy	Female	24	10.1	Yes	No	A+	Neg	(+)	780	Pos	Neg
16255	Ashanti	Rainy	Female	35	8.8	Yes	No	O+	Neg	Neg	0	Neg	Neg
16256	Ashanti	Rainy	Female	23	11.4	Yes	No	O+	Neg	Neg	0	Neg	Neg
16257	Ashanti	Rainy	Female	1	10.8	Yes	No	A+	Neg	Neg	0	Neg	Neg
16259	Ashanti	Rainy	Female	28	10.9	Yes	No	AB+	Neg	Neg	0	Neg	Neg
16260	Ashanti	Rainy	Female	49	10.5	Yes	No	O+	Neg	Neg	0	Neg	Neg
16262	Ashanti	Rainy	Female	6	13	No	No	O-	Neg	Neg	0	Neg	Neg
16264	Ashanti	Rainy	Female	68	8.4	Yes	No	O-	Neg	Neg	0	Neg	Neg
16269	Ashanti	Rainy	Female	2	10.7	Yes	No	O+	Neg	Neg	0	Neg	Neg
16272	Ashanti	Rainy	Female	5	11.1	Yes	No	B+	Neg	Neg	0	Neg	Neg
16277	Ashanti	Rainy	Female	42	12	No	No	A-	Neg	(+)	390	Pos	Pos
16282	Ashanti	Rainy	Female	19	14.1	No	No	O+	Neg	Neg	0	Neg	Pos
16280	Ashanti	Rainy	Female	80	9.1	Yes	No	A+	Neg	Neg	0	Neg	Neg
16276	Ashanti	Rainy	Female	63	11.4	Yes	No	A+	Neg	Neg	0	Neg	Neg
16267	Ashanti	Rainy	Female	8	8.6	Yes	No	A-	Neg	(+)	740	Pos	Pos
16268	Ashanti	Rainy	Female	52	9.9	Yes	No	O+	Neg	Neg	0	Neg	Neg
16275	Ashanti	Rainy	Male	7	10.9	Yes	No	O+	Neg	Neg	0	Neg	Neg
16274	Ashanti	Rainy	Female	4	12.2	No	No	O+	Pos	(+)	530	Pos	Pos
16273	Ashanti	Rainy	Female	39	11.8	Yes	No	O-	Neg	Neg	0	Neg	Neg
16287	Ashanti	Rainy	Female	16	10	Yes	No	O+	Neg	Neg	0	Neg	Neg
16292	Ashanti	Rainy	Female	22	8.5	Yes	No	A+	Neg	Neg	0	Neg	Neg
16293	Ashanti	Rainy	Female	24	9.2	Yes	No	A+	Neg	Neg	0	Neg	Neg
16397	Ashanti	Rainy	Female	33	9.3	Yes	No	O+	Pos	Neg	0	Neg	Neg
16295	Ashanti	Rainy	Female	33	11.9	Yes	No	O+	Neg	Neg	0	Neg	Neg
16294	Ashanti	Rainy	Female	54	9.9	Yes	No	A+	Neg	(+)	680	Pos	Pos
16248	Ashanti	Rainy	Female	28	10.4	Yes	No	O+	Neg	Neg	0	Neg	Neg
16242	Ashanti	Rainy	Female	5	6.2	Yes	Yes	AB+	Pos	Neg	0	Pos	Pos
16237	Ashanti	Rainy	Male	30	11.8	Yes	No	B+	Neg	Neg	0	Neg	Neg
16241	Ashanti	Rainy	Female	31	11.1	Yes	No	O+	Pos	Neg	0	Neg	Neg

209

16300	Ashanti	Rainy	Male	57	14.3	No	No	A +	Neg	(+)	920	Pos	Pos
16288	Ashanti	Rainy	Male	37	11.1	Yes	No	O +	Neg	Neg	0	Neg	Neg
16286	Ashanti	Rainy	Female	34	11.3	Yes	No	O +	Neg	Neg	0	Pos	Pos
16355	Ashanti	Rainy	Female	38	11.1	Yes	No	A +	Neg	(+)	380	Pos	Pos
16301	Ashanti	Rainy	Female	25	11.2	Yes	No	B -	Pos	Neg	0	Neg	Neg
16302	Ashanti	Rainy	Female	31	11.4	Yes	No	O +	Neg	Neg	0	Neg	Neg
16303	Ashanti	Rainy	Male	36	9.9	Yes	No	B +	Neg	Neg	0	Neg	Neg
16305	Ashanti	Rainy	Male	27	9.6	Yes	No	B +	Neg	Neg	0	Neg	Pos
16307	Ashanti	Rainy	Female	18	11.8	Yes	No	A +	Pos	(+)	190	Pos	Pos
16308	Ashanti	Rainy	Female	19	10.6	Yes	No	A -	Neg	(+)	840	Pos	Pos
16309	Ashanti	Rainy	Female	43	12	No	No	O +	Neg	Neg	0	Neg	Neg
16314	Ashanti	Rainy	Female	38	11.5	Yes	No	A +	Pos	(+)	300	Pos	Pos
16310	Ashanti	Rainy	Male	40	11.7	Yes	No	A +	Neg	Neg	0	Pos	Pos
16312	Ashanti	Rainy	Female	27	10.7	Yes	No	O +	Neg	(+)	850	Pos	Pos
16313	Ashanti	Rainy	Female	46	10.1	Yes	No	O +	Neg	Neg	0	Neg	Neg
16391	Ashanti	Rainy	Male	26	13.9	No	No	B +	Neg	Neg	0	Neg	Neg
16390	Ashanti	Rainy	Female	33	8.8	Yes	No	O +	Pos	Neg	0	Neg	Neg
16389	Ashanti	Rainy	Female	16	10.5	Yes	No	O +	Neg	Neg	0	Neg	Neg
16387	Ashanti	Rainy	Female	38	11.5	Yes	No	B +	Neg	Neg	0	Neg	Neg
16388	Ashanti	Rainy	Female	25	11.8	Yes	No	O +	Neg	Neg	0	Neg	Neg
16383	Ashanti	Rainy	Female	24	11.3	Yes	No	AB +	Pos	Neg	0	Neg	Neg
16394	Ashanti	Rainy	Female	63	11	Yes	No	A +	Neg	Neg	0	Neg	Neg
16393	Ashanti	Rainy	Female	48	11.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
16392	Ashanti	Rainy	Female	28	11.8	Yes	No	B +	Neg	Neg	0	Neg	Neg
16376	Ashanti	Rainy	Female	38	12.2	No	No	B +	Neg	Neg	0	Neg	Neg
16359	Ashanti	Rainy	Female	20	6.4	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
16364	Ashanti	Rainy	Male	19	16.8	No	No	B +	Neg	Neg	0	Neg	Neg
16366	Ashanti	Rainy	Female	38	10.7	Yes	No	B +	Neg	(+)	880	Pos	Pos
16365	Ashanti	Rainy	Female	32	11	Yes	No	O -	Neg	Neg	0	Neg	Neg
16368	Ashanti	Rainy	Female	29	11.1	Yes	No	O +	Neg	Neg	0	Neg	Neg

210

16369	Ashanti	Rainy	Female	32	11.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
16355	Ashanti	Rainy	Female	26	11.4	Yes	No	O +	Neg	Neg	0	Neg	Neg
16371	Ashanti	Rainy	Female	89	9.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
16375	Ashanti	Rainy	Female	26	9.6	Yes	No	A +	Neg	Neg	0	Neg	Neg
16374	Ashanti	Rainy	Female	34	11.8	Yes	No	A +	Neg	Neg	0	Neg	Neg
16372	Ashanti	Rainy	Female	27	10.6	Yes	No	B +	Neg	Neg	0	Neg	Neg
16378	Ashanti	Rainy	Female	60	12	No	No	O +	Neg	Neg	0	Neg	Neg
16339	Ashanti	Rainy	Female	37	11.5	Yes	No	O +	Neg	Neg	0	Neg	Neg
16337	Ashanti	Rainy	Female	79	11.7	Yes	No	O +	Neg	(+)	200	Pos	Pos
16334	Ashanti	Rainy	Female	25	10.7	Yes	No	A +	Neg	Neg	0	Neg	Neg
16332	Ashanti	Rainy	Female	30	10.1	Yes	No	O +	Neg	Neg	0	Neg	Neg
16330	Ashanti	Rainy	Female	2	7.1	Yes	No	O +	Pos	Neg	0	Neg	Neg
16328	Ashanti	Rainy	Female	3	8.6	Yes	No	B +	Neg	(+)	80	Pos	Pos
16326	Ashanti	Rainy	Female	5	8.9	Yes	No	O +	Pos	(+)	920	Pos	Pos
16280	Ashanti	Rainy	Female	29	10.7	Yes	No	O +	Neg	Neg	0	Neg	Neg
16323	Ashanti	Rainy	Female	30	10.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
16341	Ashanti	Rainy	Female	32	8.8	Yes	No	B +	Neg	Neg	0	Neg	Neg
1	Western	Rainy	Male	2	9.9	Yes	No	O +	Neg	(+)	730	Neg	Pos
2	Western	Rainy	Female	2	10.3	Yes	No	O -	Neg	Neg	0	Neg	Neg
4	Western	Rainy	Male	2	11.3	No	No	B +	Pos	Neg	0	Neg	Neg
5	Western	Rainy	Male	68	14.1	No	No	B +	Neg	Neg	0	Neg	Neg
6	Western	Rainy	Female	58	13.1	No	No	O +	Neg	Neg	0	Neg	Neg
8	Western	Rainy	Male	19	15.6	No	No	O +	Neg	Neg	0	Neg	Neg
10	Western	Rainy	Male	83	13.1	No	No	B +	Neg	Neg	0	Neg	Neg
11	Western	Rainy	Male	63	13.7	No	No	O +	Neg	Neg	0	Neg	Neg
12	Western	Rainy	Female	47	13.7	No	No	B -	Neg	Neg	0	Neg	Neg
16	Western	Rainy	Female	47	14.7	No	No	B +	Pos	Neg	0	Neg	Neg
17	Western	Rainy	Female	9	15.7	No	No	O +	Neg	(+++)	5430	Pos	Pos
18	Western	Rainy	Female	22	12.3	No	No	O +	Neg	Neg	0	Pos	Pos
19	Western	Rainy	Female	1	10.4	Yes	No	O +	Neg	Neg	0	Neg	Neg

20	Western	Rainy	Female	3	10.4	Yes	No	A +	Neg	(+)	860	Pos	Pos
21	Western	Rainy	Female	55	7.3	Yes	Yes	B +	Pos	Neg	0	Neg	Neg
22	Western	Rainy	Female	36	13	No	No	B +	Neg	Neg	0	Neg	Neg
23	Western	Rainy	Female	29	12.2	No	No	O +	Neg	Neg	0	Neg	Neg
24	Western	Rainy	Male	24	7.4	Yes	Yes	O -	Neg	(+++)	4350	Pos	Pos
25	Western	Rainy	Female	41	16.7	No	No	A -	Neg	Neg	0	Neg	Neg
26	Western	Rainy	Male	22	11.3	Yes	No	O +	Neg	Neg	0	Neg	Neg
27	Western	Rainy	Female	40	12.5	No	No	B +	Neg	Neg	0	Neg	Neg
29	Western	Rainy	Male	19	13.1	No	No	A -	Neg	Neg	0	Neg	Neg
30	Western	Rainy	Female	26	10.3	Yes	No	AB +	Neg	Neg	0	Neg	Neg
32	Western	Rainy	Female	35	12.8	No	No	B +	Neg	Neg	0	Neg	Neg
33	Western	Rainy	Female	32	11.8	Yes	No	A +	Pos	Neg	0	Neg	Neg
39	Western	Rainy	Male	28	12.3	No	No	A +	Neg	Neg	0	Neg	Neg
46	Western	Rainy	Female	2	10	Yes	No	A +	Neg	Neg	0	Neg	Neg
47	Western	Rainy	Female	28	11.3	Yes	No	A +	Neg	Neg	0	Neg	Neg
48	Western	Rainy	Female	76	11.2	Yes	No	B +	Neg	Neg	0	Neg	Neg
52	Western	Rainy	Male	40	11.1	Yes	No	A +	Neg	Neg	0	Neg	Neg
62	Western	Rainy	Female	29	10.5	Yes	No	B +	Pos	Neg	0	Neg	Neg
63	Western	Rainy	Female	24	11.1	Yes	No	O +	Neg	Neg	0	Neg	Neg
64	Western	Rainy	Female	35	14.2	No	No	B +	Pos	Neg	0	Neg	Neg
68	Western	Rainy	Female	23	12.1	No	No	B +	Neg	Neg	0	Neg	Neg
69	Western	Ralny	Male	1	11.4	No	No	O +	Neg	Neg	0	Neg	Neg
70	Western	Rainy	Male	28	14	No	No	O -	Neg	Neg	0	Neg	Neg
71	Western	Rainy	Male	49	8.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
72	Western	Rainy	Female	6	16.3	No	No	O +	Neg	Neg	0	Pos	Neg
74	Western	Rainy	Male	68	8.2	Yes	No	B +	Pos	Neg	0	Pos	Pos
75	Western	Ralny	Male	2	11.3	No	No	O -	Pos	Neg	0	Neg	Neg
80	Western	Rainy	Male	5	16.3	No	No	O +	Neg	(++)	2130	Pos	Pos
82	Western	Ralny	Female	42	16.3	No	No	B +	Neg	Neg	0	Neg	Neg
84	Western	Rainy	Female	19	9.1	Yes	No	B +	Pos	Neg	0	Neg	Neg

212

ID	Region	Season	Sex	Age	Hb			Blood					
101	Western	Rainy	Male	80	8.8	Yes	No	O +	Neg	Neg	0	Neg	Neg
103	Western	Rainy	Male	63	10.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
105	Western	Rainy	Male	8	11.1	Yes	No	A +	Neg	(+++)	3890	Pos	Pos
106	Western	Rainy	Female	52	15.9	No	No	A -	Neg	Neg	0	Pos	Pos
107	Western	Rainy	Male	21	14.9	No	No	O +	Pos	Neg	0	Neg	Neg
108	Western	Rainy	Male	60	9.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
109	Western	Rainy	Female	24	11.9	Yes	No	B +	Neg	Neg	0	Neg	Neg
110	Western	Rainy	Male	54	15.6	No	No	O +	Neg	Neg	0	Pos	Neg
111	Western	Rainy	Female	28	10.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
112	Western	Rainy	Female	28	11.3	Yes	No	O +	Neg	(+)	780	Pos	Pos
113	Western	Rainy	Female	35	13.5	No	No	A +	Pos	Neg	0	Pos	Pos
115	Western	Rainy	Male	21	15.1	No	No	A +	Neg	Neg	0	Neg	Neg
116	Western	Rainy	Female	26	9.4	Yes	No	O +	Neg	(+)	930	Pos	Pos
117	Western	Rainy	Female	24	8.8	Yes	No	O +	Neg	Neg	0	Neg	Neg
118	Western	Rainy	Female	19	11.8	Yes	No	A -	Neg	(++)	1930	Neg	Pos
120	Western	Rainy	Male	12	11.4	Yes	No	B +	Neg	(++++)	5390	Pos	Pos
121	Western	Rainy	Male	42	12.9	No	No	A +	Neg	Neg	0	Neg	Neg
123	Western	Rainy	Male	42	16.1	No	No	O -	Neg	Neg	0	Neg	Neg
124	Western	Rainy	Male	36	9.2	Yes	No	O -	Neg	(++++)	4320	Pos	Pos
127	Western	Rainy	Female	20	11.9	Yes	No	O +	Neg	Neg	0	Pos	Pos
129	Western	Rainy	Female	42	10.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
130	Western	Rainy	Male	7	3.6	Yes	Yes	O -	Neg	Neg	0	Neg	Neg
132	Western	Rainy	Female	39	10.2	Yes	No	O -	Neg	(+)	280	Pos	Pos
133	Western	Rainy	Female	29	10.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
136	Western	Rainy	Male	12	14.7	No	No	O +	Neg	Neg	0	Neg	Neg
137	Western	Rainy	Female	29	11.8	Yes	No	O +	Neg	Neg	0	Neg	Neg
138	Western	Rainy	Female	33	8.5	Yes	No	A -	Neg	Neg	0	Pos	Neg
140	Western	Rainy	Female	43	13.6	No	No	O +	Pos	Neg	0	Neg	Neg
141	Western	Rainy	Male	12	10.5	Yes	No	O -	Neg	Neg	0	Neg	Neg
142	Western	Rainy	Female	43	9.2	Yes	No	B +	Neg	Neg	0	Neg	Neg

213

ID	Region	Season	Sex	Age	Value			Blood					
143	Western	Rainy	Male	45	16.4	No	No	A -	Neg	Neg	0	Neg	Neg
144	Western	Rainy	Male	62	12.2	No	No	A +	Neg	Neg	0	Neg	Neg
146	Western	Rainy	Female	24	8.9	Yes	No	O -	Neg	Neg	0	Neg	Neg
147	Western	Rainy	Female	2	11.6	No	No	O +	Neg	Neg	0	Neg	Neg
148	Western	Rainy	Female	6	10.3	Yes	No	O -	Neg	Neg	0	Neg	Neg
149	Western	Rainy	Female	1	7.7	Yes	No	O +	Neg	Neg	0	Neg	Neg
151	Western	Rainy	Male	42	10.4	Yes	No	O -	Pos	Neg	0	Neg	Neg
152	Western	Rainy	Female	30	10.1	Yes	No	O +	Neg	Neg	0	Neg	Neg
153	Western	Rainy	Female	20	10.4	Yes	No	A +	Neg	Neg	0	Neg	Neg
154	Western	Rainy	Female	26	6.6	Yes	Yes	A -	Pos	Neg	0	Neg	Neg
158	Western	Rainy	Female	50	10.2	Yes	No	A +	Neg	Neg	0	Neg	Pos
17	Central	Rainy	Male	34	9.4	Yes	No	O +	Pos	Neg	0	Neg	Neg
934	Central	Rainy	Female	14	16.3	No	No	O -	Neg	Neg	0	Neg	Neg
952	Central	Rainy	Female	46	11.7	Yes	No	A +	Neg	Neg	0	Neg	Neg
974	Central	Rainy	Male	39	11.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
977	Central	Rainy	Female	33	10.4	Yes	No	A +	Neg	Neg	0	Neg	Neg
922	Central	Rainy	Female	76	10.9	Yes	No	O -	Neg	Neg	0	Neg	Neg
959	Central	Rainy	Male	47	10.1	Yes	No	O -	Neg	Neg	0	Neg	Neg
939	Central	Rainy	Female	62	12.8	No	No	A -	Pos	Neg	0	Neg	Neg
990	Central	Rainy	Female	4	10.2	Yes	No	O -	Neg	(+)	730	Pos	Pos
945	Central	Rainy	Male	6	13.9	No	No	O +	Pos	Neg	0	Neg	Neg
4	Central	Rainy	Male	30	11.4	Yes	No	O +	Neg	Neg	0	Neg	Neg
998	Central	Rainy	Female	2	9.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
949	Central	Rainy	Female	31	14.8	No	No	A -	Neg	Neg	0	Neg	Neg
921	Central	Rainy	Female	31	11.3	Yes	No	O +	Neg	Neg	0	Neg	Neg
994	Central	Rainy	Female	78	8	Yes	No	AB +	Neg	Neg	0	Neg	Neg
965	Central	Rainy	Male	65	9.6	Yes	No	B +	Neg	Neg	0	Neg	Neg
991	Central	Rainy	Female	0.6	15	No	No	O +	Neg	(+)	850	Pos	Pos
14	Central	Rainy	Male	4	7.9	Yes	No	B +	Neg	(++)	2280	Pos	Pos
33	Central	Rainy	Male	10	8.3	Yes	No	O +	Neg	Neg	0	Neg	Neg

