Safety around the *Plasmodium Vivax* induced blood stage malaria model

Thesis submitted in accordance with the requirements of the Liverpool School of Tropical Medicine for the degree of Doctor in Philosophy by *Anand Arjan Odedra*.

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LIST OF ABBREVIATIONS

AE	Adverse event
ASFA	American society for apheresis
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANZCTR	Australia New Zealand clinical trials registry
APACHE	Acute physiology and chronic health evaluation
ARDS	Acute respiratory distress syndrome
AST	Aspartate transaminase
CDC	Centers for disease control and prevention
CHMI	Controlled human malaria infection
CI	Confidence intervals
CK	Creatinine kinase
CMNC	Continuous mononuclear cell
CMV	Cytomegalovirus
CRP	C-reactive protein
CTCAE	Common terminology criteria for adverse events
CYP2D6	Cytochrome P450 2D6
DCQ	N-desethylchloroquine
DEC	Diethylcarbamazine
DIC	Disseminated intravascular coagulation
DILI	Drug induced liver injury
EBV	Epstein-Barr virus
ECG	Electrocardiogram
ECMO	Extracorporeal membrane oxygenation
eMFA	Enriched membrane feeding assay
EOS	End of study
FDA	Food and drug administration
G6PD	Glucose-6 phosphate dehydrogenase
GCP	Good clinical practice
GCS	Glasgow coma scale

GGT	Gamma-glutamyl transferase
GMP	Good manufacturing practices
HCT	Haematocrit
HIV	Human immunodeficiency virus
HO-1	Heme oxygenase 1
HREC	Human research ethics committee
IBSM	Induced blood stage malaria
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-8	Interleukin-8
INR	International normalised ratio
IQR	Interquartile range
IFN	Interferon
LDH	Lactate dehydrogenase
LFT	Liver function test
LLN	Lower limit of normal
MFA	Membrane feeding assay
MIC	Minimum inhibitory concentration
MiR-122	MicroRNA-122
NCE	New clinical entity
NR	Not recorded
PBMC	Peripheral blood mononuclear cells
PCB	Parasite clearance burden
$PCt_{1/2}$	Parasite clearance half-life
PD	Pharmacodynamic
PI	Principal investigator
PICF	Patient information and consent form
PK	Pharmacokinetic
PRN	Pro re nata (as needed)
PRR	Parasite reduction rate
QIMR	Queensland institute of medical research

qPCR	Quantitative polymerase chain reaction
RT-qPCR	Real time quantitative polymerase chain reaction
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
RBC	Red blood cell
RBWH	Royal Brisbane and women’s hospital
RCE	Red cell exchange
Rh	Rhesus
RhD	Rhesus D
SAE	Serious adverse event
SCID	Severe combined immunodeficiency
SD	Standard deviation
SMRU	Shoklo malaria research unit
SOP	Standard operating procedure
TNFα	Tumour necrosis factor α
ULN	Upper limit of normal
VIS	Volunteer infection studies
WBC	White blood cell
WCC	White cell count

ACADEMIC DECLARATION

I declare that this thesis has been composed by myself and that the work has not been submitted for any other degree or professional qualification. I confirm that the work submitted is my own.

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ATTRIBUTIONS

The production of standard operating procedures, conduct of laboratory experiments and assistance with the interpretation of laboratory experiment results in chapter 4 was conducted in conjunction with QIMR Berghofer researchers as summarized in Table 8.

Lachlan Webb statistician at QIMR Berghofer was involved in the production of the statistical analysis plan in chapter 5 and data analysis using Stata®.

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PUBLICATIONS DIRECTLY RESULTING FROM THIS WORK

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Odedra A, Webb L, Marquart L, Britton LJ, Chalon S, Moerhle JJ, Anstey NM, William T, Grigg MJ, Lalloo DG, Barber BE, McCarthy JS, 2020. Liver Function Test abnormalities in Experimental and Clinical Plasmodium vivax infection. Am J Trop Med Hyg. accepted for publication 5th July 2020.

ABSTRACT

Safety around the *Plasmodium Vivax* induced blood stage malaria model by Anand Odedra

Experimental malaria infection studies entailing the infection of healthy volunteers using the induced blood stage malaria (IBSM) model where blood stage *Plasmodium vivax* parasites are injected intravenously provide valuable information on parasite biology and the development of drugs and vaccines. A single serious adverse event could threaten the ongoing use of this and other experimental malaria infection models.

This thesis aimed to explore the safety of the *P. vivax* IBSM model, centring around an exploratory study using apheresis as a method to extract and concentrate all stages of *P. vivax* parasites from healthy volunteers infected with blood stage *P. vivax*. The absence of a method to culture *P. vivax* has hampered many aspects of *P. vivax* research. Apheresis harvested asexual parasites may be used to produce human malaria parasite banks, and gametocytes for cryopreservation as well as for mosquito feeding and subsequent attainment of sporozoites. The safety evaluation involved; a review of safety aspects across all malaria experimental infection models, a systematic review of the safety and efficacy of apheresis in the treatment of malaria and other infectious diseases, an assessment of the safety of apheresis for the harvesting of *P. vivax* parasites and analysis of post treatment transaminase elevations encountered in prior *P. vivax* IBSM.

No safety concerns were identified during the review of malaria VIS or the use of apheresis in the treatment of infectious diseases that were concerning enough to interfere with the plan for the exploratory study. Publications identified in the systematic review were case reports, case series, and cohort studies, thus publication bias was considered to be high. Apheresis may be a potentially useful adjunct to chemotherapy in the treatment of patients hospitalised for babesia, and prior to chemotherapy in loiasis with microfilarial count >8000/mL. The data did not support the use of apheresis in critical pertussis infection or for patients with severe *P. falciparum* malaria. No serious safety concerns were encountered in the four subjects involved in the exploratory study. Apheresis achieved a modest level of parasite

enrichment compared to whole blood sampling (4.9-fold and 1.45-fold per mL of sample for asexual parasites and gametocytes respectively) but was insufficient to meet the objectives for the collection of parasites for downstream research. Therefore apheresis should not be used as a method for harvesting *P. vivax* parasites. Post treatment transaminase elevations are transient, asymptomatic and appear to be more common in *P. vivax* IBSM than in natural infection. Evidence indicates that parasite clearance burden, haemolysis and systemic inflammation post anti-malarial treatment may be drivers for the observed transaminase elevations. However, the mechanisms underlying these abnormalities remain unclear and require further investigation.

CHAPTER 1 INTRODUCTION

In 2015 the World Health Organization (WHO) declared that the response to malaria is a global development priority and thus changed their recommendations from control to elimination[1]. This announcement came against a background of a decrease in malaria incidence and deaths over the last 17 years. However this parasitic disease still threatens almost half of the world's population and evidence suggests that since 2015 this downward trend in deaths has begun to plateau. In 2018, there were 228 million cases of malaria and 405,000 deaths[2]. Most malaria cases occurred in sub-Saharan Africa. However, Asia, Latin America, the Middle East and parts of Europe are also at risk.

Plasmodium falciparum is the most prevalent malaria parasite in Africa and is responsible for the vast majority of deaths from malaria worldwide. However, *Plasmodium vivax* has a wider geographical distribution. In 2015, 41% of the malaria cases that occurred outside the African continent were caused by *P. vivax,* resulting in an estimated 3,100 deaths. Thus without control strategies that consider *P. vivax*, global eradication will undoubtedly be impeded.

A mounting body of evidence opposes the traditional view that *P. vivax* is a benign disease. It has been estimated that 0.3% of cases are fatal, but mortality varies greatly between geographical areas, with seemingly higher numbers of severe cases in Papua New Guinea and India, and low numbers in the greater Mekong subregion, Afghanistan and Bangladesh[3, 4]. Individuals acquire immunity to *P. vivax* faster than with *P. falciparum* at any given transmission intensity[4, 5]. This is thought to explain why the majority of deaths occur in children from high transmission settings[3, 4, 6]. Deaths also occur in older patients with pre-existing co-morbidities from low transmission settings[7]. Careful consideration is required to quantify the contribution of *P. vivax* in any death, particularly in elderly patients with co-morbidities as *P. vivax* may be a contributor and not necessarily a cause of death. The number of reports of severe *P. vivax* have increased in recent years[4]. This could simply represent an increase in awareness and reporting. However, some studies suggest this may be a genuine increase in severe cases. Studies involving adults conducted in India suggest that rates of severe malaria have increased from 0.4% in 1944[8] to 2.2%

in 1982[9] to 14.8% in 2012[10]. However thrombocytopenia was used as the sole marker of severity in the majority (68%) of the severe cases in the 2012 study; this was not used in the 1944 and 1982 studies, making comparison difficult. Chloroquine resistance could explain the findings in Papua New Guinea but not necessarily the increases in severe cases in India, where chloroquine resistance is not widespread[11, 12, 13].

The morbidity that results from *P. vivax* is well recognised. Infections during pregnancy worsen outcomes for both the mother and child. Maternal anaemia from *P. vivax*[14] increases the likelihood of stillbirth, premature labour and low birth weight[15, 16]. This effect is compounded by the risk of repeated bouts of malaria in pregnancy from relapsing *P. vivax*, as primaquine is contraindicated due to the potential risk of haemolysis in a foetus whose glucose-6-phosphate dehydrogenase (G6PD) deficiency status is unknown[14, 17, 18, 19]. It is important to note that anaemia in the context of *P. vivax* is usually multifactorial with iron deficiency, pregnancy, primaquine therapy and hookworm infection all potentially contributing[20]. *P. vivax* is also responsible for severe anaemia in children[21] and often accounts for the majority of severe disease in some areas[22]. Malnutrition from *P. vivax* is another complication that may have long lasting effects, especially in childhood infections resulting in underweight children with an increased risk of stunted growth[23] and potentially lower IQ[24].

Undoubtedly malaria control and eradication efforts have been focused on *P. falciparum* with good reason. However, an argument could be made as to whether the level of attention and subsequent funding for *P. vivax* compared to other malaria species is appropriate. Between 2007 to 2009 it was estimated that only 3.1% of global malaria spending was on *P. vivax*[25]. A search exercise using the G finder public search tool database identified research and development funding on malaria of $624 million in 2017 compared with spending on *P. vivax* totalling $61 million or 10.2% of overall malaria funding[26]. Moreover as the prevalence of *P. falciparum* decreases over time, *P. vivax* could become the dominant parasite in many areas. Thus it would be prudent to invest in developing and implementing strategies and interventions now in preparation for this. To maximise potential benefits, control measures should ideally target both *P. falciparum* and *P. vivax*. Indeed better control

of *P. falciparum* may well reduce the number of *P. vivax* cases by decreasing the number of *P. vivax* relapses triggered by *P. falciparum* infection[27].

Perhaps the most striking example of the need for greater research and development in *P. vivax* is the reliance on primaquine for the eradication of liver stage hypnozoites, responsible for an estimated 70 to 80 million cases of relapsing malaria each year[28]. Primaquine has significant drawbacks including the need for 14 days of therapy to clear hypnozoites, often resulting in low adherence, and thus failure of hypnozoite eradication leading to ongoing relapses and transmission. Unobserved primaquine therapy has been documented as having success rates as low as 12%[29]. Whilst the recent introduction of tafenoquine is likely to dramatically improve adherence as eradication of hypnozoites only requires a single dose of 300mg, it is not a definitive answer. Like primaquine, tafenoquine can potentially cause life threating haemolysis in patients with certain forms of G6PD deficiency, which affects around 400 million people worldwide[30]. Furthermore, even in the setting of G6PD heterozygosity, females are at risk of significant haemolysis[31] such that some authors advise primaquine only in those with G6PD activity >30% and tafenoquine in those with >70% activity[32]. The higher threshold for tafenoquine is because any haemolytic effects due to tafenoquine cannot be limited by halting treatment as occurs with primaquine as it is a single dose regime[33]. This could result in an estimated 8-19% of women being ineligible for primaquine and 50-70% of women being ineligible for tafenoquine[34]. Furthermore the limited availability of G6PD testing in endemic areas means that clinicians are reluctant to use primaquine. Current guidelines suggest those with confirmed G6PD deficiency may be considered for weekly treatment with primaquine 0.75mg/kg for 8 weeks, with close monitoring for haemolysis[35]. However this relies upon the presence of reliable G6PD testing as well as sufficient healthcare resources and the willingness of the patient to attend follow up; all of which are prohibitive factors in the endemic setting.

Although funding is part of the issue, there are other reasons why no real acceptable replacement to primaquine has been developed in the more than 65 years since it was first licensed. One of these is the inability to reliably culture *P. vivax* in vitro. This is primarily due to *P. vivax* exclusively invading reticulocytes[36]. The result is a lack of parasite availability which hampers the ability of researchers to accumulate basic

efficacy data on treatments against *P. vivax*. To address the need for reticulocytes in culture media, reticulocyte rich blood from haemochromatosis patients has been used[37] with some success, but not sufficiently to provide the reliable supply of *P. vivax* parasites required by researchers[38]. In particular, a reliable source of gametocytes and sporozoites has remained elusive. As sporozoites are required to establish hypnozoites, this is a major restriction in the study of new hypnozoiticidal drugs. Alternatively parasites can be attained ex vivo. However, this entails an expensive, logistically complex and unreliable process where *P. vivax*-infected mosquitoes are sourced from endemic areas of Asia. In addition to the logistic issues, parasites obtained in this fashion are not genetically homogenous. Thus experiments must take into account the effects of strain variability.

The definitive step with any potential intervention is establishing safety and efficacy in the target population via a randomised control trial, but earlier phase clinical evidence is needed before a new treatment can be tested against the current gold standard. In the absence of good in vitro models, volunteer infection studies (VIS) provide an important bridge between what can be achieved in the laboratory and studies involving subjects in the endemic setting.

VIS involve the intentional infection of healthy volunteers with self-limiting or easily treatable infections. VIS has been used to collect data on pathogenicity and immune responses as well as to assess interventions in typhoid, influenza, respiratory syncytial virus and cholera to name a few[39, 40, 41, 42, 43]. The first ever cholera vaccine to be licensed by the Food and Drug administration (FDA) was approved based on evidence from VIS[42]. This circumvented the need to expose thousands of individuals in a large phase three trial, saving precious time and resources, as challenge studies can be carried out for a fraction of the cost of most large field studies[43].

VIS studies do have drawbacks. Findings in VIS may not necessarily be generalisable. For example malaria VIS often use a single clonal parasite such as the *P. falciparum* NF54 parasite or its derivative 3D7 to test new interventions[44]. The question arises as to whether the obtained results are translatable to the field, where many clones may be present in any given area[45, 46]. Another potential limitation is

that challenge studies looking at vaccine efficacy can only really be designed to assess short term protection[47].

VIS in malaria involves the introduction of malaria parasites into healthy volunteers either by mosquito bite, injection of sporozoites or by injection of parasitised red blood cells (RBCs). The latter is referred to as the induced blood stage malaria model (IBSM) and is the method used at QIMR Berghofer. *P. falciparum* VIS has been successfully employed in the investigation of new antimalarial drugs both for prophylaxis[48, 49] and treatment[50, 51], vaccine candidates[52, 53] and diagnostic tests[54, 55], in addition to facilitating research on parasite biology[56, 57] and host responses[58, 59]. IBSM studies have been established for *P. falciparum* and *P. vivax*[60]. The availability of the IBSM model offers a robust pathway to test the efficacy of vaccines and drugs using a small number of non-immune subjects, in a rapid and cost-effective manner[61, 62]. One of the advantages of the IBSM model is its ability to allow analysis of antimalarial efficacy, by providing opportunities to monitor parasite growth after challenge and decay after treatment. Moreover validation studies have shown a high correlation between endemic studies involving natural infection and malaria VIS when assessing the efficacy of new malaria vaccines or drugs[63, 64, 65].

The safety and utility of this approach for assessment of antimalarial efficacy has been augmented by the implementation of a rapid, and robust real-time quantitative polymerase chain reaction (qPCR) assay for the quantification of parasitemia[66]. More sensitive than thick blood smear, qPCR is able to detect and monitor malaria infection earlier and more accurately than blood film[67, 68]. Subjects undergo close monitoring and frequent sampling, only possible in a high-resource setting. QPCR testing allows for early detection and treatment of recrudescence in the event of vaccine or antimalarial drug treatment failure.

In addition, calculation of pharmacokinetic (PK) and pharmacodynamic (PD) parameters such as the parasite reduction rate (PRR) are possible, at much lower parasitemias than in natural infection in the field[69, 70]. Modelling of qPCR data can determine the timing of the qPCR nadir. This can be related to PK modelling to determine the concentration of test drug at the timepoint of nadir parasitemia

determined by qPCR which corresponds to the minimum inhibitory concentration (MIC). Thus IBSM is a valuable tool to help ensure that large, expensive and time-consuming phase II/III studies conducted in the endemic setting which expose subjects with potentially life threating infection to treatments other than the gold-standard, involve only the most promising candidates. If data collected during IBSM suggests a drug candidate is unsafe, or lacks the appropriate level of efficacy, development can be abandoned early and at a fraction of the cost of large phase II/III studies. The time and resources saved can be re-invested into other promising candidates. Currently there are more than a dozen potential antimalarial candidates under development[71]. Most of these compounds will eventually need to be placed into combination therapies, meaning that the number of potential combinations that require assessment is significantly higher. Hence IBSM is an ideal approach to meet demand for quick, efficient and reliable testing.