214

34	Central	Rainy	Male	55	13.9	No	No	AB +	Neg	Neg	0	Neg	Neg
993	Central	Rainy	Female	27	10.4	Yes	No	O +	(+)	Neg	80	Pos	Pos
971	Central	Rainy	Female	51	13.3	No	No	O -	Neg	Pos	0	Neg	Neg
975	Central	Rainy	Female	32	9.4	Yes	No	B +	Neg	Neg	0	Neg	Neg
135	Central	Rainy	Male	27	8.5	Yes	No	A +	(+)	Neg	900	Pos	Pos
1001	Central	Rainy	Male	3	12.8	No	No	O +	(+)	Neg	490	Pos	Pos
153	Central	Rainy	Male	26	9.5	Yes	No	O +	(++)	Neg	1980	Pos	Pos
1002	Central	Rainy	Female	48	9.6	Yes	No	A +	(+)	Neg	980	Pos	Pos
151	Central	Rainy	Male	14	9.6	Yes	No	O +	(+)	Neg	480	Pos	Pos
146	Central	Rainy	Male	26	11.4	Yes	No	B -	(++)	Neg	2120	Pos	Pos
162	Central	Rainy	Female	89	10.4	Yes	No	A +	(+)	Neg	400	Pos	Pos
84	Central	Rainy	Male	26	8.9	Yes	No	O +	(+)	Neg	650	Pos	Pos
159	Central	Rainy	Male	34	6.7	Yes	Yes	B +	(+)	Neg	850	Pos	Pos
1003	Central	Rainy	Male	0.7	5.5	Yes	Yes	A -	(+)	Neg	390	Pos	Pos
15	Central	Rainy	Female	56	6.4	Yes	Yes	O +	(+)	Neg	120	Pos	Pos
20	Central	Rainy	Male	27	6.6	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
19	Central	Rainy	Male	1	7.6	Yes	Yes	A +	Neg	Pos	0	Neg	Neg
1	Central	Rainy	Female	24	6.6	Yes	Yes	AB +	Neg	Neg	0	Neg	Neg
988	Central	Rainy	Male	36	6.5	Yes	Yes	B +	Neg	Neg	0	Neg	Neg
925	Central	Rainy	Female	53	8.7	Yes	No	O +	Neg	Neg	0	Neg	Neg
16	Central	Rainy	Male	0.8	12.3	Yes	No	B -	Neg	Pos	0	Neg	Neg
9	Central	Rainy	Female	1.5	9.6	Yes	No	A +	Neg	Neg	0	Neg	Neg
5	Central	Rainy	Female	26	11.6	Yes	No	AB -	Neg	Neg	0	Neg	Neg
964	Central	Rainy	Female	89	11.5	Yes	No	B -	Neg	Neg	0	Neg	Neg
953	Central	Rainy	Female	26	14.3	No	No	B +	Neg	Neg	0	Neg	Neg
951	Central	Rainy	Female	34	10.3	Yes	No	O +	Neg	Pos	0	Neg	Pos
983	Central	Rainy	Female	27	10.7	Yes	No	B +	Neg	Neg	0	Neg	Neg
956	Central	Rainy	Male	60	13.1	No	No	O -	Neg	Neg	0	Neg	Neg
3	Central	Rainy	Male	37	10.6	Yes	No	O +	Neg	Neg	0	Neg	Neg
914	Central	Rainy	Male	79	11.5	Yes	No	O +	Neg	Neg	0	Neg	Neg

215

ID	Region	Season	Sex	Age	Value			Blood					
917	Central	Rainy	Female	25	13.2	No	No	B+	Neg	Neg	0	Neg	Neg
911	Central	Rainy	Female	31	11.5	Yes	No	O+	Neg	Neg	0	Neg	Neg
912	Central	Rainy	Female	22	10.2	Yes	No	O+	Neg	Neg	0	Neg	Neg
913	Central	Rainy	Female	31	11.1	Yes	No	O+	Neg	Neg	0	Neg	Neg
1004	Central	Rainy	Female	33	9.7	Yes	No	O+	Neg	Neg	0	Neg	Neg
944	Central	Rainy	Male	52	9.8	Yes	No	A+	Neg	Neg	0	Neg	Neg
970	Central	Rainy	Male	26	10.9	Yes	No	O+	Neg	Neg	0	Pos	Neg
37	Central	Rainy	Male	66	10.7	Yes	No	O-	Neg	Neg	0	Neg	Neg
978	Central	Rainy	Male	3	11.4	No	No	O+	Neg	Neg	0	Neg	Neg
960	Central	Rainy	Female	50	9.5	Yes	No	B+	Neg	Neg	0	Neg	Neg
27	Central	Rainy	Female	17	10	Yes	No	O+	Neg	Neg	0	Neg	Neg
926	Central	Rainy	Female	59	11.5	Yes	No	O+	Pos	Neg	0	Neg	Neg
1005	Central	Rainy	Female	76	9.8	Yes	No	O+	Pos	Neg	0	Neg	Neg
907	Central	Rainy	Female	47	8.6	Yes	No	O+	Neg	Neg	0	Neg	Neg
919	Central	Rainy	Female	62	9.8	Yes	No	O+	Neg	Neg	0	Neg	Neg
10	Central	Rainy	Male	4	5.4	Yes	Yes	O+	Neg	Neg	0	Pos	Neg
929	Central	Rainy	Female	6	9.7	Yes	No	O-	Neg	Neg	0	Neg	Neg
955	Central	Rainy	Male	30	10.4	Yes	No	O+	Pos	Neg	0	Neg	Neg
26	Central	Rainy	Male	2	8.9	Yes	No	B+	Pos	Neg	0	Neg	Neg
0	Central	Rainy	Female	31	10.2	Yes	No	B-	Neg	(++)	1940	Pos	Pos
999	Central	Rainy	Male	31	8.7	Yes	No	A+	Neg	Neg	0	Neg	Neg
976	Central	Rainy	Female	78	8.4	Yes	No	O+	Pos	Neg	0	Neg	Neg
272	Central	Rainy	Male	65	11.7	Yes	No	A+	Neg	(+)	480	Pos	Pos
190	Central	Rainy	Male	6	10	Yes	No	A-	Pos	(+)	840	Pos	Pos
234	Central	Rainy	Male	31	3.5	Yes	Yes	O+	Neg	(+)	910	Pos	Pos
208	Central	Rainy	Female	22	13	No	No	O+	Pos	Neg	0	Neg	Neg
394	Central	Rainy	Male	31	10.6	Yes	No	O-	Pos	(++)	2280	Pos	Pos
402	Central	Rainy	Female	33	14	No	No	A-	Neg	Neg	0	Pos	Pos
386	Central	Rainy	Female	29	13	No	No	O-	Neg	(+)	830	Pos	Pos
307	Central	Rainy	Female	38	10.1	Yes	No	O+	Neg	(+)	960	Pos	Pos

216

ID	Region	Season	Sex	Age									
390	Central	Rainy	Female	42	10.9	Yes	No	A -	Neg	Neg	0	Neg	Neg
391	Central	Rainy	Female	5	15.1	No	No	O +	Neg	(+)	680	Pos	Pos
253	Central	Rainy	Female	9	9.1	Yes	No	A -	Neg	(+)	820	Pos	Pos
39	Central	Rainy	Male	50	7.2	Yes	Yes	B +	Neg	Neg	0	Neg	Neg
815	Central	Rainy	Female	73	12.1	No	No	A +	Neg	Neg	0	Neg	Neg
954	Central	Rainy	Male	33	6.8	Yes	Yes	B +	Pos	Neg	0	Neg	Neg
933	Central	Rainy	Male	31	12.8	No	No	B +	Neg	Neg	0	Pos	Pos
920	Central	Rainy	Female	24	10.2	Yes	No	O +	Pos	Neg	0	Neg	Neg
915	Central	Rainy	Male	47	9.5	Yes	No	A +	Neg	Neg	0	Neg	Neg
973	Central	Rainy	Female	45	11.2	Yes	No	O +	Neg	Neg	0	Pos	Pos
910	Central	Rainy	Female	37	12.2	No	No	A +	Neg	Neg	0	Pos	Pos
2	Central	Rainy	Female	26	11.6	Yes	No	A +	Pos	Neg	0	Neg	Neg
916	Central	Rainy	Female	26	12.3	No	No	O +	Pos	Neg	0	Neg	Neg
989	Central	Rainy	Female	48	11.7	Yes	No	O +	Pos	Neg	0	Neg	Neg
41	Central	Rainy	Male	26	11	Yes	No	A +	Neg	Neg	0	Neg	Neg
24	Central	Rainy	Female	26	9.5	Yes	No	O +	Pos	Neg	0	Neg	Neg
25	Central	Rainy	Female	15	11.4	Yes	No	O +	Neg	Neg	0	Neg	Neg
38	Central	Rainy	Female	43	13.7	No	No	B +	Neg	Neg	0	Neg	Neg
948	Central	Rainy	Female	27	8.1	Yes	No	B -	Pos	Neg	0	Neg	Neg
937	Central	Rainy	Female	32	11.9	Yes	No	B +	Neg	Neg	0	Neg	Neg
927	Central	Rainy	Male	50	11.7	Yes	No	B +	Neg	Neg	0	Neg	Neg
1006	Central	Rainy	Female	26	11.4	Yes	No	O +	Neg	Neg	0	Neg	Neg
1007	Central	Rainy	Female	4	15.5	No	No	AB +	Neg	Neg	0	Pos	Pos
43	Central	Rainy	Female	48	12.1	No	No	O +	Neg	Neg	0	Neg	Neg
42	Central	Rainy	Female	21	10.1	Yes	No	O +	Neg	Neg	0	Neg	Neg
1008	Central	Rainy	Female	32	12.5	No	No	B +	Neg	Neg	0	Neg	Neg
940	Central	Rainy	Female	50	6.9	Yes	Yes	O -	Neg	Neg	0	Pos	Neg
935	Central	Rainy	Female	16	13.7	No	No	A +	Neg	Neg	0	Neg	Neg
1009	Central	Rainy	Female	4	9.8	Yes	No	B -	Neg	Neg	0	Neg	Neg
32	Central	Rainy	Male	63	3.8	Yes	Yes	O +	Neg	Neg	0	Neg	Neg

986	Central	Rainy	Female	31	9.1	Yes	No	O +	Neg	Neg	0	Neg	Neg	Pos
21	Central	Rainy	Female	45	11.3	Yes	No	A +	Pos	Neg	0	Neg	Neg	Neg
326	Greater Accra	Rainy	Female	36	15.2	No	No	A +	Neg	Neg	0	Neg	Neg	Neg
338	Greater Accra	Rainy	Female	5	13	No	No	O +	Neg	Neg	0	Neg	Pos	Neg
339	Greater Accra	Rainy	Female	21	14.2	No	No	O +	Neg	Neg	0	Neg	Neg	Neg
340	Greater Accra	Rainy	Male	45	10.9	Yes	No	A -	Neg	Neg	0	Neg	Neg	Neg
341	Greater Accra	Rainy	Male	46	12.4	No	No	B +	Neg	Neg	0	Neg	Neg	Neg
342	Greater Accra	Rainy	Female	14	11.1	Yes	No	O +	Neg	Neg	0	Neg	Neg	Neg
343	Greater Accra	Rainy	Female	26	9.1	Yes	No	B +	Neg	(+)	120	Pos	Pos	Pos
344	Greater Accra	Rainy	Male	89	11.9	Yes	No	A +	Neg	Neg	0	Pos	Neg	Neg
345	Greater Accra	Rainy	Female	26	10.6	Yes	No	A +	Neg	Neg	0	Neg	Neg	Neg
347	Greater Accra	Rainy	Male	34	8.5	Yes	No	A +	Neg	Neg	0	Neg	Neg	Pos
348	Greater Accra	Rainy	Female	31	8.2	Yes	No	O +	Neg	Neg	0	Neg	Neg	Neg
349	Greater Accra	Rainy	Female	22	13.7	No	No	O +	Neg	(+)	50	Neg	Neg	Neg
350	Greater Accra	Rainy	Male	31	15.8	No	No	B +	Neg	Neg	0	Neg	Neg	Neg
351	Greater Accra	Rainy	Female	33	10.7	Yes	No	O +	Neg	Neg	0	Neg	Neg	Neg
352	Greater Accra	Rainy	Female	29	16.2	No	No	B -	Pos	Neg	0	Neg	Neg	Neg
359	Greater Accra	Rainy	Male	38	8.7	Yes	No	B +	Neg	Neg	0	Neg	Neg	Neg
360	Greater Accra	Rainy	Female	42	13.7	No	No	O +	Neg	Neg	0	Neg	Neg	Neg
374	Greater Accra	Rainy	Male	5.9	15.5	No	No	B +	Neg	Neg	0	Neg	Neg	Neg
375	Greater Accra	Rainy	Female	9	12.5	No	No	B +	Neg	Neg	0	Pos	Neg	Neg
380	Greater Accra	Rainy	Female	50	12.8	No	No	B -	Neg	Neg	0	Neg	Neg	Neg
381	Greater Accra	Rainy	Female	52	13.1	No	No	AB +	Neg	Neg	0	Neg	Neg	Pos
383	Greater Accra	Rainy	Female	26	15.2	No	No	B +	Neg	(+)	140	Neg	Neg	Neg
384	Greater Accra	Rainy	Male	66	11.1	Yes	No	AB +	Pos	Neg	0	Pos	Neg	Neg
386	Greater Accra	Rainy	Female	3	10.9	Yes	No	O +	Neg	Neg	0	Neg	Neg	Neg
390	Greater Accra	Rainy	Female	50	12.9	No	No	B -	Neg	Neg	0	Neg	Neg	Neg
391	Greater Accra	Rainy	Female	17	12.7	No	No	O +	Neg	Neg	0	Neg	Neg	Neg
392	Greater Accra	Rainy	Female	59	7.5	Yes	Yes	B +	Neg	Neg	0	Neg	Neg	Neg
393	Greater Accra	Rainy	Female	76	14.6	No	No	A +	Neg	Neg	0	Neg	Neg	Neg

218

394	Greater Accra	Rainy	Female	47	9.3	Yes	No	O +	Neg	(+)	780	Neg	Pos
396	Greater Accra	Rainy	Female	62	14.6	No	No	O +	Neg	(+)	960	Neg	Pos
399	Greater Accra	Rainy	Female	7	9.3	Yes	No	A +	Neg	Neg	0	Neg	Neg
403	Greater Accra	Rainy	Male	6	13.2	No	No	O +	Neg	Neg	0	Neg	Pos
418	Greater Accra	Rainy	Male	30	11.3	Yes	No	A +	Neg	Neg	0	Neg	Neg
419	Greater Accra	Rainy	Female	2	11.6	No	No	O +	Neg	Neg	0	Neg	Neg
423	Greater Accra	Rainy	Male	31	13.7	No	No	A +	Neg	Neg	0	Neg	Neg
450	Greater Accra	Rainy	Male	31	18.9	No	No	B +	Neg	Neg	0	Neg	Neg
451	Greater Accra	Rainy	Male	78	14.4	No	No	O +	Neg	(++)	2370	Pos	Pos
501	Greater Accra	Rainy	Female	65	12.3	No	No	AB -	Neg	Neg	0	Neg	Neg
612	Greater Accra	Rainy	Male	32	11.1	Yes	No	O +	Pos	Neg	0	Neg	Neg
653	Greater Accra	Rainy	Male	40	12.3	No	No	O +	Neg	Neg	0	Neg	Neg
10	Greater Accra	Rainy	Male	10	14.1	No	No	A +	Neg	Neg	0	Neg	Neg
11	Greater Accra	Rainy	Female	55	10.4	Yes	No	B +	Pos	Neg	0	Neg	Neg
7074	Greater Accra	Rainy	Female	27	10.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
120	Greater Accra	Rainy	Female	51	11.4	Yes	No	O +	Neg	Neg	0	Neg	Neg
122	Greater Accra	Rainy	Female	32	12	No	No	B +	Pos	Neg	0	Pos	Neg
138	Greater Accra	Rainy	Female	27	13.1	No	No	B +	Pos	Neg	0	Neg	Neg
142	Greater Accra	Rainy	Female	3	9.7	Yes	No	A +	Neg	Neg	0	Neg	Neg
153	Greater Accra	Rainy	Female	26	8.3	Yes	No	B +	Neg	Neg	0	Neg	Neg
154	Greater Accra	Rainy	Male	48	12.8	No	No	AB +	Neg	Neg	0	Neg	Neg
155	Greater Accra	Rainy	Female	26	9.8	Yes	No	A +	Neg	Neg	0	Neg	Neg
156	Greater Accra	Rainy	Female	26	12	No	No	O +	Neg	Neg	0	Neg	Neg
159	Greater Accra	Rainy	Male	15	11.9	Yes	No	A +	Neg	Neg	0	Neg	Neg
160	Greater Accra	Rainy	Female	43	9.5	Yes	No	B +	Neg	Neg	0	Neg	Neg
164	Greater Accra	Rainy	Female	27	11.7	Yes	No	O +	Neg	Neg	0	Neg	Neg
165	Greater Accra	Rainy	Female	32	8.1	Yes	No	O +	Neg	Neg	0	Neg	Pos
7166	Greater Accra	Rainy	Female	21	7.4	Yes	No	O +	Pos	Neg	0	Neg	Neg
167	Greater Accra	Rainy	Female	28	10.3	Yes	No	A +	Pos	Neg	0	Neg	Neg
169	Greater Accra	Rainy	Female	34	11.7	Yes	No	O +	Neg	Neg	0	Neg	Neg

219

ID	Region	Season	Sex	Age	Hb	Col1	Col2	Blood Group	Col3	Col4	Value	Col5	Col6
173	Greater Accra	Rainy	Female	68	5.7	Yes	Yes	A +	Pos	Neg	0	Neg	Neg
7180	Greater Accra	Rainy	Male	55	14.2	No	No	A +	Neg	Neg	0	Neg	Neg
242	Greater Accra	Rainy	Female	19	9.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
249	Greater Accra	Rainy	Female	35	9.2	Yes	No	B +	Neg	Neg	0	Neg	Neg
250	Greater Accra	Rainy	Male	42	12.5	No	No	O +	Neg	Neg	0	Neg	Neg
265	Greater Accra	Rainy	Female	21	9	Yes	No	O +	Neg	(+)	920	Neg	Pos
266	Greater Accra	Rainy	Female	45	13.7	No	No	O +	Neg	Neg	0	Neg	Neg
267	Greater Accra	Rainy	Female	46	6.8	Yes	Yes	O +	Pos	Neg	0	Neg	Neg
272	Greater Accra	Rainy	Male	14	10.6	Yes	No	A +	Neg	Neg	0	Neg	Neg
289	Greater Accra	Rainy	Female	26	10.5	Yes	No	O +	Neg	Neg	0	Neg	Pos
294	Greater Accra	Rainy	Male	89	15.6	No	No	A +	Neg	Neg	0	Pos	Pos
297	Greater Accra	Rainy	Male	26	13.1	No	No	O +	Neg	Neg	0	Neg	Neg
298	Greater Accra	Rainy	Male	34	13.3	No	No	AB +	Neg	Neg	0	Neg	Neg
2018	Greater Accra	Rainy	Female	0.7	11.3	No	No	O +	Pos	Neg	0	Neg	Neg
316	Greater Accra	Rainy	Female	56	14.6	No	No	B +	Neg	Neg	0	Neg	Neg
319	Greater Accra	Rainy	Female	27	12.9	No	No	O +	Neg	Neg	0	Neg	Neg
320	Greater Accra	Rainy	Male	1	12.2	No	No	O +	Neg	Neg	0	Neg	Neg
321	Greater Accra	Rainy	Female	24	14.9	No	No	O +	Pos	Neg	0	Pos	Pos
323	Greater Accra	Rainy	Male	36	9	Yes	No	A +	Neg	(++)	2410	Neg	Pos
324	Greater Accra	Rainy	Female	53	12.4	No	No	B +	Neg	Neg	0	Neg	Neg
325	Greater Accra	Rainy	Female	0.8	8.4	Yes	No	O +	Neg	(+)	1000	Neg	Pos
452	Greater Accra	Rainy	Female	1.5	11.3	No	No	AB +	Neg	Neg	0	Pos	Pos
453	Greater Accra	Rainy	Female	26	14.6	No	No	A +	Pos	Neg	0	Neg	Neg
455	Greater Accra	Rainy	Female	89	13.7	No	No	AB +	Pos	Neg	0	Neg	Neg
466	Greater Accra	Rainy	Female	26	14.9	No	No	O +	Neg	Neg	0	Neg	Neg
481	Greater Accra	Rainy	Female	34	13.1	No	No	O +	Neg	Neg	0	Neg	Neg
484	Greater Accra	Rainy	Female	27	10.6	Yes	No	O +	Neg	Neg	0	Neg	Neg
485	Greater Accra	Rainy	Female	60	11.7	Yes	No	O +	Neg	Neg	0	Neg	Neg
486	Greater Accra	Rainy	Female	37	10.7	Yes	No	A -	Neg	Neg	0	Neg	Neg
487	Greater Accra	Rainy	Female	79	10.9	Yes	No	O +	Neg	Neg	0	Neg	Neg