As well as testing drug treatment and vaccines, the IBSM model facilitates a number of basic science activities focusing on understanding parasite biology, genetics and immunology[44]. QIMR Berghofer is also developing the IBSM model to assess transmission of malaria[72]. This is on the back of recent successes in transmitting malaria from human volunteers to laboratory reared mosquitos[73], potentially offering an opportunity to assess transmission blocking interventions. However, all of these potentially beneficial outcomes are heavily dependent on the safety of the *P. vivax* IBSM model. A single death or even serious adverse event, such as a seizure or persisting renal impairment, would not only threaten the IBSM model but have implications for the use of all VIS models.

This thesis explores the safety of the *P. vivax* IBSM model, centering around an exploratory study aimed at using apheresis to harvest all stages of *P. vivax*. The exploratory study was intended to provide a potential solution to the current issue of poor availability of *P. vivax* parasites for pre-clinical and clinical research. Ultimately the aim was to generate asexual parasites to produce a human malaria parasite (HMP) bank, and gametocytes for cryopreservation as well as for mosquito feeding and subsequent attainment of sporozoites from mosquito salivary glands. The specific aims of the study were to:

a) Explore the utility of apheresis to safely harvest all life cycle stages of *P. vivax* parasites from healthy volunteers infected with blood stage *P. vivax.*
b) Review the safety and efficacy of apheresis in malaria and other infectious diseases.
c) Review of safety concerns specific to malaria VIS.
d) Assess the cause of liver function test (LFT) abnormalities encountered in prior *P. vivax* IBSM studies.

CHAPTER 2 SAFETY IN MALARIA VOLUNTEER INFECTION STUDY

INTRODUCTION

In malaria volunteer infection studies (VIS) healthy volunteers are infected with Plasmodium parasites through bites from infected mosquitos, injection of cryopreserved sporozoites or injection of blood stage parasites. I reviewed the safety risks and the techniques used to protect study subjects, staff and the wider community from these risks. Safety concerns and mitigation strategies from all methods of malaria VIS are discussed as they were thought relevant to the safe conduct of future *P. vivax* induced blood stage malaria (IBSM) studies. The safe use and containment of infectious material and the mitigation of other potentially hazardous biological risks was of relevance to the plans for the apheresis exploratory study. In addition, specific safety concerns surrounding the conduct of malaria VIS in endemic settings were also included.

TYPES OF MALARIA VOLUNTEER INFECTION STUDIES

Of the three forms of malaria VIS, bite from an infected mosquito (mosquito challenge) represents the most natural method. *Anopheles* mosquitos, typically laboratory reared *An. stephensi* are rendered infectious by feeding on in vitro cultured *P. falciparum*, or in the case of *P. vivax* by feeding on infected patients because in vitro culture is not possible[74]. A less natural mode of infection is needle-based intravenous inoculation with aseptic purified cryopreserved sporozoites. Currently, only *P. falciparum* cryopreserved sporozoites are available but it is possible cryopreserved sporozoites could become available for other plasmodium species in the future. Finally, IBSM involves the intravenous injection of malaria-infected red blood cells (RBCs). Each method has specific safety risks and mitigation strategies shown in Table 1 below.

Table 1. Summary of safety risks and mitigation strategies in malaria VIS

Safety risk	**Type of VIS in which risk occurs**	**Mitigation strategies for risk**
Transfusion-related infections	IBSM	Screening and microbial contamination testing of HMP bank and challenge agent Leukodepletion of HMP bank
Co-infection with other malaria species	Mosquito, sporozoite, IBSM	Donors screened to exclude mixed malaria infection
Co-infection with filariasis	Mosquito *P. vivax*	Donor screened for filariasis
Transfusion reaction	IBSM	Subject RBC antibody negative at screening Inoculation of subjects with compatible blood group and rhesus type Small numbers of RBCs in challenge agent
Alloimmunisation	IBSM	Inoculation of subjects with compatible blood group and rhesus type RBC antibody negative at end of study
Higher malaria inoculation dose than planned	Mosquito, sporozoite, IBSM	Manufacturing process controls for consistent inoculation dose (sporozoite[75] and IBSM)
Myocarditis	Mosquito, sporozoite, IBSM	Exclusion of subjects at increased risk of

		cardiovascular disease
Relapse from *P. vivax* hypnozoites	Mosquito, *P. vivax*	Exclusion of subjects with low CYP2D6 and G6PD activity at screening Primaquine treatment at end of study
Haemolysis from primaquine treatment	Mosquito, sporozoite, IBSM	G6PD testing at screening
Exposure of study staff to infectious materials	Sporozoite, IBSM	Standard personal protection equipment
Onward malaria transmission	Mosquito, sporozoite, IBSM	Confinement of subject Travel restriction Antimalarial drug treatment that eradicates gametocytes PCR negative at end of study Blood donation restrictions Standard personal protection equipment
Escape of infected mosquitos	Mosquito, sporozoite, IBSM (with transmission studies)	Insectary controls

HMP; human malaria parasite, IBSM; induced blood stage malaria, PCR; polymerase chain reaction, RBC; red blood cell, CYP2D6; Cytochrome P450 2D6. Table summarising the safety risks identified and mitigation techniques utilised in malaria VIS.

RISKS TO SUBJECT

CROSS INFECTION

Human malaria parasite (HMP) banks are collections of malaria parasites contained within human blood products[76] and can be used as the ultimate source of parasites

for all three methods of malaria VIS. Currently in vitro culture is only possible for *P. falciparum*[77] and *P. knowlesi*[78, 79] with the latter having not been used for VIS in the modern era. In vitro culture permits the production of *P. falciparum* HMP banks in good manufacturing practice (GMP) conditions, thereby permitting the selection of parasite strain for all types of *P. falciparum* VIS, and donor blood type for IBSM[76, 80].

HMP banks can be produced from donors (ex vivo from parasitemic volunteers infected via mosquito bite or from IBSM from existing banks, or by collection of parasitemic blood from naturally infected subjects [returned travelers with malaria]), providing donors are adequately screened. Donors should complete a lifestyle questionnaire to identify risk factors for transfusion-transmitted diseases (e.g. blood borne viruses, prion diseases, Q-fever, leptospirosis, brucellosis, and Chagas disease). At QIMR Berghofer, this questionnaire is based on eligibility criteria for blood donation in Australia. Donors for HMP banks are screened, using sensitive PCR and serology assays, for a wide range of blood borne viruses (Human immunodeficiency virus [HIV] 1 and 2, human T-lymphotropic virus, Epstein–Barr virus [EBV], cytomegalovirus [CMV], hepatitis C, hepatitis B, parvovirus B19, West Nile virus, Ross River virus, Barmah Forest virus, dengue fever, and human herpes virus 6 and 7). Leukodepletion of collected blood is routinely practiced and provides an additional level of security by removing cell-associated herpes viruses such as CMV and EBV that are carried by leukocytes[81]. This obviates the need to match the serostatus of the donor and recipient for CMV and EBV. Because *P. vivax* cannot be cultured in vitro, mosquito bite inoculation can only occur if the mosquitos have been fed on infected donors. Therefore, these infected donors may require screening for other diseases transmitted by *An. stephensi* mosquitos including lymphatic filariasis. Although filarial parasites could theoretically be transmitted by blood transfusion, when microfilariae are transmitted through blood transfusion they cannot develop into adult worms and thus the risk of harm is very unlikely[82, 83].

HMP banks produced through continuous in vitro culture must be tested for adventitious agents using validated assays as required by regulatory agencies. At QIMR Berghofer, HMP banks are tested prior to release using a process that has

been subject to regulatory review. This includes testing for microbial contamination and endotoxin. Whole genome sequencing of HMP banks can identify contaminants as well as parasite genotype, clonality and parasite polymorphisms associated with antimalarial sensitivity[84, 85].

TRANSFUSION REACTION

A transfusion reaction is a risk specific to IBSM studies. The IBSM process inevitably results in transfusion of a small number of RBCs (up to 1.5×10^9 in the case of *P. vivax* IBSM at our center), which is equivalent to less than 1 mL of whole blood. Subjects are only inoculated if they have an ABO compatible blood group (and compatible rhesus group in the case of females of childbearing potential). It is remotely possible that the presence of a donor’s RBCs could precipitate a transfusion reaction, or cause development of alloantibodies that may make future blood transfusion more difficult or result in hemolytic disease of the newborn if a woman develops alloantibodies due to this process before becoming pregnant. Acute transfusion reactions are judged to be extremely unlikely because of the very small volume of blood that is administered with the challenge agent, and because white cells are removed by leukodepletion during processing. No acute transfusion reactions have been reported in the more than 380 subjects who have been infected through IBSM[86]. Nevertheless, subjects are monitored for transfusion reactions after receiving the challenge agent. Subjects are screened before inoculation and at the end of the study for RBC alloantibodies. Two subjects have been reported to develop RBC alloantibodies in the context of IBSM. One was injected with *P. falciparum* 3D7 infected blood group O (RhD) negative RBCs and developed an anti-E antibody at the end of study blood sampling (Australian and New Zealand Clinical Trial Registry [ANZCTR] ID: ACTRN12614000781640). Adsorption studies confirmed the presence of a true anti-E alloantibody. No irregular anti-RBC antibodies were detected in a sample taken 6 weeks earlier. In addition, the RBCs in the HMP bank were documented to lack the E antigen. Although anti-E antibodies have been implicated in hemolytic transfusion reactions[87, 88], it is well established that natural anti-E antibodies may occur without transfusion[89, 90]. A transfusion medicine expert concluded it was most likely that this subject had naturally occurring low level anti-E alloantibodies and that it was unlikely that these alloantibodies were induced in the

study. The second subject participated in a vaccine study in which they were injected with 3×10^7 chemically attenuated asexual whole *P. falciparum* parasites contained within blood group O (RhD) negative RBCs[86]. Parasites were derived from cultures with 5% parasitemia, making the total number of injected RBCs 6×10^8. This subject was the only one of six subjects, who developed antibodies to the minor Rh antigen c. Whether this was related in some other way to preparation of the vaccine is unknown. It is not clear why the vaccine induced an antibody response while there have been no such cases within IBSM studies with *P. vivax* (44 subjects)[60, 72] or *P. malariae* (two subjects)[55] where a mean 6.5×10^8 and 6.8×10^8 RBCs were administered per challenge agent syringe, respectively.

DOSE OF PARASITES

The number of parasites injected, i.e. the dose, is a determinant of the starting blood stage parasitemia. When infection is induced by sporozoite inoculation, the number of infected hepatocytes will determine the starting blood stage parasitemia[91, 92], while in IBSM it is the actual number of parasites injected. Mosquito bite VIS typically involves five bites by infectious mosquitos. However, this entails uncertainty regarding the dose a subject receives, which can vary by several thousand sporozoites[93, 94, 95]. Each sporozoite that successfully establishes liver stage infection results in the production of 25,000-30,000 merozoites destined to invade RBCs. Thus, this variation can significantly impact the ultimate blood stage inoculum and the time to patency (detectable parasitemia). For studies using cryopreserved sporozoites and IBSM, it is possible to vary the dose of the challenge agent and ensure a reproducible process with reduced variation between study subjects[70, 91]. This results in a well characterised and uniform pattern of growth of parasitemia in vivo. The concentration of parasites in the HMP bank imposes practical limitations on the number of parasites inoculated. In theory, a single viable parasite is all that is needed, with the duration of the prepatent period depending on the challenge agent dose.

SERIOUS ADVERSE EVENTS

No serious adverse events have been reported, apart from an episode of myocarditis in one volunteer and abnormal blood tests suggesting cardiac inflammation in two

volunteers, all infected by mosquitoes in the Netherlands. It is not clear if these events were related to the malaria infection[96, 97, 98]. As a precaution, subjects with significant cardiovascular disease risk factors are excluded at screening in VIS. At QIMR this involves the exclusion of subjects with a 5-year cardiovascular disease risk >10%[99].

RELAPSE OF PLASMODIUM VIVAX

For *P. vivax* mosquito bite VIS, researchers must ensure no liver stage hypnozoites remain at the end of the study. Although radical cure with primaquine had been considered satisfactory for this purpose, two subjects experienced multiple relapses of *P. vivax* following challenge via mosquito bite despite chloroquine and primaquine treatment[100]. These relapses were discovered to be caused by a previously unrecognised pharmacogenetic effect of polymorphism in the human cytochrome P450 isoenzyme 2D6 (CYP2D6)[101]. Both subjects were shown to have low activity of CYP2D6 resulting in them not transforming primaquine into its active metabolite. Therefore, individuals who are poor or intermediate metabolizers of CYP2D6 should not be enrolled in such studies. In addition, individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency should be excluded from sporozoite-induced *P. vivax* VIS as they cannot receive primaquine because of the risk of haemolysis. Female heterozygotes may also experience clinically significant haemolysis if moderate deficiency is not excluded by a quantitative assay[31]. Currently, it is not clear whether poor CYP2D6 activity also affects the activity of primaquine against gametocytes[102]. Although the recently registered 8-aminoquinoline, tafenoquine may provide an alternative, like primaquine, tafenoquine can potentially cause life threating haemolysis in G6PD deficient subjects. Furthermore, because of its long half-life the haemolytic effects of tafenoquine cannot be limited by halting treatment as can be practiced with primaquine as tafenoquine is a single dose regime[33]. Thus, a higher threshold of G6PD activity in female heterozygotes for use of tafenoquine (>70%) is required compared to primaquine (>30%)[31].

RISKS TO STAFF

Risks to staff performing VIS include bites from an infected mosquito, or inadvertent exposure to infectious material such as a needle stick injury in cryopreserved sporozoite studies and IBSM studies. Staff should use appropriate personal protective equipment and have clear guidelines on how to access an infectious disease physician for advice regarding malaria-specific treatment. If the apheresis exploratory study is successful in concentrating asexual malaria parasites this may theoretically increase the likelihood of malaria transmission to staff following exposure, increasing the case for antimalarial treatment post exposure.

RISKS TO THE COMMUNITY

MALARIA TRANSMISSION

Preventing onward transmission of malaria is vital. Malaria VIS has historically been conducted at a small number of research centers in developed countries with high levels of physical containment and health infrastructure (Australia, the Netherlands, the UK, and USA). At QIMR in Brisbane, Australia, subjects are inoculated at least 8 days before receiving any therapeutic intervention. *P. vivax* produces gametocytes earlier in infection[103] than *P. falciparum*, where gametocytes typically appear in the circulation 10 days after inoculation, and are therefore infectious from an earlier stage[73]. Subjects potentially infectious to mosquitos may be confined indoors to ensure they are not bitten by vector competent *Anopheles* mosquitos. More commonly, subjects are monitored as outpatients[104] and required to adhere to travel restrictions. At our center, subjects are required to not travel in the period between inoculation and curative treatment, to malaria-endemic countries or to northern Australia where *An. farauti* (the Australian malaria vector) is present[105]. In Queensland, *An. farauti* mosquitos are not found south of Mackay, 950 km north of Brisbane. No vector-competent *Anopheles* mosquitos are found in Oxford, UK or Nijmegen, Netherlands, although these mosquitos were once endemic in both cities[106, 107]. The vector-competent species *An. quadrimaculatus* is endemic to the east coast of North America[108, 109], and could conceivably result in local transmission in the context of VIS undertaken at centers in Maryland. Climate change models have predicted the expansion of malaria transmission zones in Australia, Europe and

North America[110], so researchers must remain vigilant of such changes and alter their practices accordingly.

Mitigation strategies to prevent onward transmission may need to be applied at the end of malaria VIS if infection has resulted in the development of gametocytemia. Subjects may require treatment with an appropriate anti-gametocidal agent (e.g. primaquine) prior to exiting the trial. Parasite-negative status can be confirmed using a qPCR assay that detects both asexual parasites and gametocytes[111, 112] and which is being increasingly used to confirm parasite clearance. Additional gametocyte-specific qRT-PCR assays, such as one that targets *pfs25* the abundant female gametocyte-specific mRNA present in female gametocytes, can also be used to confirm the absence of gametocytes[113]. The 18S qPCR used at QIMR Berghofer has a limit of quantitation of 111 parasites/mL. To transmit malaria in a 1 µL mosquito blood meal, the female *Anopheles* mosquito needs to take up one male and one female gametocyte. Thus, it is extremely unlikely that a subject would transmit malaria with a negative 18S qPCR. Strict enforcement of travel restrictions and qPCR negativity prior to the end of study is essential, particularly if the study entails the deliberate induction of higher gametocyte levels to test transmission blocking interventions[73]. Study subjects are not permitted to donate blood until 6 months after the end of the study in Australia, or 3 years in the USA.