220

488	Greater Accra	Rainy	Female	25	11.1	Yes	No	B +	Neg	Neg	0	Neg	Neg
490	Greater Accra	Rainy	Female	31	9.9	Yes	No	B -	Neg	Neg	0	Neg	Neg
491	Greater Accra	Rainy	Female	22	12.9	No	No	O +	Neg	Neg	0	Neg	Neg
494	Greater Accra	Rainy	Female	31	12.4	No	No	O +	Neg	(+)	800	Neg	Pos
496	Greater Accra	Rainy	Male	33	13.3	No	No	A +	Neg	Neg	0	Neg	Neg
497	Greater Accra	Rainy	Male	29	13.6	No	No	O -	Neg	Neg	0	Neg	Neg
498	Greater Accra	Rainy	Male	38	19.9	No	No	O +	Neg	Neg	0	Neg	Neg
499	Greater Accra	Rainy	Male	42	15.6	No	No	AB +	Neg	Neg	0	Neg	Neg
500	Greater Accra	Rainy	Female	5	13.2	No	No	B +	Neg	Neg	0	Neg	Neg
502	Greater Accra	Rainy	Male	9	13.9	No	No	A +	Neg	Neg	0	Neg	Neg
504	Greater Accra	Rainy	Female	50	11.7	Yes	No	A +	Neg	(++)	1830	Neg	Pos
511	Greater Accra	Rainy	Male	73	15.6	No	No	O +	Neg	Neg	0	Pos	Pos
512	Greater Accra	Rainy	Female	33	12.6	No	No	O +	Neg	Neg	0	Neg	Neg
513	Greater Accra	Rainy	Female	31	13.7	Yes	No	B +	Pos	Neg	0	Neg	Neg
514	Greater Accra	Rainy	Female	24	11.3	Yes	No	O +	Pos	Neg	0	Neg	Neg
515	Greater Accra	Rainy	Female	47	11.1	Yes	No	B +	Neg	Neg	0	Neg	Pos
516	Greater Accra	Rainy	Male	45	14.8	No	No	A -	Neg	Neg	0	Neg	Neg
543	Greater Accra	Rainy	Male	37	14.5	No	No	O +	Neg	Neg	0	Neg	Neg
547	Greater Accra	Rainy	Female	26	13.7	No	No	O +	Neg	Neg	0	Pos	Pos
170	Greater Accra	Rainy	Female	29	11.6	Yes	No	B -	Neg	Neg	0	Neg	Neg
124	Greater Accra	Rainy	Female	35	15	No	No	A +	Neg	Neg	0	Neg	Neg
292	Greater Accra	Rainy	Female	76	7.3	Yes	Yes	O +	Neg	(+)	380	Neg	Neg
270	Greater Accra	Rainy	Female	61	12.8	No	No	B +	Neg	Neg	0	Neg	Neg
379	Greater Accra	Rainy	Male	70	14.9	No	No	O +	Neg	(+++)	3780	Neg	Pos
353	Greater Accra	Rainy	Female	43	11.1	Yes	No	O +	Pos	Neg	0	Neg	Neg
495	Greater Accra	Rainy	Female	75	12.3	No	No	B +	Neg	Neg	0	Neg	Neg
454	Greater Accra	Rainy	Female	70	12.3	No	No	B +	Neg	Neg	0	Neg	Neg
315	Greater Accra	Rainy	Female	8	8.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
492	Greater Accra	Rainy	Female	17	11.3	Yes	No	A +	Neg	Neg	0	Neg	Neg
493	Greater Accra	Rainy	Female	30	12.2	No	No	B +	Neg	Neg	0	Neg	Neg

376	Greater Accra	Rainy	Female	15	12.5	No	No	B -	Pos	Neg	0	Neg	Neg
11422	Volta	Rainy	Female	16	10.2	Yes	No	O +	Pos	Neg	0	Neg	Neg
11518	Volta	Rainy	Female	27	12.6	No	No	O +	Neg	Neg	0	Neg	Pos
11520	Volta	Rainy	Female	8	7.1	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
11473	Volta	Rainy	Female	14	12.5	No	No	O +	Neg	Neg	0	Neg	Neg
11527	Volta	Rainy	Female	53	11.5	Yes	No	B -	Pos	Neg	0	Neg	Neg
11513	Volta	Rainy	Female	29	10.7	Yes	No	O +	Neg	Neg	0	Neg	Neg
11530	Volta	Rainy	Female	50	12.7	No	No	B +	Neg	Neg	0	Neg	Neg
11507	Volta	Rainy	Female	29	8.4	Yes	No	B +	Neg	Neg	0	Pos	Neg
11556	Volta	Rainy	Female	2	8.1	Yes	Yes	O +	Pos	Neg	0	Neg	Neg
11504	Volta	Rainy	Female	36	11.1	Yes	No	A +	Neg	Neg	0	Neg	Neg
11506	Volta	Rainy	Female	24	7.9	Yes	Yes	O +	Neg	Neg	0	Pos	Neg
11487	Volta	Rainy	Female	75	9.3	Yes	No	B +	Pos	Neg	0	Neg	Neg
11494	Volta	Rainy	Male	57	14.4	No	No	B +	Pos	Neg	0	Neg	Neg
11459	Volta	Rainy	Male	19	13.8	No	No	B +	Neg	Neg	0	Neg	Neg
11498	Volta	Rainy	Female	31	11.1	Yes	No	O +	Pos	Neg	0	Neg	Neg
11502	Volta	Rainy	Female	26	11	Yes	No	A +	Neg	Neg	0	Neg	Neg
11486	Volta	Rainy	Female	50	8.8	Yes	No	O +	Neg	Neg	0	Neg	Neg
11455	Volta	Rainy	Female	19	6.9	Yes	Yes	O +	Neg	Neg	0	Neg	Pos
11493	Volta	Rainy	Male	86	13.2	No	No	O +	Neg	Neg	0	Neg	Neg
11511	Volta	Rainy	Male	76	7	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
11509	Volta	Rainy	Female	21	11.8	Yes	No	A +	Neg	Neg	0	Neg	Neg
11474	Volta	Rainy	Female	57	13.1	No	No	A +	Pos	Neg	0	Neg	Neg
11458	Volta	Rainy	Female	32	9.5	Yes	No	A +	Neg	Neg	0	Neg	Neg
11469	Volta	Rainy	Female	26	8.5	Yes	No	A +	Pos	Neg	0	Neg	Neg
11467	Volta	Rainy	Male	86	5.4	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
11522	Volta	Rainy	Male	81	12.9	No	No	O +	Neg	Neg	0	Neg	Neg
11453	Volta	Rainy	Female	6	8.9	Yes	Yes	O +	Neg	Neg	0	Neg	Pos
11434	Volta	Rainy	Female	45	9.5	Yes	No	O +	Pos	Neg	0	Neg	Neg
11428	Volta	Rainy	Female	28	11.3	Yes	No	O +	Neg	Neg	0	Pos	Neg

ID	Region	Season	Gender	Age	Hb			Blood			Value		
11459	Volta	Rainy	Female	22	13.8	No	No	B +	Neg	Neg	0	Neg	Neg
11414	Volta	Rainy	Female	27	13.4	No	No	B +	Pos	Neg	0	Neg	Neg
11463	Volta	Rainy	Male	71	10.2	Yes	No	B +	Neg	Neg	0	Neg	Neg
11405	Volta	Rainy	Female	9	9.9	Yes	No	B +	Pos	Neg	0	Neg	Neg
1	Volta	Rainy	Male	7	11.6	Yes	No	B +	Neg	(+)	770	Neg	Pos
11385	Volta	Rainy	Female	62	11.8	Yes	No	A +	Neg	Neg	0	Neg	Neg
11386	Volta	Rainy	Female	58	14.2	No	No	B +	Pos	Neg	0	Neg	Neg
11390	Volta	Rainy	Female	10	11.6	Yes	No	O +	Pos	Neg	0	Neg	Neg
11412	Volta	Rainy	Female	42	10.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
11448	Volta	Rainy	Female	78	6.3	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
11457	Volta	Rainy	Female	24	11.6	Yes	No	O +	Neg	Neg	0	Neg	Neg
11397	Volta	Rainy	Female	80	12.4	No	No	B -	Neg	Neg	0	Neg	Neg
11398	Volta	Rainy	Female	1	9.5	Yes	No	B +	Neg	(+)	500	Neg	Pos
11420	Volta	Rainy	Male	13	11.5	Yes	No	B +	Neg	Neg	0	Neg	Neg
11416	Volta	Rainy	Female	29	12.1	No	No	O +	Neg	Neg	0	Neg	Neg
11353	Volta	Rainy	Female	8	9.3	Yes	No	A +	Neg	Neg	0	Neg	Neg
11392	Volta	Rainy	Female	33	12.3	No	No	B +	Neg	Neg	0	Neg	Neg
11373	Volta	Rainy	Male	42	10.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
11374	Volta	Rainy	Male	39	5.9	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
11372	Volta	Rainy	Male	29	11.5	Yes	No	O +	Neg	Neg	0	Neg	Neg
11447	Volta	Rainy	Male	35	10.9	Yes	No	B +	Neg	Neg	0	Neg	Neg
11371	Volta	Rainy	Female	18	8.6	Yes	No	B +	Neg	(+)	930	Neg	Pos
11369	Volta	Rainy	Female	82	8.3	Yes	No	O +	Neg	Neg	0	Neg	Neg
11370	Volta	Rainy	Female	38	6.6	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
11347	Volta	Rainy	Male	38	10.3	Yes	No	B +	Neg	Neg	0	Neg	Neg
11314	Volta	Rainy	Female	15	10.6	Yes	No	AB +	Neg	Neg	0	Neg	Neg
11330	Volta	Rainy	Female	43	6.4	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
11303	Volta	Rainy	Male	8	10.1	Yes	No	A +	Neg	Neg	0	Neg	Neg
11367	Volta	Rainy	Female	12	7.6	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
11363	Volta	Rainy	Female	23	10.8	Yes	No	AB +	Neg	Neg	0	Neg	Neg

223

ID	Region	Season	Gender	Age	Hb			Blood					
11355	Volta	Rainy	Female	42	11.3	Yes	No	O+	Neg	Neg	0	Pos	Pos
11356	Volta	Rainy	Male	10	8.9	Yes	No	O-	Pos	Neg	0	Neg	Neg
11335	Volta	Rainy	Female	69	11.6	Yes	No	O-	Pos	Neg	0	Neg	Pos
11368	Volta	Rainy	Female	57	15.4	No	No	A+	Neg	Neg	0	Neg	Neg
11338	Volta	Rainy	Male	41	12.6	No	No	A+	Neg	Neg	0	Neg	Neg
11626	Volta	Rainy	Male	26	12.9	No	No	O+	Neg	Neg	0	Neg	Neg
11737	Volta	Rainy	Female	4	9.3	Yes	No	O+	Neg	Neg	0	Neg	Neg
11738	Volta	Rainy	Female	48	9.9	Yes	No	O+	Neg	Neg	0	Neg	Neg
11730	Volta	Rainy	Female	21	11.5	Yes	No	B+	Neg	Neg	0	Neg	Neg
11745	Volta	Rainy	Female	32	12.1	No	No	O+	Neg	(++)	1880	Neg	Pos
11701	Volta	Rainy	Female	50	13.3	No	No	B+	Neg	Neg	0	Neg	Neg
2	Volta	Rainy	Female	16	5.5	Yes	Yes	B+	Pos	Neg	0	Neg	Neg
11744	Volta	Rainy	Male	4	13.2	No	No	O+	Neg	Neg	0	Neg	Neg
11698	Volta	Rainy	Female	63	12.9	No	No	B+	Neg	Neg	0	Neg	Neg
11687	Volta	Rainy	Female	31	11.3	Yes	No	B+	Neg	Neg	0	Neg	Neg
11695	Volta	Rainy	Female	45	9.7	Yes	No	A+	Neg	Neg	0	Neg	Neg
11718	Volta	Rainy	Female	32	13.9	No	No	O+	Neg	(++)	2190	Neg	Pos
11719	Volta	Rainy	Male	23	6.3	Yes	Yes	A+	Pos	Neg	0	Neg	Neg
11720	Volta	Rainy	Male	80	10.2	Yes	No	A+	Neg	(+)	940	Neg	Pos
11709	Volta	Rainy	Male	6	10.7	Yes	No	O+	Neg	Neg	0	Pos	Neg
11716	Volta	Rainy	Female	75	9	Yes	No	AB+	Neg	Neg	0	Neg	Neg
11746	Volta	Rainy	Male	36	7.1	Yes	Yes	B+	Neg	Neg	0	Neg	Neg
11740	Volta	Rainy	Female	22	11.8	Yes	No	B+	Neg	Neg	0	Neg	Neg
11682	Volta	Rainy	Female	14	6.9	Yes	Yes	O+	Neg	Neg	0	Neg	Neg
11706	Volta	Rainy	Female	43	13.7	No	No	B+	Neg	Neg	0	Neg	Neg
11736	Volta	Rainy	Female	29	11.3	Yes	No	A+	Neg	Neg	0	Neg	Neg
11725	Volta	Rainy	Female	38	11.1	Yes	No	O+	Neg	Neg	0	Neg	Neg
11772	Volta	Rainy	Female	56	14.7	No	No	O+	Neg	Neg	0	Neg	Neg
11739	Volta	Rainy	Female	57	13.1	No	No	O+	Neg	Neg	0	Neg	Neg
11799	Volta	Rainy	Male	42	7.4	Yes	Yes	A+	Neg	Neg	0	Neg	Neg

224

11790	Volta	Rainy	Male	19	6.9	Yes	Yes	B +	Neg	Neg	0	Neg	Neg
11754	Volta	Rainy	Female	57	11.8	Yes	No	B +	Neg	Neg	0	Neg	Neg
11788	Volta	Rainy	Female	32	8.1	Yes	No	B +	Neg	Neg	0	Neg	Neg
11793	Volta	Rainy	Female	20	11	Yes	No	O +	Pos	Neg	0	Neg	Neg
11768	Volta	Rainy	Female	33	10.5	Yes	No	B +	Neg	Neg	0	Neg	Neg
11689	Volta	Rainy	Male	68	7	Yes	Yes	O +	Neg	(+)	230	Pos	Pos
11773	Volta	Rainy	Female	18	10	Yes	No	O +	Neg	Neg	0	Pos	Pos
11744	Volta	Rainy	Male	4	7.1	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
11789	Volta	Rainy	Male	62	15	No	No	O -	Neg	Neg	0	Neg	Neg
11782	Volta	Rainy	Male	26	15	No	No	B +	Neg	Neg	0	Neg	Neg
11708	Volta	Rainy	Male	2	12.4	No	No	O +	Neg	Neg	0	Neg	Neg
5062	Brong-Ahafo	Rainy	Female	12	9.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
5015	Brong-Ahafo	Rainy	Female	26	13	No	No	A +	Pos	Neg	0	Neg	Neg
5012	Brong-Ahafo	Rainy	Female	27	12.6	No	No	O +	Pos	Neg	0	Neg	Neg
5008	Brong-Ahafo	Rainy	Female	15	10.1	Yes	No	O +	Neg	Neg	0	Pos	Pos
5014	Brong-Ahafo	Rainy	Female	23	11.3	Yes	No	O +	Pos	Neg	0	Neg	Neg
5020	Brong-Ahafo	Rainy	Female	17	10.1	Yes	No	A +	Neg	Neg	0	Neg	Pos
4994	Brong-Ahafo	Rainy	Male	27	10.9	Yes	No	O +	Neg	(++)	1180	Pos	Pos
4998	Brong-Ahafo	Rainy	Female	21	9.6	Yes	No	B +	Neg	Neg	0	Neg	Neg
5009	Brong-Ahafo	Rainy	Female	47	12.9	No	No	A +	Neg	Neg	0	Neg	Neg
4944	Brong-Ahafo	Rainy	Male	19	9.8	Yes	No	A +	Pos	Neg	0	Neg	Neg
5059	Brong-Ahafo	Rainy	Female	28	9.5	Yes	No	O +	Neg	Neg	0	Neg	Neg
5501	Brong-Ahafo	Rainy	Female	9	9	Yes	No	O -	Pos	(+)	250	Neg	Neg
5502	Brong-Ahafo	Rainy	Female	29	13	No	No	A -	Neg	Neg	0	Neg	Neg
5055	Brong-Ahafo	Rainy	Female	20	9.4	Yes	No	O +	Neg	Neg	0	Neg	Neg
5054	Brong-Ahafo	Rainy	Female	1	10.7	Yes	No	O +	Neg	Neg	0	Neg	Neg
5052	Brong-Ahafo	Rainy	Male	37	17.6	No	No	O +	Neg	Neg	0	Neg	Neg
5503	Brong-Ahafo	Rainy	Female	32	9.4	Yes	No	A +	Neg	Neg	0	Pos	Pos
5056	Brong-Ahafo	Rainy	Female	35	11.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
4945	Brong-Ahafo	Rainy	Female	15	11.9	Yes	No	B +	Neg	(++)	2480	Pos	Pos

225

4931	Brong-Ahafo	Rainy	Female	14	13.9	No	No	A +	Neg	(+)	390	Neg	Neg
5504	Brong-Ahafo	Rainy	Male	19	10.7	Yes	No	O +	Neg	(+)	920	Pos	Pos
4192	Brong-Ahafo	Rainy	Female	22	10.2	Yes	No	B +	Neg	Neg	0	Neg	Neg
4196	Brong-Ahafo	Rainy	Female	18	12.7	No	No	A +	Neg	Neg	0	Neg	Neg
4197	Brong-Ahafo	Rainy	Female	35	10.7	Yes	No	A +	Neg	Neg	0	Neg	Neg
4199	Brong-Ahafo	Rainy	Female	18	8.7	Yes	No	A +	Neg	Neg	0	Pos	Pos
4201	Brong-Ahafo	Rainy	Female	27	12	No	No	O +	Neg	Neg	0	Neg	Neg
4203	Brong-Ahafo	Rainy	Female	17	9.6	Yes	No	B +	Pos	(+)	860	Pos	Pos
4205	Brong-Ahafo	Rainy	Female	28	8	Yes	No	O +	Neg	Neg	0	Pos	Pos
4207	Brong-Ahafo	Rainy	Female	24	11.7	Yes	No	A +	Neg	Neg	0	Neg	Neg
4208	Brong-Ahafo	Rainy	Female	23	10.7	Yes	No	A +	Neg	Neg	0	Pos	Pos
4210	Brong-Ahafo	Rainy	Female	28	10	Yes	No	A +	Pos	Neg	0	Pos	Pos
4212	Brong-Ahafo	Rainy	Female	26	10.9	Yes	No	B +	Neg	Neg	0	Neg	Neg
4213	Brong-Ahafo	Rainy	Female	25	12.1	No	No	B -	Neg	Neg	0	Neg	Pos
4214	Brong-Ahafo	Rainy	Male	43	13.6	No	No	O +	Pos	Neg	0	Neg	Neg
4216	Brong-Ahafo	Rainy	Female	29	11	Yes	No	AB +	Neg	Neg	0	Neg	Neg
4218	Brong-Ahafo	Rainy	Female	25	13.5	No	No	A -	Neg	Neg	0	Neg	Neg
4219	Brong-Ahafo	Rainy	Female	23	10	Yes	No	AB -	Neg	Neg	0	Neg	Neg
4221	Brong-Ahafo	Rainy	Female	19	11.8	Yes	No	A +	Neg	(+)	240	Pos	Pos
4231	Brong-Ahafo	Rainy	Female	25	8.7	Yes	No	O +	Neg	Neg	0	Neg	Neg
4243	Brong-Ahafo	Rainy	Female	32	11	Yes	No	A +	Neg	Neg	0	Neg	Neg
4182	Brong-Ahafo	Rainy	Female	46	11.1	Yes	No	O +	Neg	Neg	0	Neg	Neg
4128	Brong-Ahafo	Rainy	Female	31	10.2	Yes	No	O +	Pos	Neg	0	Neg	Neg
4257	Brong-Ahafo	Rainy	Female	20	12.8	No	No	O +	Neg	(++)	1790	Pos	Pos
4265	Brong-Ahafo	Rainy	Female	25	10.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
4273	Brong-Ahafo	Rainy	Female	23	12.7	No	No	B +	Neg	Neg	0	Neg	Neg
4274	Brong-Ahafo	Rainy	Female	37	14.8	No	No	O +	Pos	Neg	0	Neg	Neg
5579	Brong-Ahafo	Rainy	Female	22	10.1	Yes	No	O +	Neg	(+)	380	Pos	Pos
5583	Brong-Ahafo	Rainy	Female	32	9.2	Yes	No	B +	Neg	Neg	0	Neg	Neg
5585	Brong-Ahafo	Rainy	Female	72	12.3	No	No	B +	Neg	Neg	0	Neg	Neg