MOSQUITO ESCAPE

Escape of a malaria-infected mosquito could result in difficult-to-diagnose and potentially fatal local malaria. Further, the local establishment of an exotic malaria vector would represent a serious breach of biocontainment. Recently updated guidelines from the American Society of Tropical Medicine and Hygiene provide strategies to mitigate risk of arthropod escape[114]. The primary method is the use of an appropriately secure insectary.

GENETICALLY MODIFIED PARASITES

Additional regulations, specific for each national jurisdiction apply to the use and potential release of genetically modified pathogens. This includes genetically modified malaria parasites that have been used in clinical trials ANZCTR Trial ID: 12617000824369[115, 116]. The inadvertent release of genetically modified parasites

into local malaria vectors could lead to unforeseen or additional negative effects beyond that caused by release of a wild type organism.

RISKS OF VOLUNTEER INFECTION STUDIES IN THE ENDEMIC SETTING

The development of malaria VIS in the endemic setting represents an important advance in the study of malaria infection in naturally-exposed populations[117, 118] but its introduction also poses additional logistic and ethical concerns. Of particular concern is the unintentional release of a genetically modified parasite or a strain not endemic in the area, such as the K13 artemisinin-resistant strain *P. falciparum* recently used in an IBSM study in Brisbane (ANZCTR Trial ID: ACTRN12617000244303 and ACTRN12617001394336). Measures to mitigate the risk of onward transmission from gametocytemic subjects, with any species of malaria, and the escape of infectious or non-infectious exotic mosquitos are still required[119]. For example malaria VIS conducted in the endemic setting have been known to confine subjects from initial infection to clearance of parasitemia to mitigate the risk of transmission[117]. Going forward, it is essential to ensure the availability of equivalent containment measures and qPCR to ensure comparable volunteer safety and biocontainment to existing malaria VIS models[65, 120].

CONCLUSION

In the context of malaria VIS, it is not enough to react to safety concerns as they occur. Researchers must remain vigilant to potential risks including new ones induced by climate change, changes in regulations and new genetically modified parasites to ensure the high safety standards vital to the safe conduct of such studies. Further, maintaining an intact reputation of malaria VIS is essential for ongoing support from the scientific community, regulators, the community, and, most importantly, the subjects. No new mitigation techniques are required to protect staff or the community during the apheresis exploratory study. Subject specific concerns around the apheresis procedure are discussed in the following chapter.

CHAPTER 3 SAFETY AND EFFECTIVENESS OF APHERESIS IN THE TREATMENT OF INFECTIOUS DISEASES: A SYSTEMATIC REVIEW

INTRODUCTION

Whereas mitigation strategies against safety concerns are generally well established, the safety of apheresis in healthy volunteers infected with *P. vivax* is relatively unknown. However, there are data on the safety of apheresis in the treatment of severe *P. falciparum*. I reviewed this data and in light of the similarities between babesiosis and malaria[121, 122] I also examined the safety concerns encountered during apheresis in patients with babesiosis which were thought to be relevant to malaria. Furthermore, I conducted an assessment of efficacy, in addition to safety of apheresis in malaria and babesiosis, as well as collating data on loiasis and severe pertussis infection.

APHERESIS

Apheresis is the removal of a specific component of an individual's blood, with the remainder being returned to the individual. Apheresis may involve the removal of red blood cells (RBCs) (erythrocytapheresis), white blood cells (WBCs) (leukocytapheresis), plasma (plasmapheresis), or platelets (thrombocytapheresis). Currently, centrifugal apheresis is the preferred method whereby blood components are separated based on buoyancy. Modern automated apheresis systems are computer-controlled devices that undertake continuous removal, separation of the target component, and then return blood[123]. Advantages of apheresis over exchange transfusion include greater haemodynamic stability, lower risk of electrolyte imbalances, less chance of transfusion related complications including fluid overload, transfusion reactions and blood borne infections[124], and speed such that a whole blood volume can be exchanged in 1.5 hours[125] compared to 5 hours with an exchange transfusion[124]. The primary drawback of apheresis is its limited availability, predominantly in specialist centres, typically in high income settings.

Apheresis is generally considered to be a safe and well tolerated method of treatment as well as a method for collecting blood component donations from healthy

volunteers. Apheresis is an isovolaemic procedure hence there is thought to be little if any physiological challenge to the donor as a result of the procedure[126] therefore syncope and presyncope is less common than in exchange transfusion. The Australian haemovigilance report noted that in 2013-2014 (12 month interval), over 518,000 apheresis donations were undertaken with the total rate of adverse events of 212 per 10,000 plasmapheresis and thrombocytapheresis procedures[127]. There were no associated deaths. The most common adverse events associated with plasmapheresis were vasovagal (65/10,000), haematoma and bruising (13/10,000). Adverse events requiring hospitalisation were 2/10,000 and 4/10,000 for plasmapheresis and thrombocytapheresis respectively[127]. Most hospital attendances were brief and uneventful requiring no treatment. Except for citrate reactions which are known to be more common in continuous mononuclear cell procedures (CMNC), other adverse events are thought to be similar in frequency to other forms of apheresis. Citrate reactions are probably the most likely adverse events that may occur during an apheresis procedure. They are a result of low blood calcium levels caused by the anticoagulant citrate which is commonly used instead of heparin for apheresis due to its lower tendency to cause bleeding and its short half-life. Most patients present with mild tingling around the mouth, nose, ears and extremities[128]. Very rarely citrate reactions may be severe and can manifest with seizures and abnormal heart rhythms.

APHERESIS IN MALARIA

In the case of malaria, automated erythrocytapheresis, also known as red cell exchange (RCE), has been used with the rationale of reducing the parasitised RBC concentration by replacing Plasmodium-infected red cells with normal donor red cells. RCE, as well as exchange blood transfusion, were historically used as adjuncts to intravenous quinine therapy for severe *P. falciparum* malaria with hyperparasitemia (parasitemia >5%), as per World health organisation (WHO) recommendations prior to the availability of intravenous artesunate[129]. The rapid parasite clearance resulting from artesunate therapy has resulted in exchange transfusion and RCE falling out of favor as a treatment for malaria[130, 131]. The question arises as to whether apheresis should be reconsidered as an adjunct to

treatment for severe *falciparum* malaria in cases of hyperparasitemia with coexisting artemisinin resistance as it was during the pre-artemisinin era.

APHERESIS IN BABESIOSIS

Babesiosis is a rare potentially lethal vector-borne protozoan infection. The illness resembles malaria and, like malaria, babesia parasites infect red cells and can sequester[132]. Immunodeficient patients, particularly patients who are HIV infected, >50 years old, or with a history of malignancy and/or asplenia may develop severe babesiosis, which is a more serious illness, complicated by disseminated intravascular coagulopathy (DIC), acute renal failure and haemolytic anaemia. Most patients respond well to atovaquone and azithromycin or clindamycin and quinine (for severe cases)[133]. It is thought that RCE acts by physically removing infected red cells, thus lowering the parasite burden to a level where the immune system and antimicrobials can control the infection.

APHERESIS IN LOIASIS

Loiasis is a parasitic infection spread by vectors including the deerfly (Chrysops) and is endemic to west and central Africa. Clinical manifestations include Calabar swellings (non-tender swellings around joints in arms and legs) and eye worm (visualisation of adult worm passing across the conjunctivae). Rare complications include renal impairment, pneumonitis, painful lymphadenopathy, scrotal swellings and pleural effusions[134]. Apheresis can be used to reduce the parasite burden in loiasis prior to chemotherapy with diethylcarbamazine (DEC), with the aim to reduce the likelihood of treatment-induced side effects, particularly encephalopathy which can be fatal with high microfilarial loads (>30,000/mL)[135].

APHERESIS IN PERTUSSIS

Bordetella pertussis is spread by droplet transmission resulting in a respiratory illness. It can affect all age groups but is particularly problematic to infants. Severe pertussis is characterised by a prominent leucocytosis secondary to lymphocyte proliferation stimulated by pertussis toxin production[136]. This often leads to occlusion of the pulmonary vascular tree by abnormal leukocytes, and acute vasoconstriction mediated by endothelial dysfunction and/or toxin effects. This manifests clinically as pulmonary hypertension[137, 138, 139]. This condition has a high

mortality, even when managed with antibiotics, mechanical ventilation, inhaled nitric oxide and extracorporeal membrane oxygenation (ECMO)[140, 141]. In the acute setting it has been proposed that this occlusion could be rapidly resolved by the removal of excess WBCs. Leukapheresis, plasmapheresis and whole blood exchange transfusion have all been employed to alleviate the life threating manifestations of pertussis infection[142].

AIM OF REVIEW

The purpose of this systematic review is to investigate the safety and effectiveness of apheresis as adjuvant treatment for malaria, loiasis, babesiosis, and severe pertussis. Although in each of these conditions there is a biologically plausible rationale for the use of apheresis, ethical and logistical issues mean there is a low likelihood that randomised control trials will be undertaken to assess the safety and efficacy of apheresis in the treatment of these conditions. Thus, this systematic review of the existing literature is aimed at providing as much guidance as possible.

METHODS

The complete protocol for this systematic review can be found in appendix 1, the methodology is summarised below.

SEARCH STRATEGY AND SELECTION CRITERIA

The search strategy aimed to find both published and unpublished studies in which male or female patients of any age were treated using apheresis for the following infectious diseases: severe *falciparum* malaria (including artemisinin-resistant *P. falciparum*), loiasis, babesiosis, or severe pertussis.

Only studies in which automated apheresis (erythrocytapheresis, leukocytapheresis, and plasmapheresis) was used were included. Studies that evaluated whole blood exchange transfusion were excluded. However, in reports where exchange transfusion and apheresis outcomes were combined without any way to separate the data, the data were included.

A two-step search strategy was utilised. An initial limited search of MEDLINE, PUBMED, EMBASE and CINAHL was undertaken as follows (searches #1 and #2 were combined using AND).

Search #1

Blood Component Removal [MeSH] OR Cytapheresis [MeSH] OR Leukapheresis [MeSH] OR Plasma Exchange [MeSH] OR Plasmapheresis [MeSH] OR Plateletpheresis [MeSH] OR apheres* [ti.ab.kw] OR cytapheres* [ti.ab.kw] OR leukapheres* [ti.ab.kw] OR leukopheres* [ti.ab.kw] OR leukocytapheres* [ti.ab.kw] OR lymphopheres* [ti.ab.kw] OR lymphapheres* [ti.ab.kw] OR lymphocytapheres*[ti.ab.kw] OR lymphocytopheres* [ti.ab.kw] OR plateletpheris*[ti.ab.kw] OR thrombocytapheres*[ti.ab.kw] OR thrombocytophares*[ti.ab.kw] OR "red cell exchange" [ti.ab.kw]

Search #2

Malaria, Falciparum [MeSH] OR Loiasis [MeSH] OR Babesiosis [MeSH] OR Bordetella Pertussis [MeSH] OR "falciparum malaria"[ti.ab.kw] OR "plasmodium falciparum" [ti.ab.kw] OR loias* [ti.ab.kw] OR "Loa" [ti.ab.kw] OR babesi* [ti.ab.kw] OR pertussis [ti.ab.kw]

The indices of the following journals were hand searched: *Journal of Clinical Apheresis*, *Transfusion and Apheresis Science, Therapeutic Apheresis and Dialysis.* An analysis of the title, abstract, and keywords of all retrieved articles was undertaken to ensure the article fit inclusion criteria.

Secondly, the reference list of all identified reports and articles was searched for additional studies. Studies published in all languages were considered for inclusion in this review. Papers not written in English and identified as potentially relevant (based on their title and/or abstract) were translated as required. Because apheresis was introduced in the 1970s, searches were restricted to studies published from 1 January 1969 to 16 March 2018. Literature searches were not restricted to a type of study. Animal studies were excluded.

DATA EXTRACTION AND SYNTHESIS

The data extracted included specific details about the apheresis intervention, populations, study methods and outcomes of relevance to the review questions and objectives.

The characteristics of apheresis were recorded where documented including the apheresis protocol (e.g. number of cycles), apheresis equipment (e.g. Hemonetics™ model, COBE™ Spectra™, etc), and continuous or discontinuous removal and replacement of blood cells.

The effectiveness endpoints recorded were specific to each disease and were based upon previous studies of disease. For malaria, the endpoints were the reduction in percentage parasitemia, the clinical outcome (survival vs death), and complete recovery from severe malaria without lasting complications. For loiasis, the endpoints were the percentage reduction in microfilariae, reduction of microfilariae <8000/mL, and absence of adverse events from DEC treatment post apheresis. For babesiosis, the endpoints were the percentage reduction in parasitemia and clinical outcome (survival vs death). For pertussis, the endpoints were the percentage reduction in leukocyte count, and clinical outcome (survival vs death). The safety endpoints recorded were the number and type of adverse events and complications due to apheresis. If a range was given for parasitemia, the middle of the range was selected as the value.

All data were entered in duplicate. Effect sizes expressed as weighted mean or median differences (for continuous data) and their 95% confidence intervals [95% CI] or interquartile range [IQR] were calculated for analysis. Heterogeneity was explored using subgroup analyses based on the different study designs included in this review. Where statistical pooling was not possible, the findings were presented in narrative form including tables and figures to aid in data presentation where appropriate.

RESULTS

DESCRIPTION OF INCLUDED STUDIES

A flowchart indicating the systematic selection of publications for inclusion in this review is presented in Figure 1.

Malaria-related articles consisted of 19 case reports, 15 case series and 2 cohort studies. There were 5 studies that involved plasmapheresis only, 5 studies with plasmapheresis and RCE, and the remaining 23 studies involved RCE only.

Babesiosis related articles consisted of 14 case reports and 3 case series. Pertussis-related articles consisted of 4 case reports and 1 case series. Loiasis-related articles consisted of 10 case reports and 4 case series. Additionally, personal correspondence from Dr. Thomas Nutman from the National Institutes of Health outlining his experience with the use of apheresis in treatment of loiasis was included.

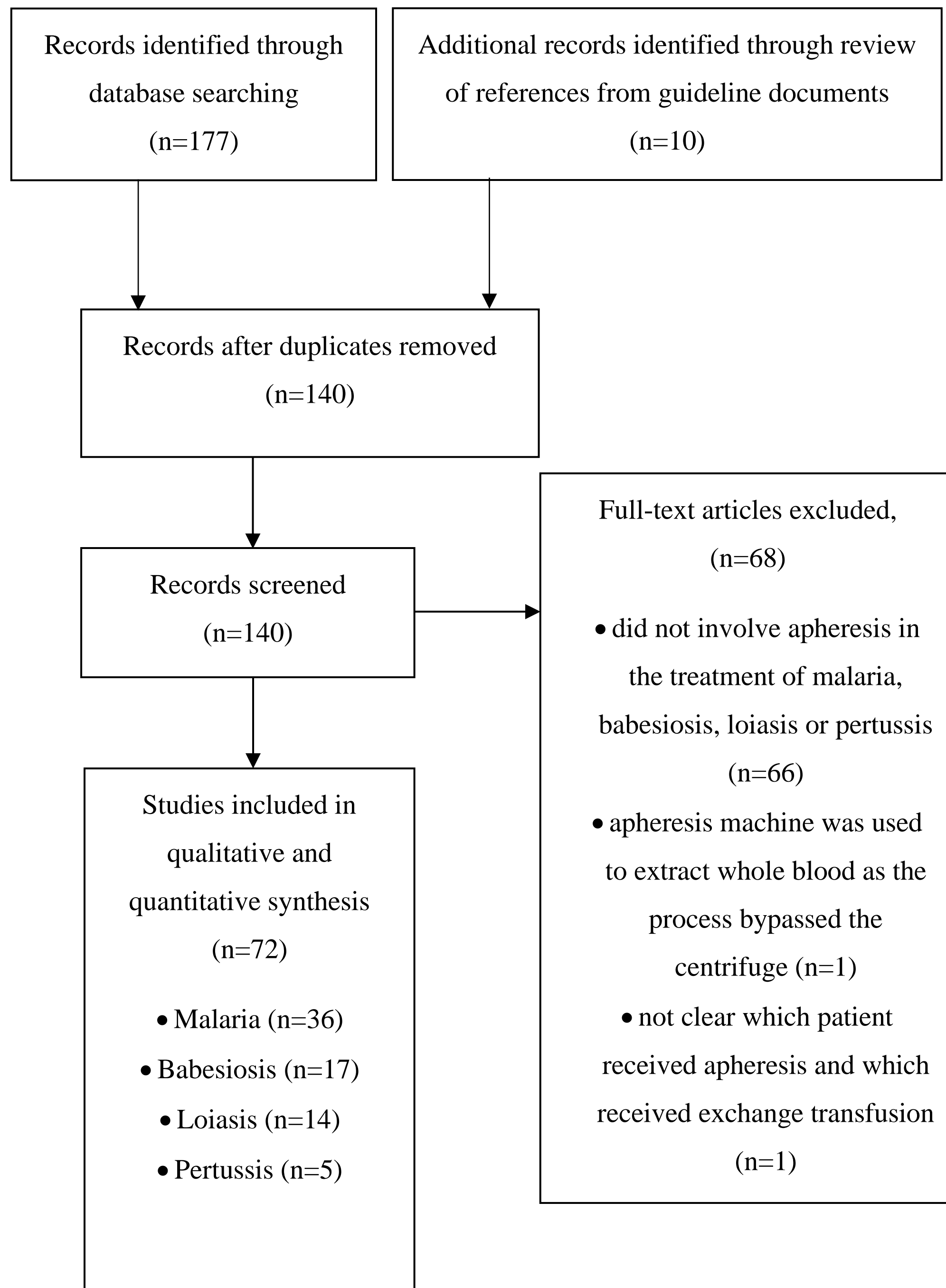
RISK OF BIAS

Apheresis is predominantly undertaken in high-resource settings, and also in institutions with sophisticated facilities and trained staff that improve the likelihood of survival (such as access to an intensive care unit and renal replacement therapy).

There are no randomised control trials assessing the use of apheresis in the treatment of any of the four diseases under study. This is primarily a reflection of the relatively low number of cases of severe *falciparum* malaria, severe babesiosis, severe pertussis and loiasis in locations, where apheresis is available. Thus, the potential bias is large as only case reports, case series and cohort studies have been published.

Publication bias will likely play a large part as studies that demonstrate perceived improvement and tolerance of apheresis, and survival, are more likely to be published. Although I included studies published in languages other than English (following translation), there may be a bias towards articles published in English as the journals where index searching was used published in English only. However, only one additional article was identified using the index searches of journals.

Figure 1. PRISMA flowchart indicating the systematic selection of publications for inclusion in this review



PRISMA flowchart indicating the systematic selection of publications for inclusion in this review.

APHERESIS IN THE TREATMENT OF MALARIA

A total of 36 suitable publications were identified that included data on 70 patients, with a total of 87 apheresis procedures. Individual data were available on 73 procedures and grouped data on 14 procedures. An additional 7 patients only underwent plasmapheresis, with a total of 14 procedures. The remainder of patients either underwent RCE alone or RCE and plasmapheresis. Of the 70 patients, 19 were from case reports, 42 were from case series and 9 were from cohort studies. All patients had confirmed severe malaria as per WHO criteria[28]. Table 2 summarises the main findings.

Table 2. Apheresis in treatment of malaria

Demographics	
Number of patients	70
Number of male patients	44 (62.9%)
Median age (IQR) in years	40 (26-49)
Number of apheresis procedures	87
Blood tests	
Median pre-apheresis haemoglobin (IQR) g/dL	9.80 (6.7-12)
Median platelet nadir (IQR) $\times 10^9$/L	27 (16-44)
Parasitemia	
Median (IQR) peak parasitemia	28% (14.5-46.5)
Median % reduction (IQR) in parasitemia for all apheresis procedures	80% (68.4-90)
Median % reduction (IQR) in parasitemia for 1st apheresis procedure per patient (n=50)	80% (68.4-90.9)
Median % reduction (IQR) in parasitemia for 2nd apheresis procedures per patient (n=44)	76.8% (42.9-90)
Log_{10} reduction (IQR) in parasitemia for all apheresis procedures	1.61 (1.2-2.3)
Log_{10} reduction (IQR) in parasitemia for 1st apheresis procedure per patient (n=50)	1.60 (1.2-2.4)
Log_{10} reduction (IQR) in parasitemia for 2nd apheresis procedure per patient (n=44)	1.46 (0.56-2.30)
Safety of apheresis	
Number of patients with AEs related to apheresis	3 (4.3%)*
Number of patients with AEs possibly related to apheresis	3 (4.3%)*
Clinical outcome	
Number of patients who made a full recovery	65 (92.9%)
Number of patients who died	2 (2.9%)
Number of patients who experienced complications	3 (4.3%)

IQR; interquartile range, AEs; adverse events

*The presence or absence of AEs was only specified for 35 patients; the percentage is based on the assumption that if AEs were not specified than none occurred.

Table detailing demographic, safety and efficacy data identified for the use of apheresis in the treatment of severe *P. falciparum* malaria.

In several studies, parasitemia estimates were not explicit (e.g. >70% or <1%); in these cases, parasitemia levels were designated as 70% and 1% respectively. There were seven patients where the peak parasitemia was described as greater than a specific value. In such cases this may be an underestimate of peak parasitemia. For a similar reason, it is possible that the level of parasitemia reduction may also have been underestimated. Of the five (7.1%) patients who were reported to not have completely recovered, two (2.9%) died. One patient had what the authors described as "minimal organ damage", one had persistent memory impairment and one developed persistent renal impairment (creatinine 165 µmol/L), peripheral neuropathy and retinopathy all thought to be secondary to malaria. One of the patients who died had a Glasgow Coma Scale (GCS) 3/15, and pinpoint pupils at presentation, suggesting that this patient's condition was unsalvageable. The patient who was reported to have persisting memory impairment, had a GCS of five prior to RCE, and required intensive supportive treatment, including mechanical ventilation, vasopressor support with norepinephrine, and dialysis.

A significant number of studies did not record (NR) whether their patients had cerebral malaria, pulmonary oedema or renal failure. Although it could be argued that these were not reported because they were absent, this cannot be stated with certainty. Hence, for cerebral malaria, pulmonary oedema and acute kidney injury, I carried out an additional analysis where NR is interpreted as not present (see Table 3). However, it must be emphasised that this additional analysis is likely to mean that I underestimate the frequency of these conditions.

The reporting of other complications, such as hyperbilirubinaemia, hyperlactataemia, hypoglycaemia and coagulopathy may have also been incomplete; they require blood testing for diagnosis. However, as the absence of reporting is less likely to represent true absence, they are not included in the additional analysis (Table 3).

Table 3. Characteristics of malaria patients treated using apheresis

	NR excluded	**NR interpreted as not present**
Cerebral malaria	33/62 (53.2%)	33/70 (47.1%)
Pulmonary oedema	24/58 (41.4%)	24/70 (34.3%)
Renal failure	39/64 (60.9%)	39/70 (55.7%)
Hyperbilirubinaemia	31/44 (70.5%)	N/A
DIC	16/39 (41.0%)	N/A
Hyperlactaemia*	12/16 (75.0%)	N/A
Hypoglycaemia	5/27 (18.5%)	N/A

NR; not recorded, DIC; disseminated intravascular coagulopathy.

*Included a cohort study with 5 patients with a median lactate of 5.1 mmol/L.

Table summarising the frequency of selected criteria for severe *P. falciparum* malaria in patients treated with apheresis. Frequency data is demonstrated both for when a severity characteristic is not recorded (NR) and is subsequently excluded and for when a severity characteristic is not recorded (NR) and is interpreted as not being present.

There were only three adverse events thought to be related to apheresis. These events were all transient hypotension; in one patient this was treated by fluid resuscitation and two others were simply monitored until resolution. Pulmonary oedema was later seen in two of these patients, although the reports suggest this was unlikely to have been related to apheresis[125, 143]. Of note, all 3 patients who had adverse events related to apheresis survived without any complications. Acute respiratory distress syndrome (ARDS) occurred post RCE in 3 of the 6 patients in a case report by Molla et al[144]. The authors comment that it is not clear if the ARDS was related to RCE or malaria; all had hyperparasitemia (23%, 58% and 80%), and drug treatment began shortly before apheresis.

It is notable that there were no adverse events reported in patients with anaemia or thrombocytopenia: Hb levels were not reported to be reduced in the 3 patients who developed hypotension (12.0 g/dL, 13.7 g/dL and NR).

APHERESIS IN THE TREATMENT OF BABESIOSIS

A total of 17 suitable publications were identified that included data on 22 patients who underwent a total of 29 RCE and 4 plasmapheresis procedures. Table 4 summarises the main findings.

Table 4. Apheresis in treatment of babesiosis

Demographics	
Number of patients	22
Number of male patients	16 (72.7%)
Median age (IQR) in years	64.5 (47-67)
Number of apheresis procedures	33
Parasitemia	
Median % Reduction (IQR) in parasitemia for all apheresis procedures	71.4% (60-91.7)
Median % Reduction (IQR) in parasitemia for 1st apheresis procedure per patient (n=15)	83.3% (57.6-96.7)
% Reduction in parasitemia for 2nd apheresis procedure per patient (n=2)	50%, 95%
Safety of apheresis	
Number of patients with AEs related to apheresis	0*
Clinical outcome	
Number of patients who died	4 (18.2%)

IQR; interquartile range, AEs; adverse events.

*The presence or absence of AEs was only specified for four patients.

Table detailing demographic, safety and efficacy data identified for the use of apheresis in the treatment of babesia.

No adverse events were reported although this was only specified for 4 out of 33 (12.1%) procedures. There were 4 deaths; one of the deaths occurred five days, and another six days after the blood film became negative for babesia. One patient had a background of chronic relapsing pancreatitis secondary to acute fulminant alcoholic pancreatitis and was due to have a total pancreatectomy or a biliary diverting procedure, but due to intra-operative complications a gastrojejunostomy was performed instead and the patient contracted transfusion related babesiosis. Babesia parasitemia improved from 30% to 6.6% following initiation of quinine and clindamycin and two RCE procedures. However, the patient's bilirubin continued to increase despite the insertion of a biliary drain and the decision was made to withdraw active treatment. In the final case the authors believed that the cause of death was myocardial infarction. In this case, babesiosis was thought to be a contributing factor. It is not possible to ascertain the true effect that babesiosis had in each of these deaths.

APHERESIS IN THE TREATMENT OF LOIASIS

A total of 14 identified publications included data on 34 patients with a total of 61 apheresis procedures. Table 5 summarises the main findings.

Table 5. Apheresis in treatment of loiasis

Demographics	
Number of patients	34
Number of male patients	25 (74%)
Median age (IQR) in years	32 (27-41)
Number of apheresis procedures	61
Microfilaraemia	
Median % reduction (IQR) in microfilaraemia per apheresis procedure for all apheresis procedures	51.7% (28.9-70.8)
Median % reduction (IQR) in microfilaraemia for 1st apheresis procedure (n=24)	60.3% (27.4-82.5)
Median % reduction (IQR) in microfilaraemia for 2nd apheresis procedure (n=9)	38.2% (32.6-50.4)
Median % reduction (IQR) in microfilaraemia for 3rd apheresis procedure (n=10)	52.2% (36.7-64.7)
Median absolute reduction (IQR) in microfilaraemia per apheresis procedure for all apheresis procedures	2400 (1250-6750) microfilariae/mL
Median absolute reduction (IQR) in microfilaraemia for 1st apheresis procedure (n=24)	2450 (1110-7100) microfilariae/mL
Median absolute reduction (IQR) in microfilaraemia for 2nd apheresis procedure (n=9)	6600 (2370-7200) microfilariae/mL
Median absolute reduction (IQR) in microfilaraemia for 3rd apheresis procedure (n=10)	1515 (1200-4900) microfilariae/mL
Number of patients with pre-apheresis microfilariae count >8000/mL with successful reduction to <8000/mL by apheresis	9/12 (75%)*
Safety of apheresis	
Number of patients with AEs related to apheresis	12 (35.3%)[#]
Clinical outcome	
Number of patients with DEC related AEs post apheresis	3 (8.8%)

IQR; interquartile range, AEs; adverse events, DEC; diethylcarbamazine.

*Only assessable in 12 patients; in the remaining patients it was not possible to confirm that this target was met as the patients either had microfilaremia <8000/mL prior to apheresis, or data on microfilaremia were incomplete.

#The presence or absence of AEs was only specified for 28 patients; the percentage is based on the assumption that if AEs were not specified than none occurred.

Table detailing demographic, safety and efficacy data identified for the use of apheresis in the treatment of loiasis.

The generally accepted aim of apheresis in loiasis is to reduce the microfilariae level to as low as possible prior to anti-filarial therapy with efforts made to target <8000/mL (although this level has been chosen relatively arbitrarily)[135, 145]. In 22 patients it was not possible to confirm that this target was met, as the patients either had microfilaremia <8000/mL prior to apheresis, or data on microfilaremia were incomplete. In the 12 patients that could be assessed, 9/12 (75%) reached this target. In the other three patients, there was a substantial reduction in parasitemia (15,000 to 10,666, 37,500 to 20,000 and 21,900 to 8,900).

A total of 12 patients were reported to have experienced adverse events related to apheresis. The most common adverse event was a reduction in platelet count. Other adverse events include reductions in lymphocyte count, haemoglobin and haematocrit. Lastly, difficultly in venous access was experienced in an obese woman (body mass index 40.1) and short-lived generalised weakness occurred following the procedure.

Personal communication from Dr Thomas Nutman from the National Institutes of Health was also received. Since 1987, 72 apheresis procedures have been carried out in 50 patients, most of whom had microfilariae levels >1000/mL. No adverse events related to apheresis have been reported. The team typically aims for a 7 L apheresis procedure focused at the monocyte interface (Buffy coat) for maximal microfilarial yield. No reliable data on reduction in parasitemia are available, given that multiple types of apheresis equipment were used over this 30-year period and standardised assessment of microfilarial counts were not performed on all individuals. It was also noted that carrying out procedures at midday and re-checking the microfilarial level

24 hours post apheresis is the only real way to provide accurate results about microfilarial clearance through apheresis because of the natural diurnal periodicity of microfilaremia.

APHERESIS IN THE TREATMENT OF PERTUSSIS

A total of 5 studies reporting patients who had undergone leukapheresis as part of treatment for severe pertussis were identified. All data were published relatively recently (from 2006 onwards). Table 6 summarises the main findings.

Table 6. Apheresis in treatment of pertussis

Demographics	
Number of patients	6
Number of male patients	0
Median age (IQR) in months	2.0 (1.3-3.0)
Number of apheresis procedures	8
Lymphocytes	
Median % Reduction (SD) in lymphocytes per apheresis procedure	61.3% (19.4)
Safety of apheresis	
Number of patients with AEs related to apheresis	2 (33.3%)*
Clinical outcome	
Number of patients who died	2 (33.3%)[#]

IQR; interquartile range, SD; standard deviation.

*The presence or absence of AEs was only specified for 4 patients; the percentage is based on the assumption that if AEs were not specified than none occurred.

[#]Survival was only specified for 5 patients; the percentage is based on the assumption that if survival was not specified than the subject did not die.

Table detailing demographic, safety and efficacy data identified for the use of apheresis in the treatment of pertussis.

All 6 patients were female, in contrast to the usual male predominance in severe pertussis disease. Two patients died; one case report did not record survival or death. The first death was due to intraventricular and respiratory hemorrhage and the second was a result of respiratory failure as a direct result of pertussis infection. Adverse events secondary to apheresis were reported in 2 patients. One patient experienced a fall in fibrinogen (270 mg/dL to 238 mg/dL) and platelet count (576×10^9/L to 163×10^9/L) and an increase in prothombin time (74 s to 85 s) and partial thromboplastin time (29.8 s to non-clotting). The other patient experienced a large drop in platelet count 706×10^9/L to 34×10^9/L. It may be inferred that these changes in clotting and bleeding tendency may have contributed to the intraventricular and respiratory hemorrhage and death.

DISCUSSION

The aim of the review was to analyse the safety and efficacy of apheresis in the treatment of severe *P. falciparum* malaria, severe babesiosis, severe pertussis and loiasis. The review highlights the relative lack of high quality, prospective studies for all four diseases.

MALARIA

Assessing the effectiveness of parasite reduction was difficult because many studies did not report parasite counts immediately before or after RCE; in some cases they were taken more than 24 hours after the procedure. This is compounded by sequestration of infected RBCs during the second half of the *P. falciparum* lifecycle, making the relative contribution of apheresis, sequestration or drug effect difficult to determine[146, 147, 148, 149, 150]. The median 80% (IQR 68.4-90) reduction in parasitemia per apheresis procedure may therefore overestimate the true reduction[151]. It should be noted that, independent of parasite clearance, other potential benefits of apheresis such as removal of toxins or cytokines[152, 153] could help improve overall prognosis; this could explain the clinical improvements experienced following plasmapheresis[153, 154, 155].

Complete recovery in 65/70 (92.9%) of patients in this analysis, was an interesting finding given that, the literature suggests, malaria patients treated with apheresis are typically more unwell than the general population with severe malaria[156, 157].