ID	Region	Season	Sex	Age	Hb			Blood					
5592	Brong-Ahafo	Rainy	Male	80	13.2	No	No	O +	Neg	Neg	0	Neg	Neg
5587	Brong-Ahafo	Rainy	Female	33	6.3	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
5	Brong-Ahafo	Rainy	Female	64	4.4	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
5598	Brong-Ahafo	Rainy	Male	72	4.2	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
5602	Brong-Ahafo	Rainy	Female	23	12.7	No	No	O +	Neg	Neg	0	Neg	Neg
5567	Brong-Ahafo	Rainy	Female	39	13.5	No	No	O +	Neg	Neg	0	Neg	Neg
5557	Brong-Ahafo	Rainy	Female	59	11	Yes	No	O +	Neg	(+)	940	Neg	Pos
5565	Brong-Ahafo	Rainy	Male	54	15.6	No	No	O +	Neg	Neg	0	Pos	Pos
5564	Brong-Ahafo	Rainy	Female	38	12.1	No	No	O -	Neg	Neg	0	Neg	Neg
5582	Brong-Ahafo	Rainy	Female	35	10.2	Yes	No	B +	Neg	Neg	0	Neg	Neg
5576	Brong-Ahafo	Rainy	Female	54	12.2	No	No	A +	Neg	Neg	0	Neg	Neg
5568	Brong-Ahafo	Rainy	Male	43	15.5	No	No	O +	Pos	Neg	0	Neg	Neg
5552	Brong-Ahafo	Rainy	Male	36	13.2	No	No	O +	Neg	Neg	0	Neg	Neg
5566	Brong-Ahafo	Rainy	Female	25	11.8	Yes	No	A +	Neg	Neg	0	Neg	Neg
5365	Brong-Ahafo	Rainy	Male	30	10.7	Yes	No	O +	Pos	Neg	0	Neg	Neg
5342	Brong-Ahafo	Rainy	Male	3	9.8	Yes	No	B -	Neg	Neg	0	Neg	Neg
5341	Brong-Ahafo	Rainy	Female	31	13.4	No	No	B +	Pos	Neg	0	Neg	Pos
5330	Brong-Ahafo	Rainy	Female	35	9.5	Yes	No	B +	Neg	Neg	0	Neg	Neg
5322	Brong-Ahafo	Rainy	Male	33	14	No	No	A +	Neg	Neg	0	Neg	Neg
5310	Brong-Ahafo	Rainy	Female	75	12.9	No	No	B +	Neg	Neg	0	Neg	Neg
5339	Brong-Ahafo	Rainy	Female	33	10.7	Yes	No	B +	Neg	Neg	0	Neg	Neg
5321	Brong-Ahafo	Rainy	Female	54	13	No	No	A +	Neg	Neg	0	Neg	Neg
5308	Brong-Ahafo	Rainy	Male	61	13.9	No	No	A -	Pos	Neg	0	Neg	Pos
6121	Brong-Ahafo	Rainy	Female	27	8.6	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
6122	Brong-Ahafo	Rainy	Female	3	11.6	No	No	O +	Neg	Neg	0	Neg	Neg
6124	Brong-Ahafo	Rainy	Male	26	16	No	No	B +	Pos	Neg	0	Neg	Neg
6129	Brong-Ahafo	Rainy	Female	48	12.9	No	No	A +	Neg	Neg	0	Neg	Neg
6130	Brong-Ahafo	Rainy	Female	26	8.8	Yes	Yes	O +	Pos	Neg	0	Neg	Neg
6131	Brong-Ahafo	Rainy	Female	26	11.6	Yes	No	O +	Neg	Neg	0	Pos	Pos
6132	Brong-Ahafo	Rainy	Male	15	12.7	No	No	O +	Neg	Neg	0	Neg	Neg

ID	Region	Season	Sex	Age	Hb			Blood					
6133	Brong-Ahafo	Rainy	Male	43	13.3	No	No	O +	Neg	Neg	0	Neg	Neg
6134	Brong-Ahafo	Rainy	Female	27	9	Yes	No	O -	Neg	Neg	0	Neg	Neg
6135	Brong-Ahafo	Rainy	Female	32	8.9	Yes	No	B +	Pos	Neg	0	Neg	Neg
6137	Brong-Ahafo	Rainy	Male	0.6	8.9	Yes	No	O +	Neg	(+)	830	Pos	Pos
6138	Brong-Ahafo	Rainy	Female	28	11.7	Yes	No	O +	Neg	Neg	0	Neg	Neg
6139	Brong-Ahafo	Rainy	Female	4	11.3	No	No	B +	Pos	Neg	0	Neg	Neg
6140	Brong-Ahafo	Rainy	Female	19	10.3	Yes	No	AB +	Neg	Neg	0	Pos	Pos
6141	Brong-Ahafo	Rainy	Female	42	12.4	No	No	O +	Neg	Neg	0	Neg	Neg
6142	Brong-Ahafo	Rainy	Female	71	12.5	No	No	O +	Neg	Neg	0	Neg	Neg
6143	Brong-Ahafo	Rainy	Male	27	14.4	No	No	O +	Pos	Neg	0	Pos	Pos
6144	Brong-Ahafo	Rainy	Male	52	5.8	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
6145	Brong-Ahafo	Rainy	Female	26	7.9	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
6146	Brong-Ahafo	Rainy	Male	66	12.8	No	No	A +	Neg	Neg	0	Neg	Neg
6147	Brong-Ahafo	Rainy	Female	3	9.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
6148	Brong-Ahafo	Rainy	Female	50	12.3	No	No	O +	Pos	Neg	0	Neg	Neg
6149	Brong-Ahafo	Rainy	Female	17	10.7	Yes	No	A +	Neg	Neg	0	Neg	Neg
6150	Brong-Ahafo	Rainy	Male	59	12.2	No	No	B +	Neg	Neg	0	Neg	Neg
6152	Brong-Ahafo	Rainy	Female	76	8.4	Yes	No	A -	Pos	Neg	0	Neg	Neg
6153	Brong-Ahafo	Rainy	Female	47	4.7	Yes	Yes	A +	Pos	Neg	0	Neg	Neg
6154	Brong-Ahafo	Rainy	Male	62	10.9	Yes	No	B +	Neg	Neg	0	Neg	Neg
6155	Brong-Ahafo	Rainy	Male	4	9.5	Yes	No	A +	Neg	Neg	0	Neg	Neg
6157	Brong-Ahafo	Rainy	Female	6	6.7	Yes	Yes	O +	Pos	Neg	0	Neg	Neg
6158	Brong-Ahafo	Rainy	Female	30	10.1	Yes	No	A +	Neg	Neg	0	Neg	Neg
6159	Brong-Ahafo	Rainy	Male	2	9.2	Yes	No	A +	Neg	Neg	0	Neg	Neg
6160	Brong-Ahafo	Rainy	Female	31	9.5	Yes	No	A +	Neg	Neg	0	Neg	Neg
6161	Brong-Ahafo	Rainy	Female	31	10.1	Yes	No	AB +	Pos	(+)	280	Pos	Pos
6162	Brong-Ahafo	Rainy	Male	78	9.8	Yes	No	B +	Neg	Neg	0	Neg	Neg
6163	Brong-Ahafo	Rainy	Female	65	12.4	No	No	O +	Neg	Neg	0	Neg	Neg
6164	Brong-Ahafo	Rainy	Male	0.6	9.7	Yes	No	A +	Neg	Neg	0	Pos	Neg
6165	Brong-Ahafo	Rainy	Female	4	11.9	No	No	A +	Neg	Neg	0	Neg	Neg

228

ID	Region	Season	Sex					Blood					
6167	Brong-Ahafo	Rainy	Female	10	12.1	No	No	O+	Neg	Neg	0	Neg	Neg
6168	Brong-Ahafo	Rainy	Female	55	12	No	No	O+	Pos	Neg	0	Neg	Neg
6169	Brong-Ahafo	Rainy	Female	27	10.9	Yes	No	O+	Neg	Neg	0	Neg	Neg
6170	Brong-Ahafo	Rainy	Female	36	12.7	No	No	O+	Neg	Neg	0	Neg	Neg
6171	Brong-Ahafo	Rainy	Female	43	11.4	Yes	No	O+	Neg	Neg	0	Pos	Neg
6172	Brong-Ahafo	Rainy	Male	0.6	6.4	Yes	Yes	O+	Neg	Neg	0	Neg	Neg
6173	Brong-Ahafo	Rainy	Male	28	10.9	Yes	No	O+	Neg	Neg	0	Neg	Neg
6174	Brong-Ahafo	Rainy	Male	4	10.7	Yes	No	O+	Pos	Neg	0	Neg	Neg
6175	Brong-Ahafo	Rainy	Male	45	11.3	Yes	No	O+	Neg	Neg	0	Neg	Neg
6176	Brong-Ahafo	Rainy	Female	0.3	9.9	Yes	Yes	O+	Neg	Neg	0	Neg	Neg
5538	Volta	Dry	Female	36	4.6	Yes	Yes	O+	Neg	Neg	0	Neg	Neg
5480	Volta	Dry	Male	9	10.4	Yes	No	AB+	Neg	(+)	890	Pos	Pos
5458	Volta	Dry	Female	50	14.5	Yes	No	B+	Pos	Neg	0	Neg	Neg
5529	Volta	Dry	Female	73	12.5	No	No	O+	Neg	(++)	2250	Pos	Pos
5459	Volta	Dry	Female	33	11.2	Yes	No	B-	Neg	Neg	0	Neg	Neg
5482	Volta	Dry	Female	31	11.5	Yes	No	O+	Neg	Neg	0	Neg	Neg
5440	Volta	Dry	Male	24	13.6	No	No	B-	Neg	(++)	1730	Pos	Pos
5531	Volta	Dry	Female	47	8.7	Yes	No	B+	Pos	Neg	0	Neg	Neg
5512	Volta	Dry	Female	35	5.1	Yes	Yes	B+	Neg	Neg	0	Neg	Pos
5454	Volta	Dry	Female	22	9.4	Yes	No	A-	Neg	Neg	0	Neg	Neg
5463	Volta	Dry	Female	50	11.9	Yes	No	B+	Neg	(++)	2380	Pos	Pos
5461	Volta	Dry	Female	63	13.4	No	No	O+	Pos	Neg	0	Neg	Neg
5486	Volta	Dry	Female	64	12.2	No	No	AB+	Neg	Neg	0	Neg	Neg
5487	Volta	Dry	Female	29	10.5	Yes	No	B+	Neg	Neg	0	Neg	Neg
5489	Volta	Dry	Female	21	8.3	Yes	No	AB-	Pos	Neg	0	Neg	Neg
5501	Volta	Dry	Male	27	11.4	Yes	No	O+	Neg	Neg	0	Neg	Neg
5490	Volta	Dry	Female	15	10.3	Yes	No	AB+	Neg	Neg	0	Neg	Neg
5520	Volta	Dry	Female	50	11.7	Yes	No	O+	Neg	Neg	0	Neg	Neg
5476	Volta	Dry	Male	1.2	11.4	No	No	O+	Neg	Neg	0	Neg	Neg
5530	Volta	Dry	Female	32	9.9	Yes	No	O+	Neg	Neg	0	Neg	Neg

229

5485	Volta	Dry	Male	28	10.8	Yes	No	B+	Neg	Neg	0	Neg	Neg
4	Volta	Dry	Female	52	10.7	Yes	No	O+	Neg	(+)	870	Pos	Pos
5534	Volta	Dry	Female	33	10.4	Yes	No	A+	Neg	(+)	1000	Pos	Pos
5513	Volta	Dry	Female	18	13.7	No	No	B+	Neg	Neg	0	Neg	Neg
5548	Volta	Dry	Female	2	11	No	No	B+	Neg	Neg	0	Neg	Neg
5546	Volta	Dry	Female	40	12.2	No	No	O-	Pos	Neg	0	Neg	Neg
5545	Volta	Dry	Female	29	12.3	No	No	AB-	Neg	(+)	740	Pos	Pos
5543	Volta	Dry	Female	21	8.8	Yes	No	A+	Neg	Neg	0	Neg	Neg
5547	Volta	Dry	Female	30	12.4	No	No	O+	Neg	(++)	1920	Pos	Pos
5549	Volta	Dry	Female	30	8.5	Yes	No	O+	Neg	(++)	2380	Pos	Pos
5495	Volta	Dry	Female	51	11.1	Yes	No	B+	Pos	Neg	0	Neg	Neg
5521	Volta	Dry	Female	73	8.9	Yes	No	O+	Neg	Neg	0	Neg	Neg
5610	Volta	Dry	Female	0.2	7.2	Yes	No	B+	Pos	Neg	0	Neg	Neg
5605	Volta	Dry	Male	80	12.8	No	No	A-	Pos	Neg	0	Neg	Neg
5604	Volta	Dry	Male	47	11	Yes	No	O+	Pos	Neg	0	Neg	Neg
5603	Volta	Dry	Male	38	7	Yes	Yes	O+	Neg	Neg	0	Neg	Neg
5613	Volta	Dry	Female	99	11.4	Yes	No	O+	Neg	Neg	0	Neg	Pos
5584	Volta	Dry	Female	64	12.2	No	No	A-	Neg	Neg	0	Neg	Neg
5550	Volta	Dry	Male	21	13.3	No	No	O+	Neg	Neg	0	Neg	Neg
5551	Volta	Dry	Male	79	13.2	No	No	O-	Neg	Neg	0	Neg	Neg
5579	Volta	Dry	Female	22	10.1	Yes	No	O+	Neg	(+)	500	Neg	Pos
5583	Volta	Dry	Female	32	9.2	Yes	No	B+	Neg	Neg	0	Neg	Neg
5585	Volta	Dry	Female	72	12.3	No	No	B+	Neg	Neg	0	Neg	Neg
5592	Volta	Dry	Male	80	13.2	No	No	O+	Neg	Neg	0	Neg	Neg
5587	Volta	Dry	Female	33	6.3	Yes	Yes	O+	Neg	Neg	0	Neg	Neg
5	Volta	Dry	Female	64	4.4	Yes	Yes	A+	Neg	Neg	0	Neg	Neg
5598	Volta	Dry	Male	72	4.2	Yes	Yes	O+	Neg	Neg	0	Neg	Neg
5602	Volta	Dry	Female	23	12.7	No	No	O+	Neg	Neg	0	Neg	Neg
5567	Volta	Dry	Female	39	13.5	No	No	O+	Neg	Neg	0	Neg	Neg
5557	Volta	Dry	Female	59	11	Yes	No	O+	Neg	(+)	990	Pos	Pos

230

5565	Volta	Dry	Male	54	15.6	No	No	O +	Neg	Neg	0	Neg	Neg
5564	Volta	Dry	Female	38	12.1	No	No	O -	Neg	Neg	0	Neg	Neg
5582	Volta	Dry	Female	35	10.2	Yes	No	B +	Neg	Neg	0	Neg	Neg
5576	Volta	Dry	Female	54	12.2	No	No	A +	Neg	Neg	0	Neg	Neg
5568	Volta	Dry	Male	43	15.5	No	No	O +	Pos	Neg	0	Neg	Neg
5552	Volta	Dry	Male	36	13.2	No	No	O +	Neg	Neg	0	Neg	Neg
5566	Volta	Dry	Female	25	11.8	Yes	No	A +	Neg	Neg	0	Neg	Neg
5365	Volta	Dry	Male	30	10.7	Yes	No	O +	Pos	Neg	0	Neg	Neg
5342	Volta	Dry	Male	3	9.8	Yes	No	B -	Neg	Neg	0	Neg	Neg
5341	Volta	Dry	Female	31	13.4	No	No	B +	Pos	Neg	0	Pos	Neg
5330	Volta	Dry	Female	35	9.5	Yes	No	B +	Neg	Neg	0	Neg	Neg
5322	Volta	Dry	Male	33	14	No	No	A +	Neg	Neg	0	Neg	Neg
5310	Volta	Dry	Female	75	12.9	No	No	B +	Neg	Neg	0	Neg	Neg
5339	Volta	Dry	Female	33	10.7	Yes	No	B +	Neg	Neg	0	Neg	Neg
5321	Volta	Dry	Female	54	13	No	No	A +	Neg	Neg	0	Neg	Neg
5308	Volta	Dry	Male	61	13.9	No	No	A -	Pos	Neg	0	Neg	Neg
5355	Volta	Dry	Female	33	6.8	Yes	Yes	O -	Neg	Neg	0	Neg	Neg
5361	Volta	Dry	Female	1.25	11.3	No	No	A +	Neg	Neg	0	Neg	Neg
5318	Volta	Dry	Female	35	10.9	Yes	No	A +	Neg	Neg	0	Neg	Neg
5315	Volta	Dry	Female	61	5.9	Yes	Yes	B +	Neg	Neg	0	Neg	Neg
5337	Volta	Dry	Female	73	11.3	Yes	No	A +	Neg	Neg	0	Neg	Neg
5323	Volta	Dry	Female	30	13	No	No	B +	Neg	Neg	0	Neg	Neg
5334	Volta	Dry	Female	42	9.5	Yes	No	O +	Neg	Neg	0	Neg	Neg
5346	Volta	Dry	Female	60	8.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
5313	Volta	Dry	Female	19	10.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
5340	Volta	Dry	Male	62	14.3	No	No	A -	Neg	Neg	0	Neg	Neg
5327	Volta	Dry	Female	42	12.9	No	No	A +	Neg	Neg	0	Neg	Neg
5335	Volta	Dry	Female	33	7.4	Yes	Yes	O -	Neg	Neg	0	Neg	Neg
5325	Volta	Dry	Female	21	12.3	No	No	A +	Neg	Neg	0	Neg	Neg
5343	Volta	Dry	Female	31	11.4	Yes	No	B +	Neg	(+)	210	Pos	Pos

231

ID	Region	Season	Gender	Age	Value	Q1	Q2	Blood	R1	R2	Value2	R3	R4
5368	Volta	Dry	Female	8	10.9	Yes	No	B +	Neg	Neg	0	Neg	Neg
5347	Volta	Dry	Female	38	12.5	No	No	O +	Neg	Neg	0	Neg	Neg
5374	Volta	Dry	Female	65	8.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
5172	Volta	Dry	Male	27	14	No	No	O +	Neg	(+)	580	Pos	Pos
5171	Volta	Dry	Male	54	5.3	Yes	Yes	B +	Pos	Neg	0	Neg	Neg
5170	Volta	Dry	Male	28	16.1	No	No	A +	Pos	Neg	0	Neg	Neg
5181	Volta	Dry	Male	5	5.8	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
5169	Volta	Dry	Female	30	11.5	Yes	No	O +	Neg	(+)	620	Pos	Pos
5226	Volta	Dry	Female	31	12.8	No	No	O -	Neg	Neg	0	Neg	Neg
5212	Volta	Dry	Female	29	12.4	No	No	B +	Neg	Neg	0	Neg	Neg
5204	Volta	Dry	Female	37	9	Yes	No	B +	Neg	Neg	0	Neg	Neg
5201	Volta	Dry	Female	34	8.8	Yes	No	A +	Neg	Neg	0	Neg	Pos
5225	Volta	Dry	Female	38	12.3	No	No	A +	Neg	Neg	0	Neg	Neg
5185	Volta	Dry	Female	25	11.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
5194	Volta	Dry	Female	31	8.7	Yes	No	B +	Neg	Neg	0	Neg	Neg
5238	Volta	Dry	Female	36	12.6	No	No	B +	Neg	Neg	0	Neg	Neg
5221	Volta	Dry	Female	27	10.4	Yes	No	A -	Neg	Neg	0	Neg	Neg
5235	Volta	Dry	Female	18	12.7	No	No	A -	Pos	Neg	0	Neg	Neg
5313	Volta	Dry	Female	19	10.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
5214	Volta	Dry	Male	43	15.6	No	No	A +	Neg	Neg	0	Neg	Neg
5208	Volta	Dry	Male	38	12.4	No	No	B +	Neg	Neg	0	Neg	Neg
5223	Volta	Dry	Female	40	6	Yes	Yes	AB +	Neg	Neg	0	Neg	Pos
5189	Volta	Dry	Female	27	12.9	No	No	O +	Neg	Neg	0	Neg	Neg
5190	Volta	Dry	Female	46	13.5	No	No	A +	Neg	Neg	0	Neg	Neg
5224	Volta	Dry	Female	38	10.2	Yes	No	B +	Pos	Neg	0	Neg	Neg
5210	Volta	Dry	Female	30	12.3	No	No	O -	Neg	Neg	0	Neg	Neg
165	Central	Dry	Female	56	13.4	No	No	O +	Neg	Neg	0	Neg	Neg
167	Central	Dry	Male	64	15.6	No	No	O +	Neg	Neg	0	Neg	Neg
168	Central	Dry	Female	63	13.4	No	No	O +	Neg	Neg	0	Neg	Neg
169	Central	Dry	Female	28	10.4	Yes	No	A +	Neg	Neg	0	Neg	Neg