Specifically, this is evidenced by a higher Acute Physiologic Assessment and Chronic Health Evaluation (APACHE) 2 score (26 versus 17)[156] and a higher initial median parasitemia (46.5% vs 7.3%) in specific cohort studies. Anecdotally, several cases describe RCE as rescuing patients who were rapidly deteriorating [158], or clinical improvement soon after RCE or plasmapheresis[154, 155]. Plasmapheresis has also been used as an adjunct to antimalarial therapy[154, 155, 159], with or without RCE and as a potential treatment for thrombotic microangiography[154] and associated renal failure[154, 159]. However, the numbers are relatively small making interpretation of therapeutic benefit very difficult.

Adverse events were rare, even in patients with thrombocytopaenia or anaemia. Some RCE procedures were complicated by transient hypotension[143, 160], with most cases resolving without intervention or following fluid resuscitation[160], all patients made a full recovery from malaria. Molla et al[144] reported a case series involving 6 patients with severe *P. falciparum* treated with RCE. Three patients developed ARDS post RCE requiring mechanical ventilation. The authors could not determine if the ARDS was due to malaria or RCE but all three were at high risk of ARDS as they had hyperparasitemia (23%, 58% and 80%) and multiple features of severe malaria including cerebral (2/3), renal (3/3) and DIC (2/3). All patients who experienced possible adverse events from apheresis completely recovered.

This review specifically focused on the use of apheresis and not whole blood exchange transfusion. The WHO and Centers for Disease Control and Prevention (CDC) changed their guidelines regarding the use of exchange transfusion in severe malaria cases with parasitemia >5% following the introduction of artesunate with its rapid parasite clearance and superior efficacy over quinine[130, 131]. Tan et al[161] completed the most extensive review to date on the use of exchange transfusion in severe malaria. The overall mortality rate from the review was 17.8% and 15.9% for exchange transfusion and non-exchange transfusion respectively (OR 0.84; 95% CI 0.44-1.60). The review assessed exchange transfusion as a whole and did not distinguish between manual and automated exchange or the relative proportions of either mechanism of exchange transfusion[161]. The inference was that the majority of cases if not all were manual exchange. The commentary regarding apheresis largely focused on the rate of adverse events reported from a study by McLeod et al[162] with

an overall complication rate of 10.3% based on a total of only 78 procedures. The review article was published in 1999, it is likely that practices have improved greatly over the last 20 years resulting in fewer adverse events. This is a large study in terms of overall numbers, but it is relatively small in terms of RCE numbers, and although important to consider it, the data does not allow for any strong decisions to be made on the safety, or efficacy of therapeutic RCE.

The only randomised control trial assessing exchange transfusion in severe malaria occurred in Harare between 1986 to 1987. 8 patients with severe malaria were randomly assigned to receive antimalarials or antimalarials and exchange transfusion. All 4 patients who received exchange transfusion survived compared to only 1/4 who did not receive exchange transfusion. The authors reported that the result was not significant on Fischer's exact testing but did not provide any statistical measures. Mean parasitemia was 4.2% and 2.5% for exchange transfusion treated and non-exchange transfusion treated patients. The authors commented this could represent the non-parasite reduction related effects of exchange transfusion such as removal of toxic metabolites or deficient clotting factors.

In the context of increasing spread of artemisinin resistance, the question arises as to whether apheresis should be used as an adjunct to antimalarial drugs in those severe malaria patients at risk of having an artemisinin resistant parasite (e.g. returned traveller from Cambodia). In a multivariate logistic-regression model completed by Ashley et al[163] demonstrated an infection associated with a *kelch13* (K13) propeller mutation was substantially more likely to have a parasite clearance half-life longer than 5 hours (odds ratio, 94.7; 95% CI, 54.6 to 164.0; P<0.001)[163]. The parasite clearance half-life for quinine is 5.15 (IQR; range) (3.83-6.68; 0.86-105.5) compared to artesunate (non-resistant parasites) which is 3.11 (2.33-4.24; 0.59-34.28) hours[164]. This suggests that even in the presence of K13 mutations artemisinin therapy may still have a similar rate of parasite killing to quinine. However, given the increase in parasite clearance half-life where there is concern over the possibility of K13 mutation infections as well as starting artesunate therapy apheresis may also be considered, especially given its good safety profile. It is important to note that different K13 mutations infer different degrees of artemisinin resistance and therefore slowed clearance, although as time has progressed the dominant form of

K13 mutation has been the variant C580Y which has relatively moderate increases in parasite clearance time compared to other mutations[165, 166].

A clinician may be faced with a patient with severe *P. falciparum* from an area of high prevalence for artemisinin resistance and a high parasitemia, or alternatively they may not have access to artesunate in a timely manner or be unable to use artesunate, for example in the case of allergy (very rare[167, 168]). Given the low rate and short-lived nature of adverse events experienced during apheresis (4.3%), in such circumstances it may be considered as adjunctive treatment. If apheresis is undertaken and hypotension occurs, it would be more appropriate to stop the procedure given the data supporting the conservative use of intravenous fluid treatment in the management of severe malaria infection[169]. RCE should be carried out at least 3-4 hours post artesunate dosing to avoid removal of artesunate and its metabolite dihydroartemisinin[157, 170, 171]. Apheresis should only be used where there is local expertise and should not delay artesunate treatment.

Overall, the published data indicate that the use of apheresis in the context of severe *P. falciparum* is safe and may have some therapeutic benefit. However, given the lack of randomised control studies, there is insufficient evidence to support the general application of apheresis as an adjunct, use of apheresis as routine treatment, or its establishment in resource limited settings solely for the treatment of severe malaria.

BABESIOSIS

The use of RCE and apheresis in babesiosis have mainly been extrapolated from evidence in malaria. Babesia-infected patients with high parasite loads have been treated with adjunctive whole blood exchange transfusion, or apheresis-mediated RCE, to reduce parasite burden. Patients hospitalised with babesiosis have a mortality of 6-9%, with case fatality rates of up to 21% in immunocompromised patients[132, 172, 173]. However, the patients in this review appear to reflect the severe end of the spectrum, with a median parasitemia pre-apheresis of 20.25% compared to median parasitemia levels of 7.6% to 15.1% in most studies[132, 172, 173]. All but one of the patients identified during this review had identifiable risk factors for immunosuppression, and 4/22 patients died.

In terms of the safety of RCE in severe babesiosis, no adverse events related to RCE were reported. Furthermore, the low mean (SD) haemoglobin level pre-apheresis 7.6 g/dl (2.2 g/dl) suggests the procedure is safe and well tolerated even at low haemoglobin levels.

Guidelines from the Infectious Diseases Society of America suggest that RCE should be carried out in anyone with renal, liver, respiratory failure, significant haemolysis or high parasitemia[174]. They advise that one RCE volume is sufficient, as it replaces 85-90% of the patient's RBCs; this is the same advice given by the American Society for Apheresis (ASFA)[175]. ASFA suggests RCE for babesia infected patients with parasitemia >10%, or with significant comorbidities such as significant haemolysis, DIC, pulmonary, renal or hepatic compromise. In babesia-infected patients who are asplenic, RCE is recommended even in asymptomatic patients. In addition, plasmapheresis is recommended for critically ill asplenic patients not responding to chemotherapy or RCE, or with severe coagulopathy[175]. The data these recommendations are based on is considered weak.

With the high reliance on case reports and lack of randomised control trials, the available data are not of sufficient quality to support the routine use of RCE in babesiosis. However, given the high mortality rate in immunosuppressed hospitalised patients, the lack of adverse events from the apheresis procedure, and the low number of babesia-related deaths identified in this review, the data suggests RCE may be considered by clinicians as a useful adjunct to standard chemotherapy in hospitalised patients with babesia infection (especially those with immunosuppression). Plasmapheresis may be considered if there is evidence of haemolytic anaemia, or if there are specific concerns regarding renal failure (e.g. in the context of renal transplant).

LOIASIS

Apheresis is aimed at reducing the microfilarial load prior to treatment with DEC, reducing the likelihood of treatment related encephalopathy[135, 145]. This review demonstrated a median (IQR) parasitemia reduction of 51.7% (28.9-70.8%) across all apheresis procedures where both pre- and post-procedure data are available. Given the diurnal variation and potential for microfilariae to migrate from the

periphery[176], it cannot be assumed that this reduction is solely due to apheresis, but it is highly likely to be contributing.

Safety data suggests apheresis is an acceptable intervention as long as subjects have relatively normal platelet and haemoglobin counts. Given the clinical benefits of reducing the parasite count prior to anti-filarial treatment, and the good safety profile, the data support apheresis for the treatment of loiasis infected patients with high microfilarial counts.

PERTUSSIS

Severe pertussis cases make up only a small minority of the overall number of pertussis cases (3.2% in the United States)[177]. Severe cases can be complicated by a prominent leukocytosis[136] which leads to occlusion of the pulmonary vascular tree manifesting clinically as pulmonary hypertension[137, 138, 139]. Pulmonary hypertension is associated with high mortality, even when managed with mechanical ventilation, inhaled nitric oxide, and extracorporeal membrane oxygenation (ECMO)[140, 141].

I identified published reports on only six patients, of which two died. At least one death appears to have been related to apheresis[178]. The combination of uncertain efficacy and serious safety concerns therefore suggest that apheresis should not be used in the treatment of severe pertussis infection.

GENERAL CONSIDERATIONS

The study endpoint was parasite clearance for malaria and babesiosis, microfilarial clearance for loiasis and lymphocyte clearance for pertussis. This does not allow any firm conclusion for other biological benefits of RCE or plasmapheresis, or indeed clinical outcome. Indeed any observed clinical improvement post apheresis could also be due to a delayed effect from the curative chemotherapy. Of course it is important to remember that non-circulating parasites (malaria, babesia and loa loa) will not be removed during apheresis, and hence return to the circulation. Thus, increases in parasite burden following apheresis may not necessarily signify failure of apheresis, and repeat apheresis should not be ruled out.

This study assessed all the original published data on the use of apheresis in the treatment of malaria, babesiosis, loiasis and pertussis. No randomised trials were identified and only cohort studies, case series and case reports were available,

reflecting the fact that most of these conditions are rare in resource rich settings where apheresis is available. Overall, the small sample sizes, lack of hard clinical endpoints and limitations of study design issues make it extremely difficult to clearly identify beneficial or harmful effects of adjunctive apheresis on clinical outcome in any of the four conditions.

Where there is the potential for apheresis to be used to treat other conditions such as sickle cell disease, and where the health and financial incentives are present, apheresis may be able to establish itself in a resource limited setting. A randomised controlled trial initiated to study the use of apheresis in severe *P. falciparum* infection or loiasis may also be warranted. However, this may not be possible due to ethical concerns given the risk of potentially fatal encephalopathy and the relative lack of loiasis infected patients in areas where apheresis is available. In the case of severe pertussis and severe babesiosis, the issue is not one of mismatch between apheresis availability and disease endemicity but rather a lack of cases making a randomised control trial logistically difficult. A more realistic option would be to establish a managed database with voluntary reporting of all apheresis procedures carried out in the treatment of severe pertussis and severe babesia. There is no doubt that standardised recording and publication of data for any patients treated with apheresis would be valuable. In particular, accurate recordings of the parasitemia pre and post apheresis, safety data and clinical outcome data should be prioritised.

CONCLUSION

Existing data suggests that apheresis may be a useful adjunct to chemotherapy in the treatment of patients hospitalised for babesia, and prior to chemotherapy in loiasis with microfilarial count >8000/mL on the basis of reduction of parasite counts, but there are no clear clinical benefits. This review does not support the use of apheresis in critical pertussis infection or currently for patients with severe *P. falciparum* malaria. No safety concerns were identified during the review that were concerning enough to interfere with the plan to use apheresis as a method of harvesting all life cycle stages from subjects with induced blood stage *P. vivax* malaria. However, based on the findings in chapters 2 and 3 it was anticipated that the combination of apheresis and *P. vivax* IBSM may increase the likelihood and severity of existing safety concerns commonly observed in both apheresis and IBSM studies including

citrate reactions, bruising and haematoma, syncope and anaemia. Techniques aimed at mitigating these risks are discussed in the next chapter.

CHAPTER 4 APHERESIS OF SUBJECTS WITH INDUCED BLOOD STAGE PLASMODIUM VIVAX

INTRODUCTION

In the absence of a method of in vitro culture for *P. vivax*, the only way to source parasites is ex vivo from infected humans. This hampers all aspects of the development of tools to study this parasite and of tools to eliminate it. For example, to test and develop new hypnozoiticidal drugs that do not have the risk of significant haemolysis[33], a reliable source of *P. vivax* sporozoites is required. Currently this entails an expensive, logistically complex and unreliable process of sourcing *P. vivax* infected mosquitoes from endemic areas. In addition to the inherent logistic issues, parasites sourced in this way are not genetically homogenous. Thus experiments must take into account the effects of strain variability.

QIMR have successfully transmitted *P. vivax* parasites from healthy volunteers to *An. stephensi* mosquitoes and subsequently harvested sporozoites[179]. In one study 75 mL of blood was pooled from 6 subjects, parasites were concentrated by density gradient centrifugation and the concentrate was fed to 6 pots of 100 *An. Stephensi* mosquitoes[179].

Based on this study, an exploratory study was designed to assess the use of apheresis as a means to harvest and concentrate all stages of *P. vivax* including gametocytes from up to 8 human subjects infected with blood stage *P. vivax* malaria. Venesection cannot be used to select a specific haematocrit and therefore purified parasites cannot be sourced as efficiently as through apheresis. The harvested parasites could then be cryopreserved; the asexual parasites could then be used to produce a human malaria parasite (HMP) bank for use in future IBSM studies, while gametocytes may be used to infect *An. stephensi* mosquitoes. Previous work has established that cryopreserved *P. vivax* gametocytes are infectious to mosquitoes[180]. These infected mosquitoes, or sporozoites derived from them, could be used to infect healthy subjects in radical cure challenge models. Additionally, these sporozoites could be used in in vitro microfluidic devices that sustain human hepatocyte culture[181], as well as in humanised mouse models such as the severe combined immunodeficiency (SCID) mouse model which has shown potential to act as a model for the hepatic stage of

malaria infection[182]. Furthermore, apheresis could transform *P. vivax* mosquito membrane feeding assays, obviating the need for a gametocytemic test subject.

This chapter reports, for the first time, how apheresis was used as a method of acquiring purified *P. vivax* parasites from healthy human subjects infected with IBSM.

HYPOTHESES

- Apheresis can be used to extract and concentrate all stages of *P. vivax* parasites to a greater extent to that which can be attained by simple blood draws.
- Parasites harvested via apheresis can be cryopreserved.
- Asexual parasites extracted by apheresis can be used to produce a *P. vivax* HMP bank for future IBSM studies.

OBJECTIVES

PRIMARY OBJECTIVES

- To further assess the safety of the *P. vivax* IBSM model following inoculation of healthy subjects with *P. vivax* isolate HMPBS02-*Pv*.
- To assess the safety of apheresis in the *P. vivax* IBSM model following inoculation of healthy subjects with *P. vivax* isolate HMPBS02-*Pv*.

SECONDARY OBJECTIVES

- To assess the feasibility of apheresis as a method of extracting and concentrating all stages of malaria parasites following inoculation of healthy subjects with *P. vivax* isolate HMPBS02-*Pv*.
- To evaluate the success of cryopreservation of all stages of *P. vivax* parasites from blood products extracted via apheresis from healthy subjects inoculated with. *P. vivax* isolate HMPBS02-*Pv*.

EXPLORATORY OBJECTIVES

- To explore the potential for apheresis to be used as a method for producing a *P. vivax* human malaria parasite (HMP) bank to be used for future IBSM studies.
- To evaluate the transmission of *P. vivax* gametocytes to mosquitoes.

MATERIALS AND METHODS

A complete version of the study plan and specific safety concerns, and mitigation techniques regarding the use of apheresis in healthy volunteers infected with blood stage *P. vivax* including citrate reactions, syncope, bruising and haematoma, and anaemia can be found in the study protocol contained in appendix 2. Below is a summary of the main considerations.

RISK MANAGEMENT OF CITRATE REACTIONS

Citrate reactions are the most likely adverse events that may occur during the apheresis procedure. They are due to low blood calcium levels (hypocalcaemia) caused by the anticoagulant citrate. To reduce the chances of citrate reactions only subjects without a history of hypocalcaemia and with normal calcium levels on screening blood tests were included. If a subject developed signs or symptoms of hypocalcaemia during apheresis they would be advised verbally to commence a high calcium diet for 24 hours post-apheresis.

RISK MANAGEMENT OF PRE-SYNCOPE AND SYNCOPE

Subjects were most likely to experience syncope and pre-syncope during venous cannulation, often exacerbated by difficult venous access. To mitigate against this, only subjects with no previous history of syncope as a result of venous cannulation or venesection, and good venous access were included. Furthermore, blood draws from veins located in the ante-cubital fossa in the lead up to apheresis were avoided in order to preserve the veins.

RISK MANAGEMENT OF BRUISING AND HAEMATOMA DURING VENOUS CANNULATION

To counter the risk of bruising and haematoma only subjects with good venous access were included and blood draws from the veins in the ante-cubital fossa in the lead up to apheresis in order to preserve the veins.

RISK MANAGEMENT OF ANAEMIA

Statistical analysis of the trends in haemoglobin during *P. vivax* IBSM studies at QIMR Berghofer demonstrated a mean haemoglobin drop 10 days post-inoculation of 3 g/L and the mean maximum fall in haemoglobin at any point during previous *P. vivax* IBSM studies was 13 g/L with a standard deviation of 7 g/L. A double red cell collection apheresis procedure was estimated to cause a maximum drop in haemoglobin of 20%, approximating to 30 g/L. All subjects included in the study had normal haemoglobin levels at screening (≥135 g/L) thus, the maximum anticipated fall of around 43 g/L would leave a haemoglobin of 92 g/L, well above the cut-off for blood transfusion (70 g/L). STUDY DESIGN

I conducted a phase 1, exploratory study designed to determine the safety and feasibility of using apheresis as a method for extracting all lifecycle stages of malaria parasites from the blood of healthy subjects experimentally infected with blood stage *P. vivax*. This study was conducted in four sequential cohorts of one subject each (ANZCTR Trial ID: ACTRN12617001502325). The study was conducted at Q-Pharm Pty Ltd, Brisbane, Australia and the apheresis unit of the Royal Brisbane and Women's Hospital (RBWH), Australia between October 2017 and May 2019.

It was anticipated during planning that modifications specific of the apheresis procedure would likely be required during the study in order to meet the objectives. The changes made in the apheresis procedure between cohorts were based on the findings of previous cohorts. A number of laboratory based experiments were conducted during the study to aid with study planning and interpretation of study results.

See appendix 2 for full study protocol.

ETHICS

The study was conducted in accordance with the protocol approved by QIMR Berghofer human research ethics committee (HREC) and the RBWH HREC, the principles of the Declaration of Helsinki (recommendations guiding medical doctors in biomedical research involving human subjects, Fortaleza, Brazil 2013), the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Notes for Guidance on Good Clinical Practice (GCP) (CPMP/ICH/135/95), as adopted by the Australian Therapeutic Goods Administration (2000). All amendments and addenda to the protocol were similarly submitted to the QIMR Berghofer HREC and the RBWH HREC for approval prior to their implementation. All subjects gave written informed consent before being included in the study.

STUDY SUBJECTS

Healthy adult males and females aged between 18 and 55 years who met all inclusion criteria and none of the exclusion criteria were eligible for participation. Subjects were required to be malaria naïve, Duffy Blood group positive and have blood type O; females had to be Rh(D) positive, and available for a safety follow up period of three months. A full list of the inclusion/exclusion criteria for this study are included in the study protocol located in appendix 2.

STUDY CONDUCT

PRE-CLINICAL COMPONENT

A pre-clinical experiment was conducted prior to the inoculation of subjects in order to confirm the feasibility of harvesting Plasmodium parasites using apheresis.

The *P. falciparum* NF54 clone was used in these experiments[183] due to limited availability of *P. vivax* parasites. *P. falciparum* infected red blood cells (RBCs) (17.6 mL; 16 mL blood with 0.1% asexual parasitemia and 1.67 mL blood with 0.01% gametocytemia) was added to 450 mL of fresh venous whole blood and subjected to ex vivo apheresis. Samples were collected from the 1%, 2%, 3%, 5% and 7% haematocrit (HCT) layers as determined by visualising the colour saturation of the apheresis product. An automated haematology analyser (Sysmex XN-3000; Sysmex

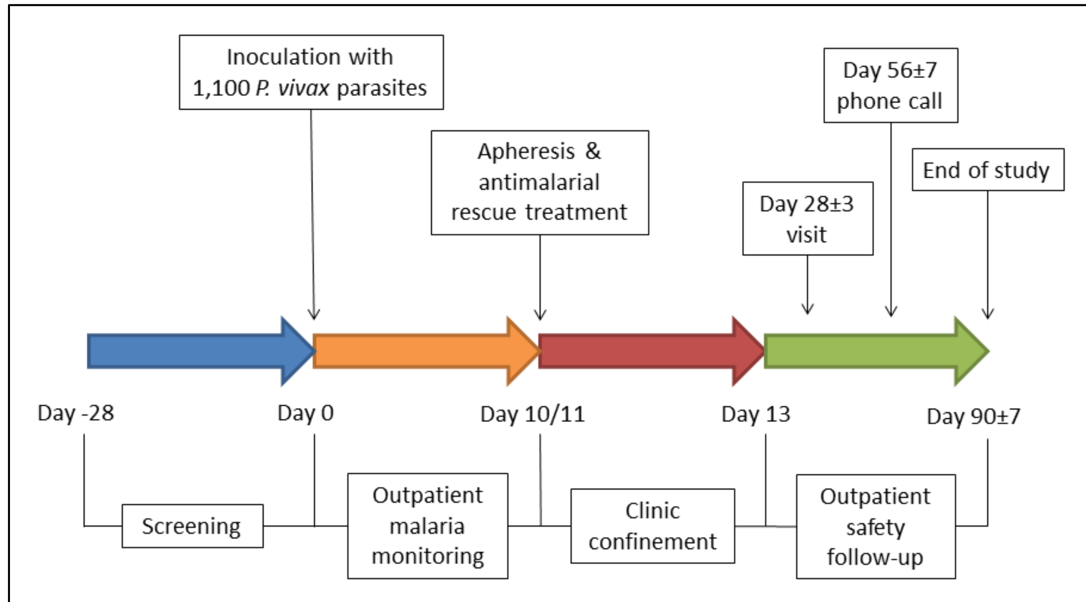
UK) was used to confirm the HCT of samples collected during apheresis. Presence of parasites was assessed in each layer by 18S qPCR[66] and microscopy.

CLINICAL COMPONENT

Following intravenous injection of *P. vivax* parasites (day 0), subjects were monitored by daily telephone calls until day 4, when subjects visited the clinical unit daily until the day of apheresis. Subjects were monitored for adverse events (AEs), signs and symptoms of malaria infection, and blood collected for 18S qPCR measurement of parasitemia.

The threshold for commencement of apheresis and treatment with artemether-lumefantrine was within 24 hours of a parasitemia >20,000 parasites/mL or the malaria clinical score reaching >6 (appendix 3) or at the Investigator's discretion. The morning that this threshold was reached (anticipated based on previous studies to be Day 10 or 11[179], subjects were admitted to the clinical unit (Q-Pharm) for initial safety assessments before being escorted to the Apheresis Unit at RBWH by Q-Pharm staff. The Apheresis Unit is located in the Haematology Department at RBWH where patients are subject to donor or therapeutic apheresis. At the Apheresis Unit the subjects underwent the apheresis procedure as per the Standard operating procedure (SOP) (appendix files 4 to 7) whilst being supervised by the apheresis specialist nurse and under the supervision of the responsible clinical haematologist. The apheresis specialist nurse was responsible for writing all SOPs. The same apheresis nurse performed the apheresis procedure for all four subjects. The apheresis procedure lasted 1-4 hours. Subjects were then escorted back to the clinical unit and began treatment with artemether-lumefantrine (Riamet®, Novartis Pharmaceuticals Australia Pty Ltd). Treatment consisted of six doses of 4 tablets at 12 hourly intervals (each tablet contains 20 mg artemether and 120 mg of lumefantrine). Subjects remained confined within the clinical unit for 48-72 hours for safety monitoring. Following release from confinement, subjects attended protocol specified visits until three months post treatment to monitor for signs of parasite recrudescence and to assess late safety signals. A schematic of the study design is shown in Figure 2.

Figure 2. Schematic of Apheresis study



Schematic illustrating the main study activities.

This study used an iterative adaptive design approach where subject safety and outcome data were analysed after each subject and modifications made to improve the chances of meeting the study objectives in the subsequent subject. A summary of the changes instituted is shown in Table 7.

Table 7. Summary of main study design differences between subjects

	Subject 1	**Subject 2**	**Subject 3**	**Subject 4**
Apheresis procedure	CMNC	CMNC	CMNC	Red cell depletion followed by CMNC on red cell depletion product
HCT layers sampled	1%, 2%, 3%, 5%, 7%	1%, 2%, 3%, 5%, 7%	0.5 %, 1%, 2%, 3%, 5%, 7%, 11%, 2-3%, 5-7%, 1-7%	From the primary apheresis (HCT): Intermediate (64%) From the secondary apheresis (HCT): Final (3%) Spare (5%) Waste (42%)
Apheresis timepoint	10	10	11 PM	11 AM
Mosquito feeding assay samples	Pre-apheresis (with-percoll enrichment), 1%, 2%, 3% HCT layers	Pre-apheresis (with-percoll enrichment)	Pre-apheresis (with and without-percoll enrichment)	Pre-apheresis (without-percoll enrichment), Intermediate, Final, Waste
Whole blood:citrate ratio during apheresis	15:1	8:1	8:1	13:1
Citrate added to apheresis collection bags	No	Yes	Yes	Yes
Biological duplicates*	No	No	Yes	Yes

CMNC; continuous mononuclear cell collection, HCT; haematocrit. Protocols for subjects 1 to 4 and all experiments can be found in appendix 4 to 11. *Biological duplicates involved repeat 18S qPCR testing from two separate blood samples from each HCT layer collected using apheresis.

Table summarising the key differences in planning across each of the four subject cohorts during the clinical component of the study.

Clinical and laboratory SOPs for cohorts 1 to 4 and all experiments can be found in appendix 4 to 11. Results for cohorts 1 to 4 and all experiments are located in the results chapter. The below table outlines the contributions provided by QIMR Berghofer researchers during the laboratory components of the study:

Table 8. Summary of contributions provided by QIMR Berghofer researchers

Researcher	Apheresis cohort laboratory activities				
	Pre-clinical	**1**	**2**	**3**	**4**
Katherine Collins (post doctoral researcher)	Transmission experiments conduct and interpretation of results	Not applicable	Not applicable	Not applicable	Not applicable
Rebecca Pawliw (research assistant)	Laboratory Protocol design	Laboratory Protocol design	Laboratory Protocol design	Laboratory Protocol design	Not applicable
Zuleima Pava Imitola (post doctoral researcher)	Not applicable	Not applicable	Transmission experiments conduct and interpretation of results	Transmission experiments conduct and interpretation of results	Transmission experiments conduct and interpretation of results Study design and execution of ex vivo culture
Maria Robelo (post doctoral researcher)	Not applicable	Not applicable	Not applicable	Not applicable	Flow cytometry conduct interpretation of results
Katherine Trenholme (post doctoral researcher)	Coulter cell counting	Coulter cell counting	Coulter cell counting	Not applicable	Not applicable
Jeremy Gower (research assistant)	Not applicable	Not applicable	Not applicable	Not applicable	Laboratory Protocol design flow cytometry conduct
Hayley Mitchell (research assistant)	Coulter cell counting transmission experiments conduct	Transmission experiments conduct and interpretation of results	Transmission experiments conduct and interpretation of results	Transmission experiments conduct and interpretation of results	Transmission experiments conduct and interpretation of results
Sean Lynch (research assistant)	Not applicable	Coulter cell counting	Coulter cell counting	Not applicable	Mosquito rearing and provision flow cytometry conduct

Table outlining the contributions provided by QIMR Berghofer researchers during the laboratory components of the study.

MALARIA INOCULUM

The *P. falciparum* NF54 clone was used in the pre-clinical stage[183]. The *P. vivax* HMP013 was derived from blood group O rhesus positive blood donated from a

returned traveller from India who presented with clinical manifestations of malaria[179]. The inoculum was prepared as previously described[184].

MEASUREMENT OF PARASITEMIA

Parasitemia was quantified using 18S qPCR targeting a highly conserved Plasmodium 18S ribosomal RNA gene[66, 185]. Quantitative reverse transcriptase PCR (qRT-PCR) assays were used to measure gametocytemia with assays targeting the *P. falciparum pfS25* and *pfMGET,* male and female gametocyte mRNA transcripts[186] respectively and *P. vivax pvS25* gametocyte mRNA transcripts[187].

MICROSCOPY

Slides were produced using Giemsa stain and examined by microscope under 100× oil immersion objective. Thick and thin slides were examined by level 1 or 2 World Health Organisation (WHO) certified microscopists. Apheresis samples were expected to have a significantly different composition in terms of proportions of red blood cells (RBCs) and white blood cells (WBCs) when compared to whole blood (e.g. RBCs make up 1% and approximately 46% of 1% HCT and whole blood samples respectively). As such traditional parasitemia measures were not feasible.

FLOW CYTOMETRY

Flow cytometry, using the CD71-APC antibody, was performed to characterise reticulocytes present in samples collected during the apheresis process in subject 4.

MOSQUITO FEEDING ASSAYS

Transmissibility of pre-apheresis samples and post-apheresis samples to *Anopheles stephensi* mosquitoes was evaluated using membrane feeding assays (MFA)[73, 179]. For enriched MFA (eMFA), gametocytes present in 80 mL of whole blood (pre-apheresis) were enriched in 70% percoll gradient. For direct MFA (DMFA), 650 µL of pellet from whole blood (pre-apheresis) or from each apheresis sample was reconstituted to 50% HCT with AB+ serum. Infection in midguts was assessed by qPCR[188] 8 days after the feeding assays. For logistic reasons, eMFA was not carried out in subject 4. Following consideration of gametocyte levels, DMFA was not carried out in subject 2.

APHERESIS PROCEDURES

Apheresis was carried out using a Spectra Optia v11.3 apheresis system (Terumo BCT, Inc Tokyo Japan).

STATISTICAL ANALYSIS

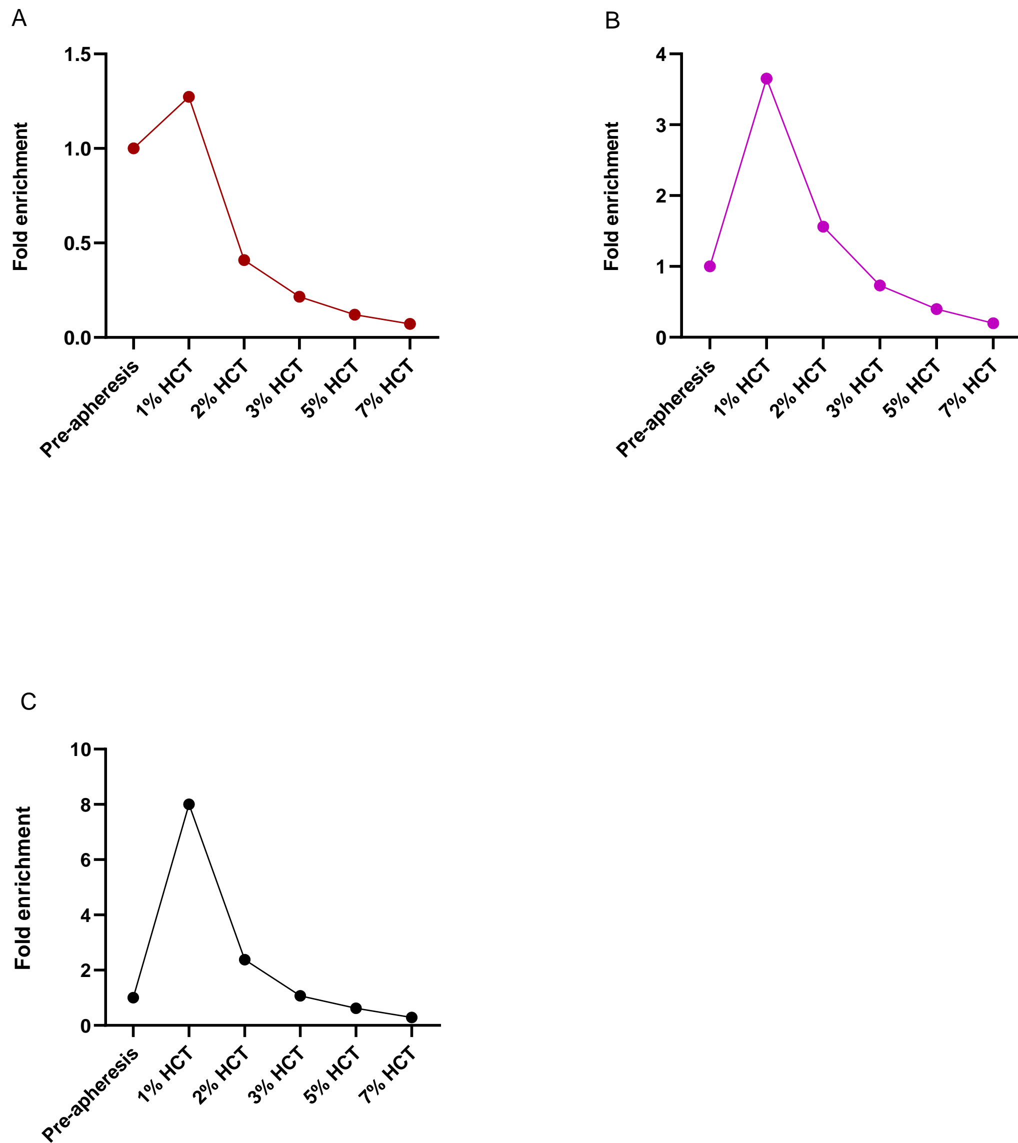
All measured variables and derived values were listed. Continuous data was summarised using descriptive statistics (mean and SD, or median and IQR). Categorical data was presented using N and % (using the number of subjects without missing data in the calculation). Unless otherwise stated, Microsoft Excel® version 1903 was used for statistical analysis. PRISM® was used for the construction of all figures.