232

ID	Region	Season	Gender	Age	Hb			Blood						
170	Central	Dry	Female	29	11.3	Yes	No	B+	Neg	Neg	Neg	0	Neg	Neg
171	Central	Dry	Male	50	13.9	No	No	O+	Neg	Neg	Neg	0	Neg	Neg
172	Central	Dry	Female	41	11.2	Yes	No	O-	Neg	Neg	Neg	0	Neg	Neg
175	Central	Dry	Female	41	12.1	No	No	A+	Neg	Neg	Neg	0	Neg	Neg
176	Central	Dry	Male	20	13.9	No	No	B+	Neg	Neg	Neg	0	Neg	Neg
177	Central	Dry	Female	32	11.9	Yes	No	O+	Neg	Neg	Neg	0	Neg	Neg
178	Central	Dry	Female	47	13.2	No	No	B-	Neg	Neg	Neg	0	Neg	Neg
180	Central	Dry	Male	12	12.6	No	No	A+	Neg	Neg	Neg	0	Neg	Neg
181	Central	Dry	Female	28	11.4	Yes	No	AB+	Neg	Neg	Neg	0	Neg	Neg
182	Central	Dry	Female	47	7.5	Yes	Yes	B-	Neg	Neg	Neg	0	Neg	Neg
185	Central	Dry	Female	64	12.2	No	No	A+	Neg	Neg	Neg	0	Neg	Neg
186	Central	Dry	Female	48	10.7	Yes	No	O-	Neg	Neg	Neg	0	Neg	Neg
187	Central	Dry	Female	64	10.3	Yes	No	O-	Neg	Neg	Neg	0	Neg	Neg
188	Central	Dry	Female	32	7.7	Yes	Yes	O+	Neg	Neg	Neg	0	Neg	Neg
189	Central	Dry	Female	26	10.2	Yes	No	O+	Neg	Neg	Neg	0	Neg	Neg
190	Central	Dry	Female	49	6	Yes	Yes	O+	Pos	Neg	Neg	0	Neg	Neg
191	Central	Dry	Male	44	10.3	Yes	No	O+	Neg	Neg	Neg	0	Neg	Neg
196	Central	Dry	Female	33	10.7	Yes	No	B+	Neg	Neg	Neg	0	Neg	Neg
199	Central	Dry	Male	4	12.6	No	No	O+	Neg	Neg	Neg	0	Neg	Neg
200	Central	Dry	Male	2	11.8	No	No	B+	Neg	Neg	Neg	0	Neg	Neg
201	Central	Dry	Male	39	10.1	Yes	No	O+	Neg	Neg	Neg	0	Neg	Neg
202	Central	Dry	Female	0.8	9.8	Yes	No	A+	Neg	Neg	Neg	0	Neg	Neg
203	Central	Dry	Female	53	9.7	Yes	Yes	B+	Neg	Neg	Neg	0	Neg	Neg
205	Central	Dry	Female	74	10.8	Yes	No	O+	Neg	Neg	Neg	0	Neg	Pos
207	Central	Dry	Male	0.3	11	Yes	No	B+	Neg	Neg	Neg	0	Neg	Pos
209	Central	Dry	Female	13	9.8	Yes	No	B+	Neg	Neg	Neg	0	Neg	Neg
211	Central	Dry	Female	26	9.1	Yes	No	O+	Neg	Neg	Neg	0	Neg	Neg
212	Central	Dry	Female	29	6.1	Yes	Yes	B+	Neg	Neg	Neg	0	Neg	Neg
213	Central	Dry	Male	40	8.9	Yes	No	B+	Neg	Neg	Neg	0	Neg	Neg
218	Central	Dry	Female	49	7.1	Yes	Yes	O+	Neg	Neg	Neg	0	Neg	Neg

219	Central	Dry	Female	8	8.2	Yes	No	B+	Pos	Neg	0	Neg	Neg
221	Central	Dry	Female	36	11.6	Yes	No	O+	Neg	Neg	0	Neg	Neg
225	Central	Dry	Female	21	12.2	No	No	O+	Neg	Neg	0	Neg	Neg
226	Central	Dry	Female	35	12.1	No	No	A+	Neg	Neg	0	Neg	Neg
227	Central	Dry	Female	31	10.3	Yes	No	O-	Pos	Neg	0	Neg	Neg
229	Central	Dry	Female	33	12	No	No	O+	Neg	Neg	0	Neg	Neg
230	Central	Dry	Male	35	6.7	Yes	Yes	A+	Pos	Neg	0	Neg	Neg
231	Central	Dry	Female	0.1	10.5	Yes	No	B+	Neg	Neg	0	Neg	Neg
232	Central	Dry	Male	16	8.3	Yes	No	O+	Pos	Neg	0	Neg	Neg
233	Central	Dry	Female	29	13.5	No	No	O-	Neg	Neg	0	Neg	Neg
234	Central	Dry	Female	30	11.9	Yes	No	A+	Neg	Neg	0	Neg	Neg
235	Central	Dry	Male	1.5	7.5	Yes	No	AB+	Neg	Neg	0	Neg	Neg
237	Central	Dry	Female	39	11.4	Yes	No	AB+	Neg	(+)	780	Pos	Pos
239	Central	Dry	Female	24	10.4	Yes	No	O+	Neg	Neg	0	Neg	Neg
240	Central	Dry	Female	37	11.2	Yes	No	B+	Pos	Neg	0	Neg	Neg
241	Central	Dry	Female	17	9.9	Yes	No	A+	Neg	Neg	0	Pos	Neg
242	Central	Dry	Male	28	14.1	No	No	B+	Pos	Neg	0	Neg	Neg
243	Central	Dry	Female	56	9.6	Yes	No	A+	Neg	(+)	1000	Pos	Pos
244	Central	Dry	Female	64	8.2	Yes	No	O+	Neg	Neg	0	Neg	Neg
250	Central	Dry	Female	63	9.8	Yes	No	O+	Neg	Neg	0	Neg	Neg
251	Central	Dry	Female	28	9.6	Yes	No	O+	Pos	Neg	0	Pos	Neg
252	Central	Dry	Female	31	10.5	Yes	No	O+	Neg	Neg	0	Neg	Neg
254	Central	Dry	Female	31	11	Yes	No	B+	Neg	Neg	0	Neg	Neg
255	Central	Dry	Male	33	14.3	No	No	AB+	Neg	Neg	0	Neg	Neg
256	Central	Dry	Female	35	8.6	Yes	No	AB+	Neg	Neg	0	Pos	Neg
257	Central	Dry	Male	45	10.9	Yes	No	AB+	Pos	Neg	0	Neg	Neg
260	Central	Dry	Female	29	9.6	Yes	No	O+	Neg	Neg	0	Neg	Neg
261	Central	Dry	Female	38	11.3	Yes	No	O+	Neg	Neg	0	Neg	Neg
263	Central	Dry	Female	9	9.8	Yes	No	A+	Neg	Neg	0	Neg	Neg
266	Central	Dry	Female	20	8.9	Yes	No	A+	Neg	(+)	990	Pos	Pos

234

267	Central	Dry	Female	18	12.8	No	No	B+	Neg	Neg	0	Neg	Neg
268	Central	Dry	Female	28	12.6	No	No	O+	Neg	Neg	0	Neg	Neg
269	Central	Dry	Male	0.5	9.3	Yes	No	O+	Pos	(++)	2260	Pos	Pos
270	Central	Dry	Female	32	10	Yes	No	A+	Neg	Neg	0	Neg	Neg
271	Central	Dry	Male	54	14.5	No	No	B+	Neg	Neg	0	Neg	Neg
272	Central	Dry	Female	71	12.8	No	No	A+	Neg	Neg	0	Pos	Pos
273	Central	Dry	Female	38	11.4	Yes	No	O+	Pos	Neg	0	Neg	Neg
274	Central	Dry	Male	56	12.9	No	No	O+	Neg	Neg	0	Neg	Neg
275	Central	Dry	Female	33	11	Yes	No	A+	Neg	Neg	0	Neg	Neg
277	Central	Dry	Female	48	12.4	No	No	O+	Neg	Neg	0	Neg	Neg
280	Central	Dry	Female	48	13	No	No	A+	Neg	Neg	0	Neg	Neg
283	Central	Dry	Male	27	9.3	Yes	No	B+	Neg	Neg	0	Neg	Neg
286	Central	Dry	Female	78	11.7	Yes	No	O+	Neg	Neg	0	Neg	Neg
290	Central	Dry	Male	0.8	10.1	Yes	No	O+	Neg	Neg	0	Neg	Neg
292	Central	Dry	Female	37	11.3	Yes	No	O+	Neg	Neg	0	Neg	Neg
294	Central	Dry	Male	79	11.9	Yes	No	B+	Neg	Neg	0	Neg	Neg
295	Central	Dry	Female	25	12.2	No	No	O+	Neg	Neg	0	Neg	Neg
298	Central	Dry	Male	31	12.7	No	No	O+	Neg	Neg	0	Neg	Neg
299	Central	Dry	Female	22	5.1	Yes	Yes	O+	Neg	(+)	850	Pos	Pos
300	Central	Dry	Female	31	9.6	Yes	No	A+	Neg	Neg	0	Neg	Neg
302	Central	Dry	Male	33	13.9	No	No	O+	Neg	Neg	0	Neg	Neg
304	Central	Dry	Female	29	11.3	Yes	No	O-	Neg	Neg	0	Neg	Neg
305	Central	Dry	Male	60	8.1	Yes	No	O+	Neg		0	Neg	Neg
311	Central	Dry	Female	42	13.3	No	No	B+	Neg		0	Neg	Neg
312	Central	Dry	Female	28	11.7	Yes	No	O+	Neg		0	Neg	Neg
315	Central	Dry	Female	10	11.5	Yes	No	O+	Pos		0	Neg	Neg
316	Central	Dry	Female	29	8.8	Yes	No	A+	Pos		0	Neg	Neg
317	Central	Dry	Female	27	10.8	Yes	No	O+	Neg		0	Neg	Neg
318	Central	Dry	Female	8	11.8	No	No	B+	Neg		0	Neg	Neg
319	Central	Dry	Male	7	9.9	Yes	No	A+	Neg	Neg	0	Neg	Neg

235

ID	Region	Season	Sex	Age	Value	Q1	Q2	Blood	C1	C2	Num	R1	R2
326	Central	Dry	Female	21	7.7	Yes	Yes	A +	Neg	Neg	0	Pos	Pos
327	Central	Dry	Female	53	5.8	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
328	Central	Dry	Male	102	13.5	No	No	A +	Neg	Neg	0	Neg	Neg
336	Central	Dry	Female	30	11.7	Yes	No	O +	Pos	Neg	0	Neg	Neg
338	Central	Dry	Male	25	11.3	Yes	No	B +	Neg	Neg	0	Neg	Neg
339	Central	Dry	Male	26	11.6	Yes	No	O +	Pos	Neg	0	Neg	Neg
100	Greater Accra	Dry	Male	28	12.6	No	No	O +	Neg	Neg	0	Neg	Neg
101	Greater Accra	Dry	Female	47	10.5	Yes	No	B +	Neg	Neg	0	Neg	Neg
102	Greater Accra	Dry	Male	12	15.3	No	No	A +	Pos	Neg	0	Neg	Neg
103	Greater Accra	Dry	Female	28	14.3	No	No	A -	Neg	Neg	0	Neg	Neg
104	Greater Accra	Dry	Male	47	14.6	No	No	A +	Neg	Neg	0	Neg	Neg
105	Greater Accra	Dry	Female	64	12.7	No	No	A -	Neg	Neg	0	Neg	Neg
106	Greater Accra	Dry	Female	35	10.1	Yes	No	A +	Neg	(+)	820	Pos	Pos
107	Greater Accra	Dry	Female	31	12.7	No	No	O +	Neg	Neg	0	Neg	Neg
108	Greater Accra	Dry	Male	3	13	No	No	AB +	Neg	Neg	0	Neg	Neg
109	Greater Accra	Dry	Male	50	14.5	No	No	O +	Neg	Neg	0	Neg	Neg
110	Greater Accra	Dry	Female	73	13	No	No	B +	Neg	Neg	0	Neg	Neg
111	Greater Accra	Dry	Male	33	6.8	Yes	Yes	O +	Neg	(+)	960	Pos	Pos
112	Greater Accra	Dry	Male	31	15	No	No	O +	Neg	Neg	0	Neg	Neg
113	Greater Accra	Dry	Female	24	12.9	No	No	B -	Neg	Neg	0	Neg	Neg
114	Greater Accra	Dry	Female	47	16.5	No	No	A +	Neg	Neg	0	Pos	Pos
115	Greater Accra	Dry	Female	35	12.4	No	No	O +	Neg	Neg	0	Neg	Neg
116	Greater Accra	Dry	Male	22	16	No	No	B +	Neg	Neg	0	Neg	Neg
117	Greater Accra	Dry	Female	27	11.8	Yes	No	O +	Neg	Neg	0	Neg	Neg
118	Greater Accra	Dry	Female	63	13.3	No	No	B +	Neg	Neg	0	Neg	Neg
119	Greater Accra	Dry	Male	64	18.1	No	No	O +	Neg	Neg	0	Neg	Neg
120	Greater Accra	Dry	Female	66	8.9	Yes	No	A +	Pos	Neg	0	Neg	Neg
121	Greater Accra	Dry	Male	3	15.1	No	No	A +	Neg	(+)	920	Pos	Pos
122	Greater Accra	Dry	Female	50	11.2	Yes	No	AB +	Pos	Neg	0	Neg	Neg
123	Greater Accra	Dry	Female	17	12.6	No	No	O +	Neg	Neg	0	Neg	Neg

236

124	Greater Accra	Dry	Male	59	14.5	No	No	O +	Neg	Neg	0	Neg	Neg
125	Greater Accra	Dry	Female	76	13.6	No	No	O +	Pos	Neg	0	Neg	Neg
126	Greater Accra	Dry	Male	47	10.2	Yes	No	AB +	Neg	Neg	0	Neg	Neg
127	Greater Accra	Dry	Male	62	13.5	No	No	A +	Pos	Neg	0	Neg	Neg
128	Greater Accra	Dry	Male	4	16	No	No	B -	Neg	(+)	120	Neg	Neg
129	Greater Accra	Dry	Male	6	14.5	No	No	B +	Neg	(+)	610	Pos	Pos
130	Greater Accra	Dry	Female	30	16.1	No	No	B +	Neg	Neg		Neg	Neg
131	Greater Accra	Dry	Female	2	6.9	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
132	Greater Accra	Dry	Male	31	15	No	No	B +	Pos	Neg	0	Neg	Neg
133	Greater Accra	Dry	Female	31	13.6	No	No	B +	Neg	Neg	0	Neg	Neg
134	Greater Accra	Dry	Male	78	15.5	No	No	O +	Pos	Neg	0	Neg	Neg
135	Greater Accra	Dry	Female	65	9.2	Yes	No	AB +	Neg	Neg	0	Neg	Neg
136	Greater Accra	Dry	Male	7	13.9	No	No	O +	Neg	(++)	2320	Pos	Pos
137	Greater Accra	Dry	Male	4	12.4	No	No	B +	Pos	Neg	0	Neg	Neg
138	Greater Accra	Dry	Female	39	12.1	No	No	O +	Pos	Neg	0	Neg	Neg
139	Greater Accra	Dry	Male	10	11.8	Yes	No	O +	Neg	Neg	0	Neg	Neg
140	Greater Accra	Dry	Male	53	14.8	No	No	O +	Neg	Neg	0	Neg	Neg
141	Greater Accra	Dry	Female	74	14.3	No	No	B +	Neg	Neg	0	Neg	Neg
142	Greater Accra	Dry	Female	18	13.7	No	No	O +	Pos	Neg	0	Neg	Neg
143	Greater Accra	Dry	Female	36	7.9	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
144	Greater Accra	Dry	Male	26	9.8	Yes	No	B +	Pos	Neg	0	Neg	Neg
145	Greater Accra	Dry	Female	29	12.2	No	No	B +	Pos	Neg	0	Neg	Neg
146	Greater Accra	Dry	Male	40	13.1	No	No	O +	Pos	Neg	0	Neg	Neg
147	Greater Accra	Dry	Male	49	12.3	No	No	O +	Neg	Neg	0	Neg	Neg
148	Greater Accra	Dry	Male	8	13.1	No	No	B +	Neg	Neg	0	Neg	Neg
149	Greater Accra	Dry	Female	30	11.1	Yes	No	O +	Neg	Neg	0	Neg	Neg
150	Greater Accra	Dry	Female	29	13.8	No	No	B +	Neg	Neg	0	Neg	Neg
151	Greater Accra	Dry	Female	35	10.3	Yes	No	B +	Pos	Neg	0	Neg	Neg
152	Greater Accra	Dry	Female	31	11.6	Yes	No	B +	Neg	Neg	0	Neg	Neg
153	Greater Accra	Dry	Female	33	9.2	Yes	No	AB +	Neg	Neg	0	Neg	Neg

237

154	Greater Accra	Dry	Male	35	13.7	No	No	O +	Neg	Neg	0	Neg	Neg
155	Greater Accra	Dry	Male	7	17.8	No	No	O +	Pos	Neg	0	Neg	Neg
156	Greater Accra	Dry	Male	16	13.4	No	No	O +	Neg	Neg	0	Neg	Neg
157	Greater Accra	Dry	Male	29	14.4	No	No	O +	Neg	Neg	0	Neg	Neg
158	Greater Accra	Dry	Male	30	13.5	No	No	O +	Neg	Neg	0	Neg	Neg
159	Greater Accra	Dry	Male	74	15.8	No	No	B -	Neg	Neg	0	Neg	Neg
160	Greater Accra	Dry	Male	18	13.8	No	No	O +	Neg	Neg	0	Neg	Neg
161	Greater Accra	Dry	Male	36	15.1	No	No	A +	Neg	Neg	0	Neg	Neg
162	Greater Accra	Dry	Male	26	16.3	No	No	B +	Neg	Neg	0	Neg	Neg
163	Greater Accra	Dry	Female	29	11.9	Yes	No	O +	Neg	(+)	210	Neg	Neg
164	Greater Accra	Dry	Female	40	13.5	No	No	O +	Neg	Neg	0	Neg	Neg
165	Greater Accra	Dry	Female	49	6.8	Yes	Yes	B +	Neg	Neg	0	Neg	Neg
166	Greater Accra	Dry	Female	8	13.3	No	No	O +	Neg	Neg	0	Neg	Neg
167	Greater Accra	Dry	Female	36	1.3	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
168	Greater Accra	Dry	Male	29	17	No	No	B +	Neg	Neg	0	Neg	Neg
169	Greater Accra	Dry	Male	35	14.1	No	No	O +	Neg	Neg	0	Neg	Pos
170	Greater Accra	Dry	Male	31	12.7	No	No	B +	Pos	Neg	0	Neg	Neg
171	Greater Accra	Dry	Female	33	11	Yes	No	A +	Neg	Neg	0	Neg	Neg
172	Greater Accra	Dry	Female	35	13.6	No	No	O +	Neg	Neg	0	Neg	Neg
173	Greater Accra	Dry	Male	7	13.2	No	No	O +	Neg	(+)	0	Neg	Neg
174	Greater Accra	Dry	Male	16	10.2	Yes	Yes	A -	Pos	Neg	0	Neg	Neg
175	Greater Accra	Dry	Female	29	13.3	No	No	B +	Neg	Neg	0	Neg	Neg
176	Greater Accra	Dry	Male	31	15.8	No	No	B +	Neg	(++)	1290	Pos	Pos
177	Greater Accra	Dry	Female	78	11.8	Yes	No	O +	Pos	Neg	0	Neg	Neg
178	Greater Accra	Dry	Female	65	11.9	Yes	No	O +	Neg	(+)	80	Neg	Neg
179	Greater Accra	Dry	Male	17	3.3	Yes	Yes	A -	Neg	Neg	0	Neg	Neg
180	Greater Accra	Dry	Female	24	5	Yes	Yes	O +	Pos	Neg	0	Neg	Neg
181	Greater Accra	Dry	Female	49	12.5	No	No	O +	Neg	Neg	0	Neg	Neg
182	Greater Accra	Dry	Male	10	13.4	No	No	O +	Neg	Neg	0	Neg	Neg
183	Greater Accra	Dry	Male	53	10.6	Yes	No	O +	Neg	Neg	0	Neg	Neg