RESULTS

PRE-CLINICAL EXPERIMENT

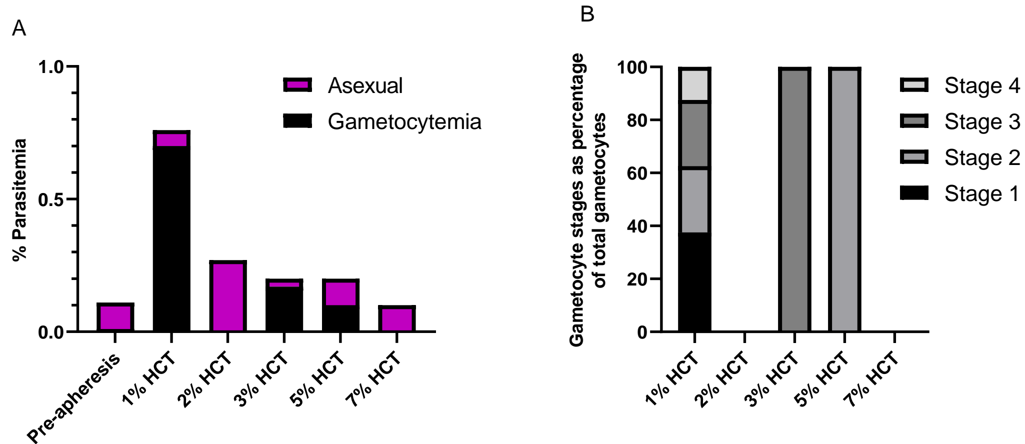
The feasibility of extracting Plasmodium parasites from blood using apheresis was initially assessed using cultured *P. falciparum* parasites. The 1% HCT layer contained the greatest concentration of all parasites as determined by 18S qPCR, with a 1.3-fold concentration of all parasites and a 3.7 and 8-fold concentration for female and male gametocytes, compared to pre-apheresis (whole blood)(Figure 3). The 2% HCT layer contained the greatest concentration of asexual parasites by microscopy (2.7-fold concentration; Figure 4A). The highest concentration of gametocytes detected by microscopy was seen in the 1% HCT layer (76-fold ; Figure 4B). Both asexual parasites and gametocytes were also visualised by microscopy in the 1%, 3% and 5% HCT layers (Figure 4B). These results demonstrated the technical feasibility of the approach and the experiment was allowed to proceed to the clinical stage.

Figure 3. Pre-clinical experiment



HCT; haematocrit. Fold enrichment of qPCR seen in samples taken during apheresis compared to the pre-apheresis sample. 3A 18S qPCR/mL for asexual *P. falciparum* parasites, 3B *pfS25* qPCR/mL for female *P. falciparum* gametocytes and 3C *pfMGET* qPCR/mL for male *P. falciparum* gametocytes.

Figure 4. Pre-clinical experiment



HCT; haematocrit. 4A microscopy of samples taken during apheresis showing levels of asexual parasites and gametocytes compared to the pre-apheresis sample. 4B staging of gametocytes identified during microscopy of samples taken during apheresis. Pre-apheresis sample did not contain any gametocytes.

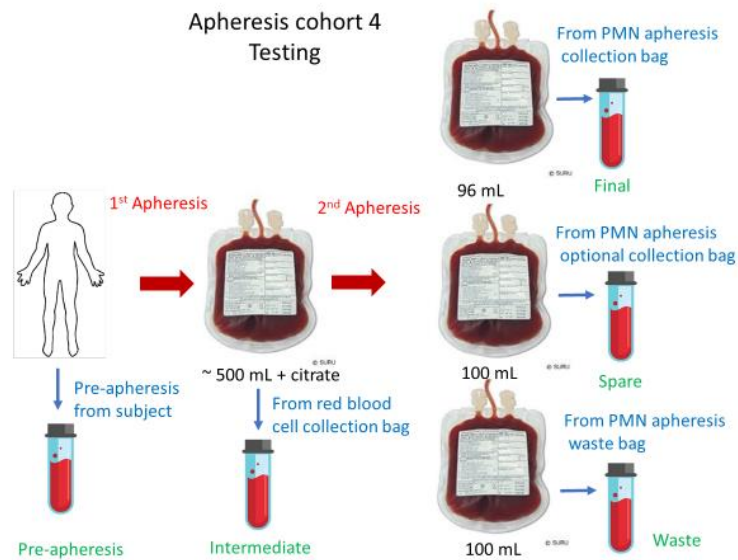
CLINICAL EXPERIMENT

All subjects became PCR negative for parasites within 72 hours of treatment initiation.

In order to meet the study objectives an iterative approach to study design was implemented. A summary of the key differences in the planning of the clinical trial in each of the four subject cohorts is shown in Table 7. Changes to study design for the subsequent subject cohort were made following review of previous subject data. To prevent sample clotting observed in apheresis collection bags from subject 1, subjects 2, 3 and 4 received higher concentrations of citrate during apheresis and citrate was directly added to apheresis collection bags pre-apheresis. Subjects 1 and 2 were treated with artemether-lumefantrine 10 days post malaria inoculation. In an attempt to augment pre-apheresis parasitemia, treatment of subjects 3 and 4 was delayed to day 11. Biological duplicates were employed in subject cohorts 3 and 4 to improve the validity of results based on low level parasitemia findings in subjects 1 and 2. Sampling below 1% HCT and above 7% HCT layer occurred in subject 3 as these layers had not been sampled previously. A red cell collection stage was added

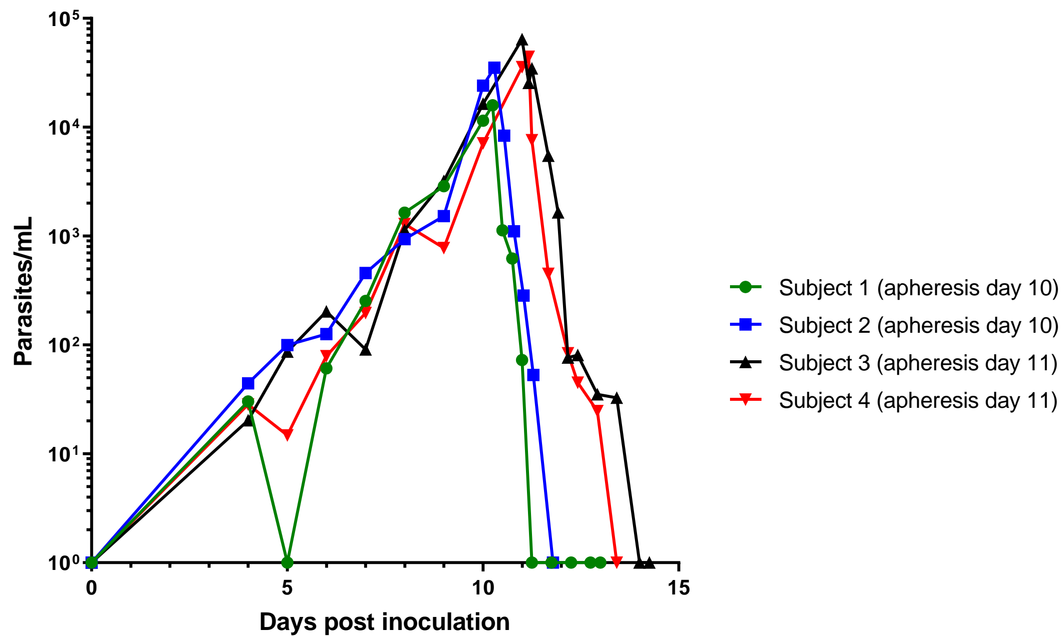
to the apheresis procedure in subject 4. During cohort 4 a RBC depletion was carried out in an attempt to target greater reticulocyte concentration, producing an intermediate bag sample, followed by a second apheresis procedure on the red cell depletion product. The second apheresis procedure involved sampling of ~100 mL of the lowest HCT layers of the sample (final bag) followed by ~100 mL of the subsequent lowest HCT layers (spare bag) and then the remainder (waste bag) (Figure 5). A schematic of the sampling that occurred during cohort 4 is shown below.

Figure 5. Schematic of cohort 4 sampling



PMN; polymorphonuclear. Schematic illustrating the origins of the pre-apheresis, intermediate, final, spare and waste samples collected during cohort 4. Cohort 4 involved two consecutive apheresis procedures. The first was a RBC collection conducted on the human subject. A continuous mononuclear cell collection (CMNC) was then conducted on the RBC collection bag.

Figure 6. Parasite growth curves



Parasitemia as measured by 18S qPCR in all four subjects presented using a logarithmic scale. Day 0 represents the day of inoculation. Apheresis occurred on day 10 for subjects 1 and 2, and day 11 for subjects 3 and 4.

SAFETY FINDINGS

A total of 68 AEs occurred in the 4 subjects (Table 9). No Serious AEs were reported. The majority of AEs were mild or moderate. Five severe AEs occurred in 4 subjects: one episode of neutropenia (0.68 × 10^9/L [0.45 × LLN]; duration 8 days), two of lymphopenia (0.43 × 10^9/L [0.43 × LLN] and 0.33 × 10^9/L [0.33 × LLN]; both lasting 3 days), and two of fever (both 40.2°C; duration 30 minutes and 25 minutes). All severe AEs were transient and resolved by the end of the study. The majority of AEs (54/68; 79.4%) were attributed to malaria, while 9/68 (13.2%) were attributed to apheresis. These included neutropenia (one subject nadir 0.68 × 10^9/L) which was recorded as three separate AEs due to changes in the common terminology criteria for adverse events (CTCAE) grading, two cases of lymphopenia (nadir 0.33 × 10^9/L and 0.75 × 10^9/L) and two cases of leukopenia (nadir 2.2 × 10^9/L and 2.4 × 10^9/L). One subject had an episode of herpes labialis (herpes simplex

virus-1 PCR positive). One subject experienced mild hypophosphatemia (0.70 mmol/L) of two days duration.

Table 9. Summary of the main safety findings

	Subject 1	Subject 2	Subject 3	Subject 4	Total AEs
SAEs	0	0	0	0	0
AEs	20	13	15	20	68
AEs related to malaria	13	8	14	19	54
AEs related to apheresis	2	3	0	4	9
Max temp °C	40.2	38.8	40.2	39.6	N/A
Max malaria clinical score	8	1	2	7	N/A
Ibuprofen use	400 mg × 5	400 mg × 5	400 mg × 5	nil	N/A
Acetaminophen use	1 g × 4; 500 mg × 1	1 g × 2	1 g × 4	500 mg × 2; 1 g × 2	N/A
Peak ALT (IU/L)	111	118	47	80	N/A
Peak AST (IU/L)	83	57	42	44	N/A
Platelet Nadir ($\times 10^9$/L)	119	98	99	75	N/A
Maximum drop in haemoglobin from baseline (g/L)	17	9	25	20	N/A
Lymphocyte Nadir ($\times 10^9$/L)	0.33	0.43	0.58	0.75	N/A
Peak parasitemia (parasites/mL)	15,943	35,156	64,243	44,431	N/A

SAE; serious adverse event, AE; adverse event, ALT; alanine transaminase, AST; aspartate transaminase.

Summary of the main safety findings encountered during the study.

APHERESIS CHARACTERISTICS

The RBC counts of the various HCT layers were generally in alignment with what would be expected (Table 10), except in two subjects. In subject 2 the RBC counts of the 2% HCT layer were closer to what would be expected from a 3% HCT layer and vice versa, and the 8% HCT sample in subject 3 had a HCT of 11%. The cell composition of samples collected using apheresis in subjects 1 to 3 (Figure 7A) showed an ~60 to 170-fold decrease in the RBC:WBC ratio from pre-apheresis samples compared to apheresis samples. Among samples collected by apheresis from subject 4, where a double apheresis process was undertaken, the RBC:WBC ratio was close to that of the pre-apheresis sample (Figure 7B), with the exception of the final bag sample (3% HCT). Reticulocyte counts measured on the Sysmex analyser were highest in subject 2: 0.23×10^9/L (reference range for whole blood: 25-120 $\times 10^9$/L).

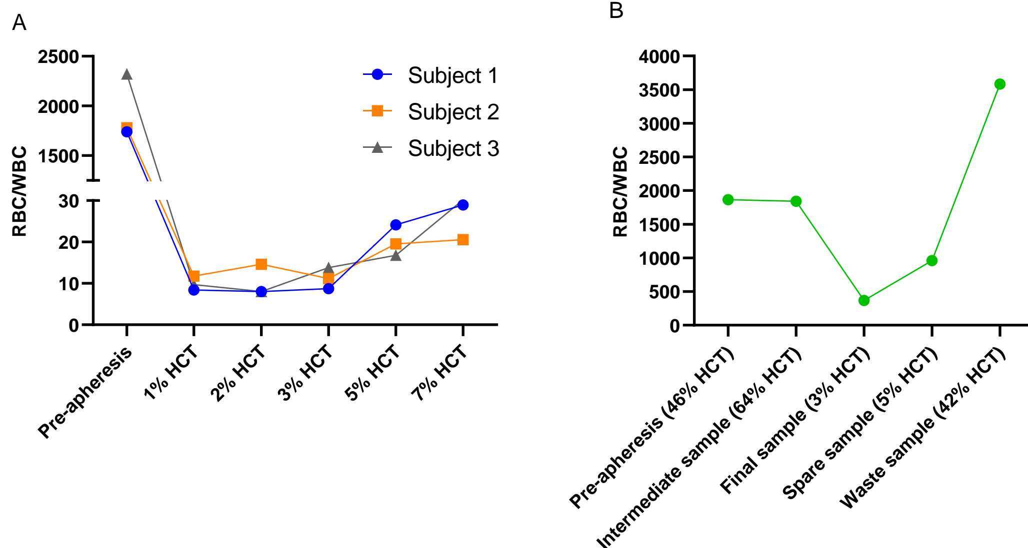
Table 10. HCT and RBC counts

Subject	Sample Type	HCT	RBC Count ($\times 10^6$/L)
1	Pre-apheresis	0.38	4.35×10^3
	1% HCT	0.01	1.40×10^2
	2% HCT	0.02	1.90×10^2
	3% HCT	0.03	3.00×10^2
	5% HCT	0.04	4.30×10^2
	7% HCT	0.05	5.90×10^2
2	Pre-apheresis	0.41	4.81×10^3
	1% HCT	0.01	2.00×10^2
	2% HCT	0.03	3.50×10^2
	3%HCT	0.03	2.60×10^2
	5% HCT	0.03	4.30×10^2
	7% HCT	0.05	5.00×10^2
3	Pre-apheresis	0.44	5.34×10^3
	0.5% HCT	0.01	7.00×10
	1% HCT	0.01	1.40×10^2
	2% HCT	0.01	1.50×10^2
	3% HCT	0.03	3.50×10^2
	5% HCT	0.04	4.40×10^2
	7% HCT	0.07	7.70×10^2
	*8% HCT	0.11	1.23×10^3
	2-3% HCT	0.02	2.60×10^2
	5-7% HCT	0.05	6.10×10^2
	0.5-8%* HCT	0.04	4.50×10^2
4	Pre-apheresis	0.46	5.22×10^3
	Intermediate sample	0.64	7.18×10^3
	Final sample	0.03	2.90×10^2
	Spare sample	0.05	4.80×10^2
	Waste sample	0.42	3.94×10^3

HCT; haematocrit, RBC; red blood cell

*8% HCT was subsequently renamed as 11% HCT given the Sysmex HCT result. HCT and RBC count results for samples taken pre-apheresis and during apheresis in subjects 1 to 4.