238

184	Greater Accra	Dry	Female	74	12	No	No	O +	Neg	Neg	0	Neg	Neg
185	Greater Accra	Dry	Male	18	6.6	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
186	Greater Accra	Dry	Male	36	16.9	No	No	B +	Neg	Neg	0	Neg	Neg
187	Greater Accra	Dry	Male	26	13.2	No	No	O +	Neg	Neg	0	Neg	Neg
188	Greater Accra	Dry	Male	19	14.6	No	No	A +	Neg	Neg	0	Neg	Neg
189	Greater Accra	Dry	Male	40	13	No	No	A +	Neg	Neg	0	Neg	Neg
190	Greater Accra	Dry	Male	38	16.7	No	No	O -	Neg	Neg	0	Neg	Neg
191	Greater Accra	Dry	Female	40	13.3	No	No	A -	Neg	(+)	460	Neg	Pos
192	Greater Accra	Dry	Male	27	12.9	No	No	B +	Neg	Neg	0	Neg	Neg
193	Greater Accra	Dry	Male	46	15.4	No	No	O +	Neg	Neg	0	Neg	Neg
194	Greater Accra	Dry	Female	38	12.2	No	No	A +	Neg	Neg	0	Neg	Neg
195	Greater Accra	Dry	Female	30	14.8	No	No	A +	Neg	Neg	0	Neg	Neg
196	Greater Accra	Dry	Male	22	10.3	Yes	No	A +	Neg	Neg	0	Neg	Neg
197	Greater Accra	Dry	Male	36	16.6	No	No	B -	Neg	Neg	0	Neg	Neg
1	Western	Dry	Female	51	10.8	Yes	No	B -	Neg	Neg	0	Neg	Neg
2	Western	Dry	Male	34	13.7	No	No	O +	Neg	Neg	0	Neg	Neg
4	Western	Dry	Male	68	13	Yes	No	B -	Neg	Neg	0	Neg	Neg
5	Western	Dry	Female	55	11.5	Yes	No	A +	Neg	(+)	900	Pos	Pos
7	Western	Dry	Female	19	13.6	No	No	A +	Neg	Neg	0	Neg	Neg
8	Western	Dry	Female	35	11.4	Yes	No	B +	Neg	Neg	0	Neg	Neg
10	Western	Dry	Female	5	12.6	No	No	O +	Neg	Neg	0	Neg	Neg
12	Western	Dry	Female	21	16.1	No	No	O +	Neg	Neg	0	Neg	Neg
13	Western	Dry	Female	45	11.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
14	Western	Dry	Female	46	12.9	No	No	O +	Neg	Neg	0	Neg	Neg
15	Western	Dry	Female	14	9.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
16	Western	Dry	Female	26	11.3	Yes	No	O -	Pos	Neg	0	Neg	Neg
17	Western	Dry	Male	89	11.3	Yes	No	O -	Neg	Neg	0	Neg	Neg
18	Western	Dry	Female	26	9.9	Yes	No	B +	Neg	Neg	0	Neg	Neg
19	Western	Dry	Female	34	11.1	Yes	No	B +	Neg	Neg	0	Neg	Neg
22	Western	Dry	Male	0.7	9.5	Yes	No	O +	Neg	(+)	490	Neg	Pos

239

23	Western	Dry	Male	56	11.6	Yes	No	B +	Neg	Neg	0	Neg	Neg
24	Western	Dry	Male	27	13.4	No	No	A +	Neg	Neg	0	Neg	Neg
25	Western	Dry	Female	1	10.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
26	Western	Dry	Female	24	12.6	No	No	O +	Neg	Neg	0	Neg	Neg
29	Western	Dry	Female	36	10.3	Yes	No	O +	Neg	Neg	0	Neg	Neg
30	Western	Dry	Female	53	11.4	Yes	No	A -	Neg	Neg	0	Neg	Neg
33	Western	Dry	Male	0.8	11.3	Yes	No	B -	Neg	Neg	0	Neg	Neg
34	Western	Dry	Female	1.5	13.1	No	No	B +	Neg	Neg	0	Neg	Pos
39	Western	Dry	Female	26	11.6	Yes	No	B +	Neg	Neg	0	Neg	Neg
41	Western	Dry	Female	89	13.2	No	No	B +	Pos	Neg	0	Neg	Neg
42	Western	Dry	Male	26	14.3	No	No	B +	Neg	Neg	0	Neg	Neg
43	Western	Dry	Female	34	12.3	No	No	O +	Neg	Neg	0	Neg	Neg
47	Western	Dry	Male	27	12.8	No	No	O +	Pos	Neg	0	Neg	Neg
49	Western	Dry	Female	60	11.9	Yes	No	O +	Pos	Neg	0	Neg	Neg
50	Western	Dry	Male	37	11.7	Yes	No	O +	Neg	Neg	0	Neg	Neg
51	Western	Dry	Male	79	12.3	No	No	A +	Pos	Neg	0	Neg	Neg
52	Western	Dry	Male	25	11.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
53	Western	Dry	Male	31	11.4	Yes	No	O +	Neg	Neg	0	Neg	Neg
54	Western	Dry	Male	22	11.5	Yes	No	B +	Neg	Neg	0	Neg	Neg
55	Western	Dry	Male	31	12.6	No	No	O +	Neg	Neg	0	Neg	Neg
56	Western	Dry	Male	33	8.4	Yes	No	B +	Neg	Neg	0	Neg	Neg
57	Western	Dry	Female	29	11.9	Yes	No	B +	Neg	Neg	0	Neg	Neg
58	Western	Dry	Female	38	9.6	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
59	Western	Dry	Female	42	7.4	Yes	Yes	B +	Neg	Neg	0	Neg	Neg
60	Western	Dry	Male	5	6.7	Yes	Yes	O +	Pos	Neg	0	Neg	Neg
61	Western	Dry	Female	9	6.9	Yes	Yes	O +	Neg	Neg	0	Pos	Pos
63	Western	Dry	Female	50	8.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
64	Western	Dry	Male	73	9	Yes	No	O +	Pos	(+)	730	Pos	Pos
65	Western	Dry	Female	33	8.5	Yes	No	A +	Pos	Neg	0	Neg	Neg
67	Western	Dry	Female	31	11.1	Yes	No	B +	Neg	Neg	0	Neg	Neg

240

68	Western	Dry	Male	24	10.2	Yes	No	O +	Neg	Neg	0	Neg	Pos
69	Western	Dry	Female	47	9.7	Yes	No	A +	Neg	Neg	0	Neg	Neg
70	Western	Dry	Male	45	9.3	Yes	No	B +	Neg	Neg	0	Neg	Neg
71	Western	Dry	Female	37	5.4	Yes	Yes	AB +	Neg	Neg	0	Neg	Neg
74	Western	Dry	Female	26	9.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
76	Western	Dry	Female	29	11.4	Yes	No	O +	Neg	Neg	0	Neg	Neg
79	Western	Dry	Female	35	10.2	Yes	No	AB +	Neg	Neg	0	Neg	Neg
101	Western	Dry	Male	76	12.8	No	No	B +	Neg	Neg	0	Neg	Neg
102	Western	Dry	Male	61	14.5	No	No	O +	Neg	Neg	0	Neg	Neg
105	Western	Dry	Female	70	11.4	Yes	No	O -	Neg	Neg	0	Neg	Neg
106	Western	Dry	Female	43	10.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
108	Western	Dry	Female	75	9.8	Yes	No	O +	Neg	Neg	0	Neg	Neg
109	Western	Dry	Male	70	13.4	No	No	A +	Neg	Neg	0	Neg	Neg
113	Western	Dry	Female	8	9.9	Yes	No	A +	Neg	Neg	0	Neg	Neg
114	Western	Dry	Male	17	10.9	Yes	No	A +	Neg	Neg	0	Neg	Neg
115	Western	Dry	Female	30	12.3	No	No	A +	Neg	Neg	0	Neg	Neg
117	Western	Dry	Male	50	8.5	Yes	No	O +	Neg	Neg	0	Neg	Neg
118	Western	Dry	Female	41	10.3	Yes	No	O +	Neg	Neg	0	Pos	Pos
119	Western	Dry	Female	51	11.2	Yes	No	A +	Neg	Neg	0	Neg	Neg
120	Western	Dry	Male	32	14	No	No	O +	Pos	Neg	0	Neg	Neg
121	Western	Dry	Female	27	8.6	Yes	No	O +	Neg	Neg	0	Neg	Neg
122	Western	Dry	Female	3	11.6	No	No	O +	Neg	Neg	0	Neg	Neg
124	Western	Dry	Male	26	16	No	No	B +	Pos	Neg	0	Neg	Neg
129	Western	Dry	Female	48	12.9	No	No	A +	Neg	Neg	0	Neg	Neg
130	Western	Dry	Female	26	8.8	Yes	No	O +	Pos	Neg	0	Neg	Neg
131	Western	Dry	Female	26	11.6	Yes	No	O +	Neg	Neg	0	Neg	Neg
132	Western	Dry	Male	15	12.7	No	No	O +	Neg	Neg	0	Neg	Neg
133	Western	Dry	Male	43	13.3	No	No	O +	Neg	Neg	0	Neg	Neg
134	Western	Dry	Female	27	9	Yes	No	O -	Neg	Neg	0	Neg	Neg
135	Western	Dry	Female	32	8.9	Yes	No	B +	Pos	Neg	0	Neg	Neg

241

ID													
137	Western	Dry	Male	0.6	8.9	Yes	No	O+	Neg	Neg	0	Pos	Pos
138	Western	Dry	Female	28	11.7	Yes	No	O+	Neg	Neg	0	Neg	Pos
139	Western	Dry	Female	4	11.3	No	No	B+	Pos	Neg	0	Neg	Neg
140	Western	Dry	Female	19	10.3	Yes	No	AB+	Neg	Neg	0	Neg	Neg
141	Western	Dry	Female	42	12.4	No	No	O+	Neg	Neg	0	Neg	Neg
142	Western	Dry	Female	71	12.5	No	No	O+	Neg	Neg	0	Neg	Neg
143	Western	Dry	Male	27	14.4	No	No	O+	Pos	Neg	0	Neg	Neg
144	Western	Dry	Male	52	5.8	Yes	Yes	A+	Neg	Neg	0	Neg	Neg
145	Western	Dry	Female	26	7.9	Yes	Yes	A+	Neg	Neg	0	Neg	Neg
146	Western	Dry	Male	66	12.8	No	No	A+	Neg	Neg	0	Neg	Neg
147	Western	Dry	Female	3	9.9	Yes	No	O+	Neg	Neg	0	Neg	Neg
148	Western	Dry	Female	50	12.3	No	No	O+	Pos	Neg	0	Neg	Neg
149	Western	Dry	Female	17	10.7	Yes	No	A+	Neg	Neg	0	Neg	Neg
150	Western	Dry	Male	59	12.2	No	No	B+	Neg	Neg	0	Pos	Pos
152	Western	Dry	Female	76	8.4	Yes	No	A-	Pos	Neg	0	Neg	Neg
153	Western	Dry	Female	47	4.7	Yes	Yes	A+	Pos	Neg	0	Neg	Neg
154	Western	Dry	Male	62	10.9	Yes	No	B+	Neg	Neg	0	Neg	Neg
155	Western	Dry	Male	4	9.5	Yes	No	A+	Neg	Neg	0	Neg	Neg
157	Western	Dry	Female	6	6.7	Yes	Yes	O+	Pos	Neg	0	Neg	Neg
158	Western	Dry	Female	30	10.1	Yes	No	A+	Neg	Neg	0	Neg	Neg
159	Western	Dry	Male	2	9.2	Yes	No	A+	Neg	Neg	0	Neg	Neg
160	Western	Dry	Female	31	9.5	Yes	No	A+	Neg	Neg	0	Neg	Neg
161	Western	Dry	Female	31	10.1	Yes	No	AB+	Pos	(+)	530	Pos	Pos
162	Western	Dry	Male	78	9.8	Yes	No	B+	Neg	Neg	0	Neg	Neg
163	Western	Dry	Female	65	12.4	No	No	O+	Neg	Neg	0	Neg	Neg
164	Western	Dry	Male	0.6	9.7	Yes	No	A+	Neg	Neg	0	Neg	Neg
165	Western	Dry	Female	4	11.9	Yes	No	A+	Neg	Neg	0	Neg	Neg
167	Western	Dry	Female	10	12.1	No	No	O+	Neg	Neg	0	Neg	Neg
168	Western	Dry	Female	55	12	No	No	O+	Pos	Neg	0	Neg	Neg
169	Western	Dry	Female	27	10.9	Yes	No	O+	Neg	Neg	0	Neg	Neg

242

ID	Region	Season	Sex	Age	Value			Blood					
170	Western	Dry	Female	36	12.7	No	No	O +	Neg	Neg	0	Neg	Neg
171	Western	Dry	Female	43	11.4	Yes	No	O +	Neg	Neg	0	Pos	Neg
172	Western	Dry	Male	0.6	6.4	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
173	Western	Dry	Male	28	10.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
174	Western	Dry	Male	4	10.7	Yes	No	O +	Pos	Neg	0	Neg	Neg
175	Western	Dry	Male	45	11.3	Yes	No	O +	Neg	Neg	0	Neg	Neg
176	Western	Dry	Female	0.3	9.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
177	Western	Dry	Female	23	7.5	Yes	Yes	O +	Neg	Neg	0	Pos	Pos
178	Western	Dry	Female	33	8.6	Yes	No	B +	Neg	Neg	0	Neg	Neg
180	Western	Dry	Female	26	12.3	No	No	O +	Neg	(+)	950	Pos	Pos
181	Western	Dry	Female	14	10.3	Yes	No	O +	Neg	(+++)	4780	Pos	Pos
183	Western	Dry	Female	0.2	9.7	Yes	No	O +	Neg	Neg	0	Neg	Neg
184	Western	Dry	Female	26	6.7	Yes	Yes	O -	Neg	Neg	0	Neg	Neg
185	Western	Dry	Female	26	2.9	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
186	Western	Dry	Female	15	5.1	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
187	Western	Dry	Female	43	8.7	Yes	No	B +	Neg	Neg	0	Neg	Neg
189	Western	Dry	Female	27	7.4	Yes	Yes	O -	Neg	Neg	0	Neg	Neg
191	Western	Dry	Female	32	8	Yes	No	O -	Neg	Neg	0	Neg	Neg
4087	Ashanti	Dry	Female	25	8.3	Yes	No	A +	Neg	Neg	0	Pos	Neg
4089	Ashanti	Dry	Male	38	13.6	No	No	B +	Neg	(+)	980	Neg	Pos
4091	Ashanti	Dry	Female	23	10.4	Yes	No	A +	Neg	Neg	0	Neg	Neg
4092	Ashanti	Dry	Female	21	11.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
4093	Ashanti	Dry	Female	19	9.8	Yes	No	O +	Neg	Neg	0	Neg	Neg
4096	Ashanti	Dry	Female	19	6.5	Yes	Yes	B +	Neg	Neg	0	Neg	Pos
4100	Ashanti	Dry	Male	7	6.3	Yes	Yes	A +	Neg	Neg	0	Neg	Neg
4102	Ashanti	Dry	Female	25	9.2	Yes	No	O +	Neg	Neg	0	Neg	Neg
4103	Ashanti	Dry	Female	28	12	No	No	O +	Neg	Neg	0	Neg	Neg
4104	Ashanti	Dry	Female	24	11.7	Yes	No	B +	Neg	Neg	0	Neg	Neg
4105	Ashanti	Dry	Female	20	10.5	Yes	No	A +	Neg	Neg	0	Pos	Pos
4107	Ashanti	Dry	Female	26	9.8	Yes	No	O +	Pos	Neg	0	Neg	Neg

243

ID	Region	Season	Sex	Age				Blood					
4108	Ashanti	Dry	Female	30	12.8	No	No	O+	Neg	Neg	0	Neg	Neg
4111	Ashanti	Dry	Female	18	10.9	Yes	No	O+	Neg	Neg	0	Neg	Neg
4112	Ashanti	Dry	Female	26	12.2	No	No	A+	Neg	Neg	0	Neg	Neg
4113	Ashanti	Dry	Female	28	9.3	Yes	No	O+	Neg	Neg	0	Pos	Pos
4114	Ashanti	Dry	Female	25	9.6	Yes	No	O+	Pos	Neg	0	Neg	Neg
4115	Ashanti	Dry	Male	25	14.8	No	No	A+	Neg	Neg	0	Pos	Neg
4117	Ashanti	Dry	Male	32	8	Yes	No	O+	Neg	Neg	0	Neg	Neg
4119	Ashanti	Dry	Female	18	8.9	Yes	No	O+	Neg	Neg	0	Neg	Neg
4120	Ashanti	Dry	Female	24	12.3	No	No	A+	Neg	Neg	0	Neg	Neg
4121	Ashanti	Dry	Female	29	9.8	Yes	No	B+	Neg	Neg	0	Neg	Neg
4122	Ashanti	Dry	Female	20	5.9	Yes	Yes	B+	Pos	Neg	0	Neg	Pos
4123	Ashanti	Dry	Female	22	11	Yes	No	B+	Neg	Neg	0	Neg	Neg
4125	Ashanti	Dry	Female	19	9.4	Yes	No	O+	Neg	Neg	0	Pos	Neg
4126	Ashanti	Dry	Female	18	7.6	Yes	Yes	A-	Neg	Neg	0	Pos	Pos
4127	Ashanti	Dry	Female	20	10.1	Yes	No	O+	Neg	(+)	290	Pos	Pos
4129	Ashanti	Dry	Female	33	8	Yes	No	A+	Neg	Neg	0	Neg	Neg
4141	Ashanti	Dry	Female	27	10.9	Yes	No	A+	Neg	Neg	0	Neg	Neg
4147	Ashanti	Dry	Female	19	9.1	Yes	No	O+	Pos	Neg	0	Neg	Neg
4148	Ashanti	Dry	Female	24	10.3	Yes	No	A+	Neg	(+)	420	Pos	Pos
4150	Ashanti	Dry	Male	42	9.4	Yes	No	A+	Pos	Neg	0	Neg	Neg
4151	Ashanti	Dry	Female	18	10.4	Yes	No	A+	Neg	Neg	0	Neg	Neg
4144	Ashanti	Dry	Female	52	10.4	Yes	No	O+	Neg	Neg	0	Neg	Neg
4156	Ashanti	Dry	Female	23	11	Yes	No	O+	Neg	Neg	0	Neg	Neg
4157	Ashanti	Dry	Male	31	10.5	Yes	No	A+	Neg	(+)	710	Pos	Pos
4158	Ashanti	Dry	Female	20	7.7	Yes	Yes	O+	Neg	Neg	0	Neg	Neg
4160	Ashanti	Dry	Female	32	11.3	Yes	No	A-	Neg	(+)	590	Pos	Pos
4162	Ashanti	Dry	Male	4	10.8	Yes	No	O+	Neg	Neg	0	Neg	Neg
4170	Ashanti	Dry	Female	20	10.4	Yes	No	B+	Neg	Neg	0	Neg	Neg
4174	Ashanti	Dry	Female	39	6.4	Yes	Yes	O+	Pos	Neg	0	Pos	Neg
4176	Ashanti	Dry	Male	39	13.3	No	No	O+	Neg	Neg	0	Neg	Neg

244

ID	Region	Season	Sex	Age				Blood					
4177	Ashanti	Dry	Female	32	11	Yes	No	O +	Neg	Neg	0	Neg	Neg
4179	Ashanti	Dry	Male	25	11.3	Yes	No	O +	Neg	(+)	900	Pos	Pos
4180	Ashanti	Dry	Female	38	8.9	Yes	No	O +	Neg	Neg	0	Neg	Neg
4185	Ashanti	Dry	Female	15	6	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
4186	Ashanti	Dry	Female	26	12.7	No	No	B +	Neg	Neg	0	Pos	Pos
4189	Ashanti	Dry	Female	33	10.2	Yes	No	B +	Neg	Neg	0	Neg	Neg
4190	Ashanti	Dry	Female	28	8.3	Yes	No	O +	Neg	Neg	0	Neg	Neg
4191	Ashanti	Dry	Female	27	12.9	No	No	O +	Neg	Neg	0	Neg	Neg
4192	Ashanti	Dry	Female	22	10.2	Yes	No	B +	Neg	Neg	0	Neg	Neg
4196	Ashanti	Dry	Female	18	12.7	No	No	A +	Neg	Neg	0	Neg	Neg
4197	Ashanti	Dry	Female	35	10.7	Yes	No	A +	Neg	Neg	0	Neg	Neg
4199	Ashanti	Dry	Female	18	8.7	Yes	No	A +	Neg	Neg	0	Pos	Pos
4201	Ashanti	Dry	Female	27	12	No	No	O +	Neg	Neg	0	Neg	Neg
4203	Ashanti	Dry	Female	17	9.6	Yes	No	B +	Pos	(+)	1010	Pos	Pos
4205	Ashanti	Dry	Female	28	8	Yes	No	O +	Neg	Neg	0	Pos	Pos
4207	Ashanti	Dry	Female	24	11.7	Yes	No	A +	Neg	Neg	0	Neg	Neg
4208	Ashanti	Dry	Female	23	10.7	Yes	No	A +	Neg	Neg	0	Pos	Neg
4210	Ashanti	Dry	Female	28	10	Yes	No	A +	Pos	Neg	0	Pos	Neg
4212	Ashanti	Dry	Female	26	10.9	Yes	No	B +	Neg	Neg	0	Neg	Neg
4213	Ashanti	Dry	Female	25	12.1	No	No	B -	Neg	Neg	0	Neg	Neg
4214	Ashanti	Dry	Male	43	13.6	No	No	O +	Pos	Neg	0	Neg	Pos
4216	Ashanti	Dry	Female	29	11	Yes	No	AB +	Neg	Neg	0	Neg	Neg
4218	Ashanti	Dry	Female	25	13.5	No	No	A -	Neg	Neg	0	Neg	Neg
4219	Ashanti	Dry	Female	23	10	Yes	No	AB -	Neg	Neg	0	Neg	Neg
4221	Ashanti	Dry	Female	19	11.8	Yes	No	A +	Neg	(+)	430	Pos	Pos
4231	Ashanti	Dry	Female	25	8.7	Yes	No	O +	Neg	Neg	0	Neg	Neg
4243	Ashanti	Dry	Female	32	11	Yes	No	A +	Neg	Neg	0	Neg	Neg
4182	Ashanti	Dry	Female	46	11.1	Yes	No	O +	Neg	Neg	0	Neg	Neg
4128	Ashanti	Dry	Female	31	10.2	Yes	No	O +	Pos	Neg	0	Neg	Neg
4257	Ashanti	Dry	Female	20	12.8	No	No	O +	Neg	(++)	2430	Pos	Pos

245

ID	Region	Season	Sex	Age	Hb	Q1	Q2	Blood	R1	R2	Count	R3	R4	R5
4265	Ashanti	Dry	Female	25	10.9	Yes	No	O+	Neg	Neg	0	Neg	Neg	Neg
4273	Ashanti	Dry	Female	23	12.7	No	No	B+	Neg	Neg	0	Neg	Neg	Neg
4274	Ashanti	Dry	Female	37	14.8	No	No	O+	Pos	Neg	0	Neg	Neg	Neg
4275	Ashanti	Dry	Female	17	12.1	No	No	B+	Neg	Neg	0	Neg	Neg	Neg
4278	Ashanti	Dry	Female	28	10	Yes	No	O+	Neg	Neg	0	Pos	Pos	Pos
4281	Ashanti	Dry	Female	40	9.1	Yes	No	B+	Neg	Neg	0	Neg	Neg	Neg
4285	Ashanti	Dry	Female	29	9.6	Yes	No	A+	Neg	Neg	0	Neg	Neg	Neg
4287	Ashanti	Dry	Female	19	10.9	Yes	No	B+	Neg	(+)	890	Pos	Pos	Neg
4288	Ashanti	Dry	Female	24	12.2	No	No	B+	Neg	Neg	0	Neg	Neg	Pos
4289	Ashanti	Dry	Female	7	11.1	Yes	No	B+	Neg	Neg	0	Neg	Neg	Neg
4290	Ashanti	Dry	Female	33	10.1	Yes	No	A+	Neg	(+)	320	Pos	Pos	Pos
4291	Ashanti	Dry	Female	35	10	Yes	No	O+	Neg	Neg	0	Neg	Neg	Neg
4293	Ashanti	Dry	Female	22	10.9	Yes	No	O+	Neg	Neg	0	Neg	Neg	Neg
4296	Ashanti	Dry	Male	17	11.7	Yes	No	AB+	Neg	Neg	0	Neg	Neg	Neg
4298	Ashanti	Dry	Female	28	10.1	Yes	No	A+	Neg	Neg	0	Neg	Neg	Neg
4300	Ashanti	Dry	Female	23	11.2	Yes	No	B+	Neg	Neg	0	Neg	Neg	Neg
4301	Ashanti	Dry	Male	54	13.9	No	No	O+	Neg	(+)	710	Neg	Neg	Pos
4303	Ashanti	Dry	Female	22	8.7	Yes	No	A+	Neg	Neg	0	Neg	Neg	Neg
4304	Ashanti	Dry	Female	23	10.5	Yes	No	B+	Neg	Neg	0	Neg	Neg	Neg
4308	Ashanti	Dry	Female	26	10.1	Yes	No	O+	Pos	Neg	0	Pos	Pos	Neg
4309	Ashanti	Dry	Female	17	11.9	Yes	No	B+	Neg	(+)	580	Pos	Pos	Pos
4310	Ashanti	Dry	Female	30	8.4	Yes	No	A+	Pos	Neg	0	Neg	Neg	Neg
4311	Ashanti	Dry	Female	11	12.3	No	No	B+	Neg	Neg	0	Neg	Neg	Neg
4312	Ashanti	Dry	Female	31	11.1	Yes	No	O+	Neg	Neg	0	Pos	Neg	Neg
4313	Ashanti	Dry	Female	24	8.8	Yes	No	A+	Neg	Neg	0	Neg	Neg	Neg
4314	Ashanti	Dry	Female	8	12.6	No	No	A+	Neg	Neg	0	Neg	Neg	Neg
4315	Ashanti	Dry	Female	27	11.5	Yes	No	B+	Neg	Neg	0	Neg	Neg	Neg
4316	Ashanti	Dry	Female	24	10.2	Yes	No	O+	Neg	Neg	0	Neg	Neg	Pos
4317	Ashanti	Dry	Female	22	11.7	Yes	No	A+	Neg	Neg	0	Neg	Neg	Neg
4318	Ashanti	Dry	Female	20	8.3	Yes	No	B+	Neg	Neg	0	Neg	Neg	Neg

246

ID	Region	Season	Sex	Age	Hb			Blood						
4322	Ashanti	Dry	Female	27	11.6	Yes	No	O+	Neg	Neg	0	Neg	Neg	Neg
4323	Ashanti	Dry	Female	9	11.8	No	No	O+	Neg	Neg	0	Neg	Neg	Neg
4324	Ashanti	Dry	Female	29	7.3	Yes	Yes	A+	Neg	Neg	0	Neg	Neg	Neg
4325	Ashanti	Dry	Female	36	9.5	Yes	No	O+	Pos	Neg	0	Neg	Neg	Neg
4326	Ashanti	Dry	Female	25	10.3	Yes	No	O+	Neg	Neg	0	Neg	Neg	Neg
4329	Ashanti	Dry	Female	23	10.6	Yes	No	O+	Neg	Neg	0	Neg	Neg	Neg
4330	Ashanti	Dry	Female	22	9.9	Yes	No	O+	Neg	Neg	0	Neg	Neg	Neg
4331	Ashanti	Dry	Female	27	11.4	Yes	No	B+	Neg	Neg	0	Neg	Neg	Neg
4333	Ashanti	Dry	Female	36	10.3	Yes	No	A+	Neg	(+)	90	Neg	Neg	Neg
4334	Ashanti	Dry	Female	32	11.4	Yes	No	B-	Neg	Neg	0	Neg	Neg	Neg
4335	Ashanti	Dry	Female	38	13.2	No	No	A+	Neg	Neg	0	Neg	Neg	Neg
4336	Ashanti	Dry	Female	26	16.1	No	No	A+	Neg	Neg	0	Neg	Neg	Neg
4337	Ashanti	Dry	Female	20	10.9	Yes	No	O+	Neg	Neg	0	Neg	Neg	Neg
4338	Ashanti	Dry	Male	3	10.7	Yes	No	O+	Neg	(+)	830	Pos	Pos	Pos
4339	Ashanti	Dry	Female	17	10.1	Yes	No	O+	Neg	(+)	260	Pos	Pos	Pos
4340	Ashanti	Dry	Female	32	11.9	Yes	No	B-	Neg	Neg	0	Neg	Neg	Neg
4222	Ashanti	Dry	Female	23	10.6	Yes	No	AB+	Neg	Neg	0	Neg	Pos	Neg
4224	Ashanti	Dry	Female	24	11.2	Yes	No	B+	Pos	Neg	0	Neg	Neg	Neg
4226	Ashanti	Dry	Female	25	9.5	Yes	No	O+	Neg	Neg	0	Neg	Neg	Neg
4227	Ashanti	Dry	Female	37	12.3	No	No	A+	Neg	Neg	0	Neg	Neg	Neg
4228	Ashanti	Dry	Male	35	13.4	No	No	O+	Pos	Neg	0	Neg	Neg	Neg
4230	Ashanti	Dry	Female	1	12.4	No	No	O+	Neg	Neg	0	Neg	Neg	Neg
4232	Ashanti	Dry	Female	20	10.8	Yes	No	A+	Neg	Neg	0	Neg	Neg	Neg
4233	Ashanti	Dry	Female	36	11.2	Yes	No	O+	Neg	(+)	450	Pos	Pos	Pos
4234	Ashanti	Dry	Female	23	10.9	Yes	No	O+	Neg	Neg	0	Neg	Pos	Neg
4235	Ashanti	Dry	Female	26	12.2	No	No	B-	Neg	Neg	0	Neg	Neg	Neg
4236	Ashanti	Dry	Female	26	10	Yes	No	A-	Neg	Neg	0	Neg	Neg	Neg
4239	Ashanti	Dry	Female	29	10.3	Yes	No	A+	Neg	Neg	0	Neg	Neg	Neg
4238	Ashanti	Dry	Female	46	11.1	Yes	No	O+	Pos	Neg	0	Neg	Neg	Neg
4241	Ashanti	Dry	Male	9	10.1	Yes	No	O+	Neg	Neg	0	Pos	Neg	Neg

4244	Ashanti	Dry	Female	23	9.3	Yes	No	O+	Neg	Neg	0	Neg	Neg
5062	Brong-Ahafo	Dry	Female	12	9.2	Yes	No	O+	Neg	Neg	0	Neg	Neg
5015	Brong-Ahafo	Dry	Female	26	13	No	No	A+	Pos	Neg	0	Neg	Neg
5012	Brong-Ahafo	Dry	Female	27	12.6	No	No	O+	Pos	Neg	0	Neg	Neg
5008	Brong-Ahafo	Dry	Female	15	10.1	Yes	No	O+	Neg	Neg	0	Pos	Neg
5014	Brong-Ahafo	Dry	Female	23	11.3	Yes	No	O+	Pos	Neg	0	Neg	Neg
5020	Brong-Ahafo	Dry	Female	17	10.1	Yes	No	A+	Neg	Neg	0	Neg	Neg
4994	Brong-Ahafo	Dry	Male	27	10.9	Yes	No	O+	Neg	(++)	1630	Pos	Pos
4998	Brong-Ahafo	Dry	Female	21	9.6	Yes	No	B+	Neg	Neg	0	Neg	Neg
5009	Brong-Ahafo	Dry	Female	47	12.9	No	No	A+	Neg	Neg	0	Neg	Neg
4944	Brong-Ahafo	Dry	Male	19	9.8	Yes	No	A+	Pos	Neg	0	Neg	Pos
5059	Brong-Ahafo	Dry	Female	28	9.5	Yes	No	O+	Neg	Neg	0	Neg	Neg
5501	Brong-Ahafo	Dry	Female	9	9	Yes	No	O-	Pos	(+)	390	Neg	Pos
5502	Brong-Ahafo	Dry	Female	29	13	No	No	A-	Neg	Neg	0	Neg	Neg
5055	Brong-Ahafo	Dry	Female	20	9.4	Yes	No	O+	Neg	Neg	0	Neg	Neg
5054	Brong-Ahafo	Dry	Female	1	10.7	Yes	No	O+	Neg	Neg	0	Neg	Pos
5052	Brong-Ahafo	Dry	Male	37	17.6	No	No	O+	Neg	Neg	0	Neg	Neg
5503	Brong-Ahafo	Dry	Female	32	9.4	Yes	No	A+	Neg	Neg	0	Pos	Pos
5056	Brong-Ahafo	Dry	Female	35	11.2	Yes	No	O+	Neg	Neg	0	Neg	Neg
4945	Brong-Ahafo	Dry	Female	15	11.9	Yes	No	B+	Neg	(++)	2250	Neg	Pos
4931	Brong-Ahafo	Dry	Female	14	13.9	No	No	A+	Neg	(+)	530	Neg	Pos
5504	Brong-Ahafo	Dry	Male	19	10.7	Yes	No	O+	Neg	(+)	250	Pos	Neg
4973	Brong-Ahafo	Dry	Female	28	7.1	Yes	Yes	O+	Neg	Neg	0	Neg	Neg
4957	Brong-Ahafo	Dry	Female	27	12.3	No	No	A+	Neg	Neg	0	Neg	Neg
4966	Brong-Ahafo	Dry	Female	14	8.6	Yes	No	AB+	Neg	Neg	0	Neg	Neg
4932	Brong-Ahafo	Dry	Female	19	8	Yes	No	O+	Pos	Neg	0	Neg	Neg
4975	Brong-Ahafo	Dry	Female	29	11.3	Yes	No	AB+	Neg	Neg	0	Neg	Neg
4952	Brong-Ahafo	Dry	Male	21	15.3	No	No	O+	Neg	(+)	550	Pos	Pos
4937	Brong-Ahafo	Dry	Female	29	10.8	Yes	No	B+	Neg	Neg	0	Neg	Neg
4963	Brong-Ahafo	Dry	Female	7	13.6	No	No	B+	Neg	Neg	0	Neg	Neg

248

4983	Brong-Ahafo	Dry	Female	16	9.6	Yes	No	B +	Neg	(+)	700	Pos	Pos
4958	Brong-Ahafo	Dry	Female	44	8.3	Yes	No	O +	Neg	Neg	0	Neg	Neg
5048	Brong-Ahafo	Dry	Female	18	13.4	No	No	O +	Neg	(++)	2430	Pos	Pos
5047	Brong-Ahafo	Dry	Male	83	12.3	No	No	O +	Neg	Neg	0	Neg	Neg
5505	Brong-Ahafo	Dry	Female	31	8.8	Yes	No	O +	Neg	Neg	0	Pos	Pos
5034	Brong-Ahafo	Dry	Male	31	15	No	No	B +	Neg	Neg	0	Neg	Neg
5035	Brong-Ahafo	Dry	Female	44	12.2	No	No	A +	Neg	Neg	0	Neg	Neg
4940	Brong-Ahafo	Dry	Female	24	9.7	Yes	No	B +	Pos	(+)	800	Neg	Pos
4943	Brong-Ahafo	Dry	Male	80	15	No	No	O +	Neg	(+)	430	Neg	Pos
5038	Brong-Ahafo	Dry	Female	29	10.3	Yes	No	B +	Neg	Neg	0	Neg	Neg
5040	Brong-Ahafo	Dry	Male	22	13.3	No	No	A +	Neg	Neg	0	Neg	Neg
5039	Brong-Ahafo	Dry	Male	5	14	No	No	A +	Pos	(+)	1000	Neg	Pos
5032	Brong-Ahafo	Dry	Male	17	15	No	No	O +	Neg	(++)	2100	Pos	Pos
5030	Brong-Ahafo	Dry	Female	20	12.3	No	No	O +	Neg	Neg	0	Neg	Neg
5029	Brong-Ahafo	Dry	Female	20	12.5	No	No	B +	Neg	Neg	0	Neg	Neg
5033	Brong-Ahafo	Dry	Female	11	13.4	No	No	O +	Neg	Neg	0	Neg	Neg
5028	Brong-Ahafo	Dry	Male	22	15.7	No	No	B +	Neg	Neg	0	Pos	Pos
5036	Brong-Ahafo	Dry	Female	31	11.8	Yes	No	B +	Neg	Neg	0	Neg	Neg
5037	Brong-Ahafo	Dry	Female	42	12.8	No	No	O +	Pos	Neg	0	Neg	Neg
4949	Brong-Ahafo	Dry	Female	40	12.5	No	No	A -	Neg	Neg	0	Neg	Neg
4979	Brong-Ahafo	Dry	Male	20	6	Yes	Yes	AB +	Pos	Neg	0	Neg	Neg
4976	Brong-Ahafo	Dry	Female	30	12.9	No	No	A +	Neg	(+)	810	Pos	Pos
4985	Brong-Ahafo	Dry	Male	40	16.5	No	No	B -	Neg	Neg	0	Neg	Neg
4936	Brong-Ahafo	Dry	Female	16	13	No	No	O -	Neg	Neg	0	Neg	Neg
4987	Brong-Ahafo	Dry	Male	27	3.5	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
4989	Brong-Ahafo	Dry	Male	63	15	No	No	B +	Neg	Neg	0	Neg	Neg
5001	Brong-Ahafo	Dry	Female	18	12.4	No	No	O +	Neg	Neg	0	Neg	Neg
4961	Brong-Ahafo	Dry	Female	31	4	Yes	Yes	AB +	Neg	Neg	0	Pos	Pos
4965	Brong-Ahafo	Dry	Female	31	12.9	No	No	B +	Neg	Neg	0	Neg	Neg
4951	Brong-Ahafo	Dry	Female	31	8.3	Yes	No	B +	Neg	Neg	0	Neg	Neg

249

4969	Brong-Ahafo	Dry	Female	25	13.3	No	No	A +	Neg	Neg	0	Neg	Neg
4995	Brong-Ahafo	Dry	Female	25	13.4	No	No	O +	Neg	Neg	0	Pos	Neg
4933	Brong-Ahafo	Dry	Female	25	16.1	No	No	AB +	Pos	Neg	0	Neg	Neg
4972	Brong-Ahafo	Dry	Male	70	11.3	Yes	No	B +	Neg	Neg	0	Pos	Pos
4988	Brong-Ahafo	Dry	Female	27	12.4	No	No	A +	Neg	Neg	0	Neg	Neg
4991	Brong-Ahafo	Dry	Female	25	14.2	No	No	A +	Neg	Neg	0	Neg	Neg
5006	Brong-Ahafo	Dry	Female	18	12.4	No	No	B +	Neg	Neg	0	Neg	Neg
5004	Brong-Ahafo	Dry	Female	26	12.4	No	No	B +	Neg	(+)	240	Neg	Neg
5013	Brong-Ahafo	Dry	Male	36	14	No	No	A -	Neg	Neg	0	Neg	Neg
4999	Brong-Ahafo	Dry	Female	20	9.6	Yes	No	A +	Neg	Neg	0	Neg	Neg
5003	Brong-Ahafo	Dry	Male	44	15.3	No	No	B +	Neg	(+)	1050	Neg	Pos
4996	Brong-Ahafo	Dry	Female	30	13.2	No	No	O +	Neg	Neg	0	Pos	Neg
4982	Brong-Ahafo	Dry	Female	29	14.3	No	No	B -	Neg	Neg	0	Neg	Neg
5010	Brong-Ahafo	Dry	Female	21	12	No	No	B +	Neg	Neg	0	Neg	Neg
5005	Brong-Ahafo	Dry	Female	52	12.6	No	No	O +	Neg	Neg	0	Neg	Neg
4980	Brong-Ahafo	Dry	Female	33	13.4	No	No	B +	Neg	Neg	0	Neg	Neg
5506	Brong-Ahafo	Dry	Female	10	16.1	No	No	O +	Pos	(+)	430	Neg	Neg
5021	Brong-Ahafo	Dry	Female	21	15.3	No	No	A +	Neg	Neg	0	Neg	Neg
5026	Brong-Ahafo	Dry	Female	26	12.8	No	No	O +	Neg	Neg	0	Neg	Neg
5166	Brong-Ahafo	Dry	Female	32	9.7	Yes	No	O +	Neg	Neg	0	Neg	Neg
5170	Brong-Ahafo	Dry	Female	35	12	No	No	O -	Pos	Neg	0	Neg	Pos
5176	Brong-Ahafo	Dry	Female	16	12.4	No	No	O +	Neg	Neg	0	Neg	Neg
5024	Brong-Ahafo	Dry	Female	20	8.8	Yes	No	A +	Neg	(+)	630	Pos	Pos
5164	Brong-Ahafo	Dry	Female	33	10.8	Yes	No	A +	Neg	(+)	1000	Neg	Neg
5174	Brong-Ahafo	Dry	Female	24	7.4	Yes	Yes	O +	Neg	Neg	0	Neg	Neg
5173	Brong-Ahafo	Dry	Female	22	14.3	No	No	O -	Pos	(+)	510	Pos	Pos
5507	Brong-Ahafo	Dry	Female	22	13.2	Yes	No	A +	Neg	Neg	0	Neg	Neg
5163	Brong-Ahafo	Dry	Female	5	11.7	Yes	No	O +	Neg	Neg	0	Neg	Neg
5200	Brong-Ahafo	Dry	Female	10	12.9	No	No	O +	Neg	(+)	420	Neg	Neg
5201	Brong-Ahafo	Dry	Female	22	7.5	Yes	Yes	O +	Neg	(+)	570	Pos	Pos

250

ID	Region	Season	Sex	Age	Value	Col7	Col8	Blood	Col10	Col11	Num	Col13	Col14
5180	Brong-Ahafo	Dry	Male	2	10.8	Yes	No	B+	Neg	Neg	0	Neg	Neg
5179	Brong-Ahafo	Dry	Female	30	9.4	Yes	No	O+	Neg	Neg	0	Neg	Neg
5190	Brong-Ahafo	Dry	Male	4	11.1	No	No	O+	Neg	Neg	0	Neg	Neg
5199	Brong-Ahafo	Dry	Female	86	8.3	Yes	No	A+	Neg	(+)	370	Neg	Neg
5185	Brong-Ahafo	Dry	Female	24	13.3	No	No	A+	Neg	Neg	0	Neg	Neg
5023	Brong-Ahafo	Dry	Female	30	9.5	Yes	No	A+	Neg	Neg	0	Pos	Pos
5191	Brong-Ahafo	Dry	Female	27	12	No	No	A+	Neg	Neg	0	Neg	Neg
5205	Brong-Ahafo	Dry	Male	1	11.4	No	No	B+	Neg	Neg	0	Pos	Pos
5186	Brong-Ahafo	Dry	Female	32	12	No	No	O-	Neg	Neg	0	Neg	Neg
5208	Brong-Ahafo	Dry	Female	32	8.5	Yes	No	O+	Neg	Neg	0	Neg	Neg
5211	Brong-Ahafo	Dry	Female	8	11.3	Yes	No	O+	Neg	Neg	0	Neg	Neg
5198	Brong-Ahafo	Dry	Female	10	10	Yes	No	B+	Pos	Neg	0	Neg	Neg
5212	Brong-Ahafo	Dry	Female	21	14.3	No	No	B+	Pos	Neg	0	Pos	Pos
5203	Brong-Ahafo	Dry	Female	7	12.6	No	No	B+	Neg	Neg	0	Neg	Neg
4398	Brong-Ahafo	Dry	Female	27	12.4	No	No	A+	Neg	Neg	0	Neg	Neg
5217	Brong-Ahafo	Dry	Female	41	6.1	Yes	Yes	O+	Neg	Neg	0	Neg	Neg
5206	Brong-Ahafo	Dry	Male	8	12.5	No	No	O+	Neg	Neg	0	Neg	Neg
5216	Brong-Ahafo	Dry	Female	26	11.3	Yes	No	A+	Pos	Neg	0	Pos	Pos
5218	Brong-Ahafo	Dry	Male	32	10.7	Yes	No	B-	Pos	Neg	0	Neg	Neg
5219	Brong-Ahafo	Dry	Male	20	8.1	Yes	No	B+	Neg	(+)	300	Pos	Pos
5210	Brong-Ahafo	Dry	Female	58	10.1	Yes	No	B+	Neg	(+)	650	Pos	Pos
5214	Brong-Ahafo	Dry	Female	43	11.7	Yes	No	B+	Pos	Neg	0	Neg	Neg
5220	Brong-Ahafo	Dry	Female	76	11.4	Yes	No	O+	Neg	Neg	0	Neg	Neg
5215	Brong-Ahafo	Dry	Female	24	6.3	Yes	Yes	AB+	Pos	Neg	0	Neg	Neg
5204	Brong-Ahafo	Dry	Female	2	10	Yes	No	B+	Neg	(++)	1840	Pos	Pos
5223	Brong-Ahafo	Dry	Female	29	10.2	Yes	No	O+	Neg	Neg	0	Neg	Neg
5221	Brong-Ahafo	Dry	Female	50	11.4	Yes	No	O+	Pos	Neg	0	Pos	Pos
5202	Brong-Ahafo	Dry	Female	19	11.4	Yes	No	O+	Neg	Neg	0	Pos	Pos
5222	Brong-Ahafo	Dry	Female	55	9.6	Yes	No	O+	Neg	(+)	90	Neg	Neg
5236	Brong-Ahafo	Dry	Male	65	14.2	No	No	B+	Neg	Neg	0	Neg	Neg

251

5237	Brong-Ahafo	Dry	Female	77	10.3	Yes	No	O +	Neg	Neg	0	Neg	Neg
5235	Brong-Ahafo	Dry	Female	37	9.1	Yes	No	B +	Neg	Neg	0	Neg	Neg
5234	Brong-Ahafo	Dry	Female	32	11.1	Yes	No	O +	Neg	(+)	560	Pos	Pos

252

APPENDIX III

DATA ON SNPs OF PfMDR1, PfCRT AND PfATPase6

Sample ID	crt 76	mdr 86	mdr 184	mdr 1034	mdr 1042	mdr 1246	ATP 263	ATP 289	ATP 431	ATP 623	ATP 769
343	K	Y	F	S	N	D	L	D	E	A	S
347	K	N	F	S	N	D	L	D	E	A	S
383	K	N	Y	S	N	D	L	D	E	A	S
394	K	N	Y	S	N	D	L	D	E	A	S
396	T	N	Y	S	N	D	L	D	E	A	S
403	T	Y	F	S	N	D	L	D	E	A	S
451	K	N	F	S	N	D	L	D	E	A	S
165	T	Y	F	S	N	D	L	D	E	A	S
265	K	N	Y	S	N	D	L	D	E	A	S
289	T	Y	F	S	N	D	L	D	E	A	S
294	T	N	F	S	N	D	L	D	E	A	S
321	T	Y	Y	S	N	D	L	D	E	A	S
323	K	N	F	S	N	D	L	D	E	A	S
325	K	N	Y	S	N	D	L	D	E	A	S
452	K	N	F	S	N	D	L	D	E	A	S
494	T	N	F	S	N	D	L	D	E	A	S
504	T	Y	F	S	N	D	L	D	E	A	S
511	T	Y	F	S	N	D	L	D	E	A	S
515	K	N	Y	S	N	D	L	D	E	A	S
547	T	Y	F	S	N	D	L	D	E	A	S
379	K	N	F	S	N	D	L	D	E	A	S
106	T	Y	F	S	N	D	L	D	E	A	S
111	K	N	Y	S	N	D	L	D	E	A	S
114	K	N	F	S	N	D	L	D	E	A	S

253

No.			S	N	D	L	D	E	A	S
121	T	N	F	S	N	D	L	D	E	A	S
129	T	Y	F	S	N	D	L	D	E	A	S
136	K	N	Y	S	N	D	L	D	E	A	S
169	T	Y	Y	S	N	D	L	D	E	A	S
176	K	N	F	S	N	D	L	D	E	A	S
191	T	N	F	S	N	D	L	D	E	A	S
990	K	N	Y	S	N	D	L	D	E	A	S
991	K	N	Y	S	N	D	L	D	E	A	S
14	T	Y	F	S	N	D	L	D	E	A	S
993	K	N	F	S	N	D	L	D	E	A	S
135	T	N	F	S	N	D	L	D	E	A	S
1001	K	N	Y	S	N	D	L	D	E	A	S
153	T	N	Y	S	N	D	L	D	E	A	S
1002	T	N	F	S	N	D	L	D	E	A	S
151	K	N	F	S	N	D	L	D	E	A	S
146	T	N	F	S	N	D	L	D	E	A	S
162	T	N	F	S	N	D	L	D	E	A	S
84	K	N	F	S	N	D	L	D	E	A	S
159	K	N	Y	S	N	D	L	D	E	A	S
1003	K	N	Y	S	N	D	L	D	E	A	S
15	K	N	F	S	N	D	L	D	E	A	S
951	T	N	F	S	N	D	L	D	E	A	S
0	K	Y	F	S	N	D	L	D	E	A	S
272	T	N	F	S	N	D	L	D	E	A	S
190	K	N	Y	S	N	D	L	D	E	A	S
234	T	N	F	S	N	D	L	D	E	A	S
394	T	N	F	S	N	D	L	D	E	A	S
402	T	N	F	S	N	D	L	D	E	A	S
386	T	N	F	S	N	D	L	D	E	A	S
307	T	N	F	S	N	D	L	D	E	A	S

254

391	T	N	F	S	N	D	L	D	E	A	S
253	K	N	F	S	N	D	L	D	E	A	S
933	T	N	F	S	N	D	L	D	E	A	S
973	T	Y	F	S	N	D	L	D	E	A	S
910	K	N	Y	S	N	D	L	D	E	A	S
1007	T	N	F	S	N	D	L	D	E	A	S
986	T	Y	Y	S	N	D	L	D	E	A	S
205	K	N	Y	S	N	D	L	D	E	A	S
207	T	N	Y	S	N	D	L	D	E	A	S
237	T	N	F	S	N	D	L	D	E	A	S
243	K	N	Y	S	N	D	L	D	E	A	S
266	T	Y	F	S	N	D	L	D	E	A	S
269	T	N	F	S	N	D	L	D	E	A	S
272	T	N	Y	S	N	D	L	D	E	A	S
299	K	N	F	S	N	D	L	D	E	A	S
326	T	N	Y	S	N	D	L	D	E	A	S
1	K	N	Y	S	N	D	L	D	E	A	S
17	T	Y	F	S	N	D	L	D	E	A	S
18	T	N	F	S	N	D	L	D	E	A	S
20	T	N	F	S	N	D	L	D	E	A	S
24	T	N	Y	S	N	D	L	D	E	A	S
74	K	N	F	S	N	D	L	D	E	A	S
80	K	N	F	S	N	D	L	D	E	A	S
105	K	Y	F	S	N	D	L	D	E	A	S
106	K	N	F	S	N	D	L	D	E	A	S
112	T	Y	F	S	N	D	L	D	E	A	S
113	T	N	F	S	N	D	L	D	E	A	S
116	K	N	F	S	N	D	L	D	E	A	S
118	T	N	Y	S	N	D	L	D	E	A	S
120	T	N	Y	S	N	D	L	D	E	A	S

255

ID											
124	T	N	F	S	N	D	L	D	E	A	S
127	T	N	F	S	N	D	L	D	E	A	S
132	K	N	F	S	N	D	L	D	E	A	S
158	T	N	Y	S	N	D	L	D	E	A	S
5	K	N	F	S	N	D	L	D	E	A	S
17	T	N	F	S	N	D	L	D	E	A	S
22	T	Y	F	S	N	D	L	D	E	A	S
34	K	N	F	S	N	D	L	D	E	A	S
61	K	N	F	S	N	D	L	D	E	A	S
64	T	N	F	S	N	D	L	D	E	A	S
68	T	N	Y	S	N	D	L	D	E	A	S
118	K	N	Y	S	N	D	L	D	E	A	S
137	T	N	F	S	N	D	L	D	E	A	S
138	K	N	F	S	N	D	L	D	E	A	S
150	T	Y	F	S	N	D	L	D	E	A	S
161	T	N	F	S	N	D	L	D	E	A	S
171	K	N	F	S	N	D	L	D	E	A	S
177	K	N	Y	S	N	D	L	D	E	A	S
180	T	Y	F	S	N	D	L	D	E	A	S
181	T	N	F	S	N	D	L	D	E	A	S
11518	K	N	F	S	N	D	L	D	E	A	S
11455	K	N	F	S	N	D	L	D	E	A	S
11453	K	Y	F	S	N	D	L	D	E	A	S
1	T	Y	F	S	N	D	L	D	E	A	S
11398	T	N	F	S	N	D	L	D	E	A	S
11371	K	N	Y	S	N	D	L	D	E	A	S
11355	K	N	Y	S	N	D	L	D	E	A	S
11335	K	N	Y	S	N	D	L	D	E	A	S
11745	K	N	Y	S	N	D	L	D	E	A	S
11718	T	N	F	S	N	D	L	D	E	A	S

256

11720	K	N	Y	S	N	D	L	D	E	A	S
11689	T	N	Y	S	N	D	L	D	E	A	S
11773	T	N	F	S	N	D	L	D	E	A	S
5480	T	N	F	S	N	D	L	D	E	A	S
5529	K	N	F	S	N	D	L	D	E	A	S
5440	T	N	F	S	N	D	L	D	E	A	S
5512	K	N	Y	S	N	D	L	D	E	A	S
5463	T	Y	F	S	N	D	L	D	E	A	S
4	K	N	F	S	N	D	L	D	E	A	S
5534	T	N	F	S	N	D	L	D	E	A	S
5545	T	N	F	S	N	D	L	D	E	A	S
5547	T	N	F	S	N	D	L	D	E	A	S
5549	T	N	F	S	N	D	L	D	E	A	S
5613	T	N	F	S	N	D	L	D	E	A	S
5579	K	N	Y	S	N	D	L	D	E	A	S
5557	T	N	Y	S	N	D	L	D	E	A	S
5341	T	N	Y	S	N	D	L	D	E	A	S
5343	T	N	F	S	N	D	L	D	E	A	S
5172	K	N	Y	S	N	D	L	D	E	A	S
5169	T	Y	F	S	N	D	L	D	E	A	S
5201	T	N	F	S	N	D	L	D	E	A	S
5223	T	N	F	S	N	D	L	D	E	A	S
16220	K	N	F	S	N	D	L	D	E	A	S
16211	K	N	F	S	N	D	L	D	E	A	S
16214	T	Y	F	S	N	D	L	D	E	A	S
16239	T	N	F	S	N	D	L	D	E	A	S
16246	K	N	F	S	N	D	L	D	E	A	S
16131	K	Y	F	S	N	D	L	D	E	A	S
16230	T	Y	F	S	N	D	L	D	E	A	S
16254	T	N	F	S	N	D	L	D	E	A	S

ID											
16277	T	N	F	S	N	D	L	D	E	A	S
16282	K	N	F	S	N	D	L	D	E	A	S
16267	T	N	F	S	N	D	L	D	E	A	S
16274	K	Y	F	S	N	D	L	D	E	A	S
16294	K	N	Y	S	N	D	L	D	E	A	S
16242	K	N	F	S	N	D	L	D	E	A	S
16300	T	N	F	S	N	D	L	D	E	A	S
16286	T	Y	F	S	N	D	L	D	E	A	S
16355	K	N	F	S	N	D	L	D	E	A	S
16305	T	N	F	S	N	D	L	D	E	A	S
16307	K	Y	F	S	N	D	L	D	E	A	S
16308	T	N	F	S	N	D	L	D	E	A	S
16314	K	N	F	S	N	D	L	D	E	A	S
16310	K	N	F	S	N	D	L	D	E	A	S
16312	K	Y	F	S	N	D	L	D	E	A	S
16366	T	N	F	S	N	D	L	D	E	A	S
16337	K	Y	F	S	N	D	L	D	E	A	S
16328	T	Y	F	S	N	D	L	D	E	A	S
16326	T	Y	F	S	N	D	L	D	E	A	S
4089	T	Y	F	S	N	D	L	D	E	A	S
4105	K	N	F	S	N	D	L	D	E	A	S
4113	K	N	Y	S	N	D	L	D	E	A	S
4122	T	N	F	S	N	D	L	D	E	A	S
4126	T	N	F	S	N	D	L	D	E	A	S
4127	T	Y	Y	S	N	D	L	D	E	A	S
4148	K	N	F	S	N	D	L	D	E	A	S
4157	K	N	Y	S	N	D	L	D	E	A	S
4160	K	N	F	S	N	D	L	D	E	A	S
4179	K	N	Y	S	N	D	L	D	E	A	S
4186	T	N	Y	S	N	D	L	D	E	A	S

258

ID											
4199	T	N	F	S	N	D	L	D	E	A	S
4203	K	N	Y	S	N	D	L	D	E	A	S
4205	T	N	Y	S	N	D	L	D	E	A	S
4214	K	Y	Y	S	N	D	L	D	E	A	S
4221	T	Y	F	S	N	D	L	D	E	A	S
4257	T	N	Y	S	N	D	L	D	E	A	S
4278	T	Y	F	S	N	D	L	D	E	A	S
4288	K	N	F	S	N	D	L	D	E	A	S
4290	T	Y	F	S	N	D	L	D	E	A	S
4301	T	N	F	S	N	D	L	D	E	A	S
4309	T	N	F	S	N	D	L	D	E	A	S
4316	T	N	F	S	N	D	L	D	E	A	S
4338	T	Y	F	S	N	D	L	D	E	A	S
4339	K	N	F	S	N	D	L	D	E	A	S
4233	T	Y	F	S	N	D	L	D	E	A	S
5008	T	N	Y	S	N	D	L	D	E	A	S
5020	T	N	F	S	N	D	L	D	E	A	S
4994	K	N	F	S	N	D	L	D	E	A	S
5503	T	Y	F	S	N	D	L	D	E	A	S
4945	T	N	Y	S	N	D	L	D	E	A	S
5504	T	Y	F	S	N	D	L	D	E	A	S
4199	K	N	F	S	N	D	L	D	E	A	S
4203	K	N	F	S	N	D	L	D	E	A	S
4205	T	N	F	S	N	D	L	D	E	A	S
4208	T	Y	Y	S	N	D	L	D	E	A	S
4210	K	N	F	S	N	D	L	D	E	A	S
4213	T	N	F	S	N	D	L	D	E	A	S
4221	K	N	F	S	N	D	L	D	E	A	S
4257	T	Y	F	S	N	D	L	D	E	A	S
5579	T	N	F	S	N	D	L	D	E	A	S

259

S	A	E	D	L	D	N	S	F	N	T	55S7
S	A	E	D	L	D	N	S	F	N	K	5565
S	A	E	D	L	D	N	S	F	Y	K	5341
S	A	E	D	L	D	N	S	F	N	T	5308
S	A	E	D	L	D	N	S	Y	Y	K	6131
S	A	E	D	L	D	N	S	F	N	K	6137
S	A	E	D	L	D	N	S	F	N	T	6140
S	A	E	D	L	D	N	S	F	Y	T	6143
S	A	E	D	L	D	N	S	F	Y	T	6161
S	A	E	D	L	D	N	S	Y	N	K	4994
S	A	E	N	L	D	N	S	F	Y	T	4944
S	A	E	D	L	D	N	S	F	N	T	5501
S	A	E	N	L	D	N	S	Y	N	T	5054
S	A	E	D	L	D	N	S	F	N	T	5503
S	A	E	D	L	D	N	S	F	Y	T	4945
S	A	E	D	L	D	N	S	F	N	T	4931
S	A	E	D	L	D	N	S	F	Y	T	4952
S	A	E	D	L	D	N	S	Y	N	K	4983
S	A	E	D	L	D	N	S	Y	N	K	5048
S	A	E	N	L	D	N	S	F	Y	T	5505
S	A	E	D	L	D	N	S	F	Y	T	4940
S	A	E	D	L	D	N	S	F	N	K	4943
S	A	E	D	L	D	N	S	F	N	T	5039
S	A	E	D	L	D	N	S	F	N	T	5032
S	A	E	D	L	D	N	S	F	N	T	5028
S	A	E	D	L	D	N	S	F	N	T	4976
S	A	E	D	L	D	N	S	Y	Y	K	4961
S	A	E	D	L	D	N	S	F	N	K	4972
S	A	E	D	L	D	N	S	F	N	T	4996
S	A	E	N	L	D	N	S	Y	N	K	5024

260

	T/K		F/Y	S	N	D	L	D	E	A	S
5173	T	N	F	S	N	D	L	D	E	A	S
5201	T	N	Y	S	N	D	L	D	E	A	S
5023	T	Y	F	S	N	D	L	D	E	A	S
5205	K	N	Y	S	N	N	L	N	E	A	S
5212	T	N	F	S	N	D	L	D	E	A	S
5216	K	N	Y	S	N	D	L	D	E	A	S
5219	T	Y	F	S	N	D	L	D	E	A	S
5210	K	N	F	S	N	D	L	D	E	A	S
5204	T	Y	F	S	N	D	L	D	E	A	S
5221	T	Y	F	S	N	D	L	N	E	A	S
5202	T	N	F	S	N	D	L	D	E	A	S
5234	K	N	F	S	N	D	L	D	E	A	S

261