Figure 7. RBC/WBC ratio

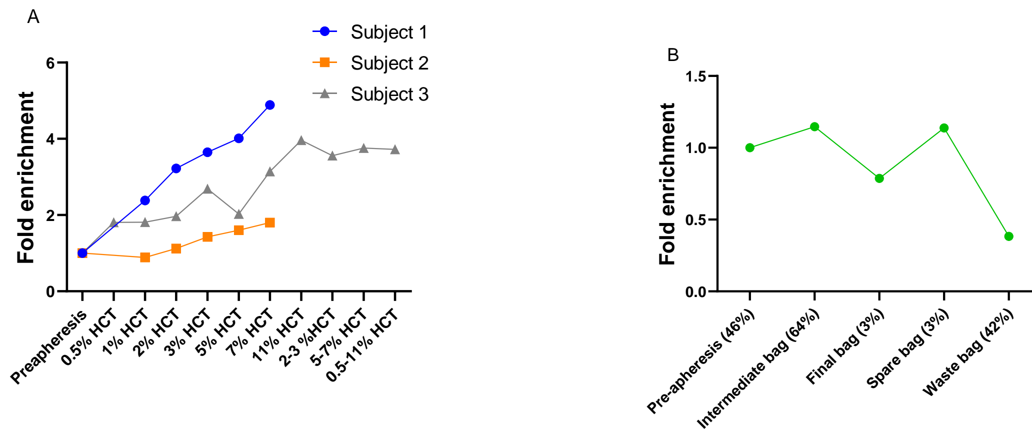


HCT; haematocrit, RBC; red blood cell, WBC; white blood cell. RBC/WBC ratio results for samples taken pre-apheresis and from the 1% HCT, 2% HCT, 3% HCT, 5% HCT and 7% HCT layers during apheresis from subjects 1 to 3 (7A). The apheresis samples selected for the figure were those that were sampled across all 3 subjects. Subject 4 was not included as the composition of samples differed significantly from subjects 1 to 3. RBC/WBC ratio results for samples taken pre-apheresis and during apheresis from subject 4 (7B).

CONCENTRATION OF ASEXUAL PARASITES

No single HCT layer contained >40% of all the recovered *P. vivax* asexual parasites (Figure 8). An increase in parasite concentration occurred as HCT increased in subjects 1 to 3 (Figure 8A), with some variation in relative enrichment of parasites in apheresis samples compared to pre-apheresis samples at any given HCT (Figure 8A). The highest concentration achieved was a 4.9-fold increase in parasite density in the 7% HCT layer in subject 1 (Figure 8A and appendix 12 Table 1). There was no apparent concentration of parasites when the procedure was modified to include a second apheresis process (subject 4; Figure 8B and appendix 12 Table 1).

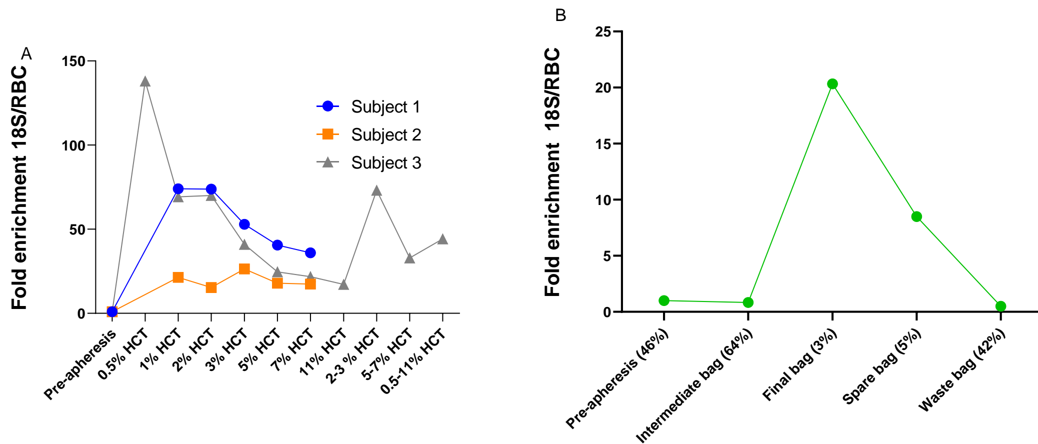
Figure 8. Fold enrichment of parasites/mL determined by 18S qPCR



HCT; haematocrit. Fold enrichment of parasites as determined by 18S qPCR in samples collected using apheresis compared to the pre-apheresis samples in subjects 1 to 3 (8A) and subject 4 (8B).

When parasite concentration was adjusted for RBC count, all apheresis samples collected from subjects 1 to 3 demonstrated enrichment for asexual parasites compared to pre-apheresis (Figure 9A). In general, when parasite counts were corrected for RBC count, parasite enrichment was highest in the low HCT samples with the highest enrichment detected in the 0.5% HCT sample in subject 3 (138-fold compared to pre-apheresis; Figure 9A and appendix 12 Table 1). The relative concentration of parasites from subject 4, where the second apheresis procedure was performed, was observed in the lowest HCT samples. In particular, the final bag (3% HCT) and the spare bag (5% HCT) samples had relative enrichment levels of 20 and 8-fold respectively compared to pre-apheresis (Figure 5B and appendix 12 Table 1).

Figure 9. Fold enrichment of parasites determined by 18S qPCR per RBC



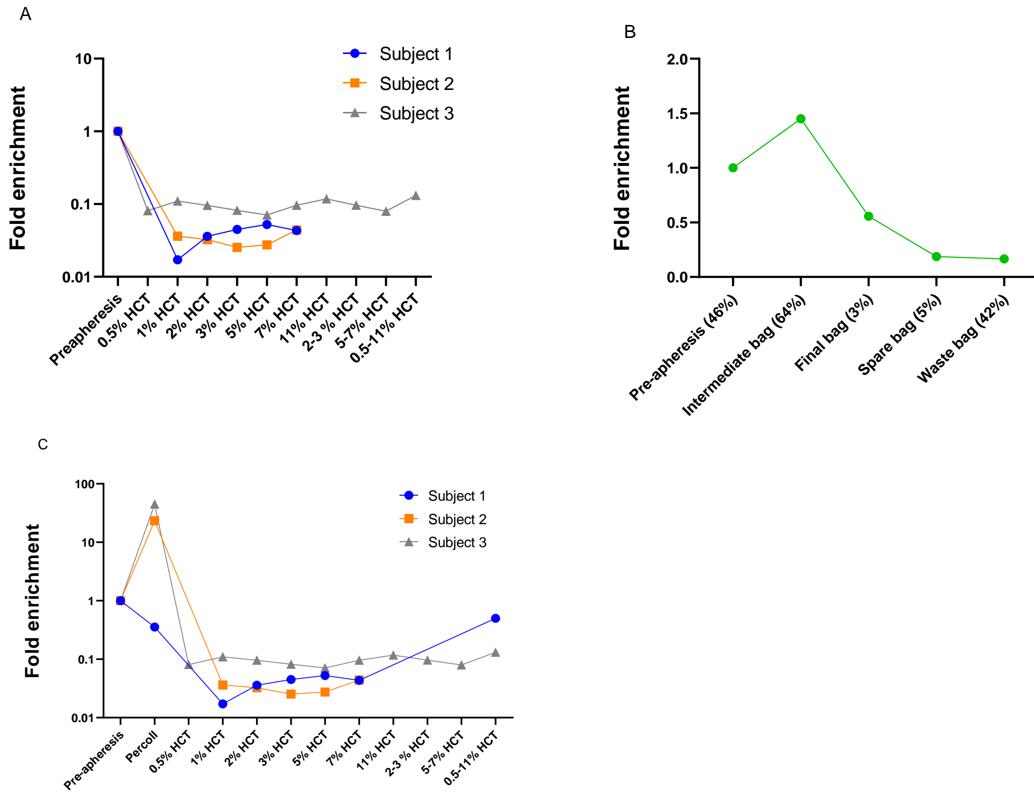
HCT; haematocrit, RBC; red blood cell. Fold enrichment of parasites corrected for RBC counts as determined by 18S qPCR in samples collected using apheresis compared to the pre-apheresis sample in subjects 1, 2 and 3 (9A) and subject 4 (9B).

CONCENTRATION OF GAMETOCYTES

Analysis of the apheresis samples from subject 4, where the double apheresis process was undertaken, demonstrated an increase in the level of female gametocytes of 1.45-fold compared to pre-apheresis, as determined by levels of the gametocyte-specific transcript *pvS25* qRT PCR (Figure 10B and appendix 12 Table 2).

Subjects 1, 2 and 3 demonstrated a reduction in the level of female gametocytes compared to pre-apheresis (Figure 10A and appendix 12 Table 2). In most cases the reduction was >10-fold. Percoll concentration of whole blood taken pre-apheresis resulted in a significant (up to 45-fold in subject 3) enrichment of *pvS25* compared to pre-apheresis samples not treated with percoll (Figure 10C and appendix 12 table 2).

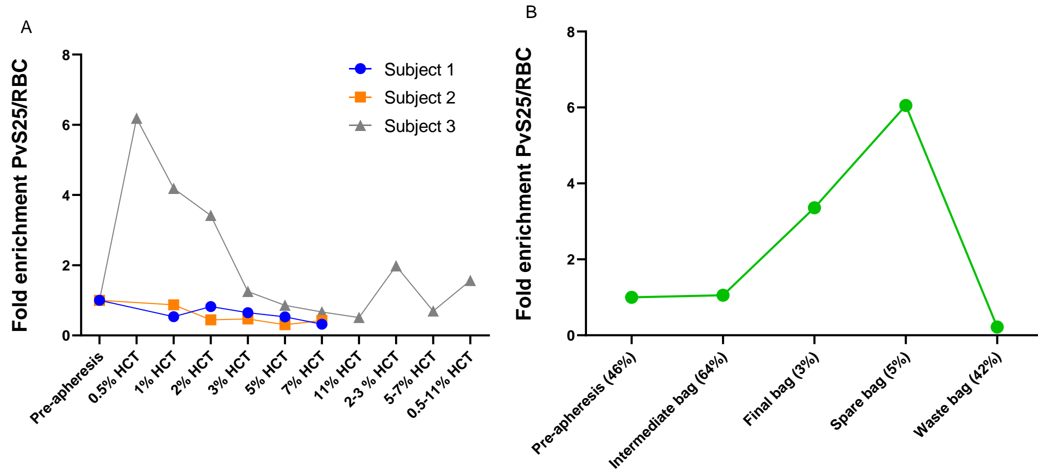
Figure 10. Fold Enrichment female *P. vivax* gametocytes/mL determined by *pvS25*



HCT; haematocrit. Fold enrichment of female *P. vivax* gametocytes determined by *pvS25*/mL in samples collected using apheresis compared to the pre-apheresis sample in subjects 1, 2 and 3 (10A) and subject 4 (10B). Fold enrichment of female *P. vivax* gametocytes determined by *pvS25*/mL in samples collected using apheresis compared to the pre-apheresis sample and pre-apheresis samples treated with percoll in subjects 1, 2 and 3 (10C).

When gametocyte concentrations were corrected for RBC counts, enrichment levels were generally higher in the lower HCT samples (Figure 11A and 11B and appendix 12 Table 2). Relative enrichment of gametocytes in subjects 1, 2 and 3 was lower than for total parasites, with a maximum enrichment of 6.2-fold in the 0.5% HCT layer (appendix 12 Table 2). In subjects 3 and 4 enrichment relative to RBC count was observed (Figure 11A and 11B). In subject 4, the greatest enrichment of gametocyte transcripts in the double apheresis process was observed in the spare bag (5% HCT) with an enrichment of 6.1-fold (Figure 11B and appendix 12 Table 2).

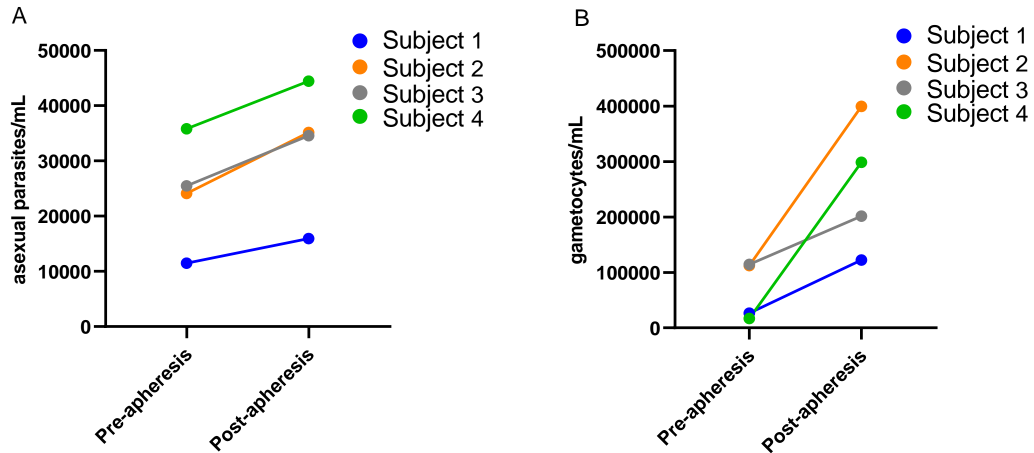
Figure 11. Fold Enrichment female *P. vivax* gametocytes determined by *pvS25* per RBC



HCT; haematocrit, RBC; red blood cell. Fold enrichment of female *P. vivax* gametocytes corrected for RBC counts determined by *pvS25* of samples collected using apheresis compared to the pre-apheresis sample in Subjects 1 , 2 and 3 (11A) and subject 4 (11B).

Asexual parasites and gametocytes were increased in whole blood samples taken post apheresis compared to pre-apheresis across all cohorts (Figure 12).

Figure 12. asexual parasites/mL and gametocytes/mL pre and post apheresis determined by qPCR



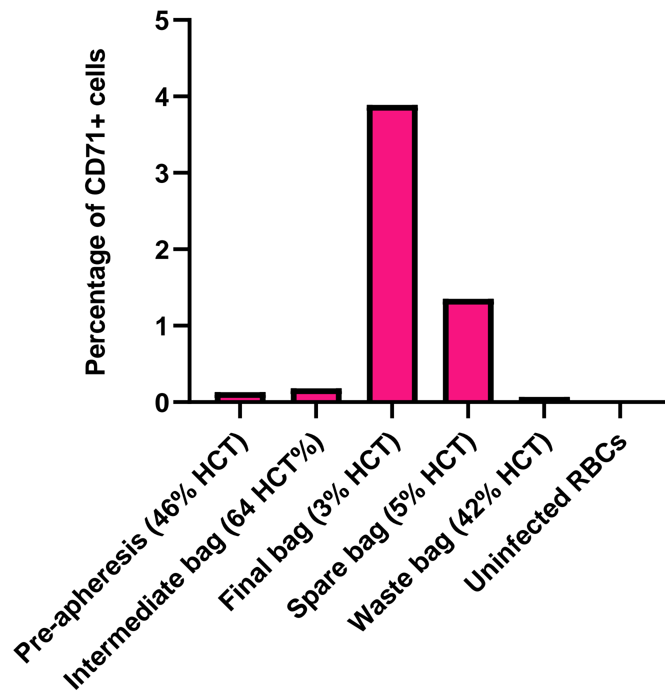
Change in 18S qPCR/mL (12A) and *pvS25* qPCR/mL (12B) from whole blood samples taken pre- and post-apheresis.

All subjects experienced an increase in asexual parasitemia and gametocytemia between samples taken pre- and post-apheresis.

FLOW CYTOMETRY

In subject 4, where CD71 antibody staining was used to identify reticulocytes, the highest levels of reticulocytes (CD71+ cells) were observed in the final bag (3% HCT) and the spare bag (5% HCT) (Figure 13). Microscopic analysis of these samples suggested that the reticulocyte population detected by flow cytometry consisted of uninfected reticulocytes. No parasitised RBCs or reticulocytes could be detected by flow cytometry.

Figure 13. Flow cytometry for reticulocytes in subject 4



HCT; haematocrit, RBC; red blood cell. The percentage of (CD71+) reticulocytes from subject 4.

MICROSCOPY

The high concentration of WBCs in apheresis samples meant many of the thick and thin blood smears were extremely difficult to read (Tables 11 and 12). Among samples where it was possible to read the films, parasite counts were low, and in alignment with the counts obtained by qPCR (Tables 11 and 12). Notwithstanding the technical difficulties in reading the slides and the low parasite counts, no apparent concentration of parasites was observed in any of the apheresis samples compared to pre-apheresis. The 3% HCT sample in subject 3 contained 7 parasitised cells compared to 0 in the pre-apheresis sample and the 1% HCT sample in subject 1 demonstrated a 4-fold increase in the number of visualised parasites compared to pre-apheresis. In general, higher parasite numbers were seen in the lower HCT layers. The vast majority of parasitised RBCs contained ring form parasites, with trophozoites and gametocytes observed in samples from subjects 3 and 4 only (Tables 11 and 12).

Table 11. Microscopy findings for subjects 1 to 3

Sample	Subject 1		Subject 2				*Subject 3	
			Reader 1		Reader 2			
	Thick film	Thin film	Thick film	Thin film	Thick film	Thin film	Thick film	Thin film
Pre-apheresis	unreadable	2 rings	2	0	3	0	17	0
Percoll Feed	unreadable	1 ring	n/a	0	n/a	0	70	unreadable
0.5% HCT	unreadable	n/a	n/a	n/a	n/a	n/a	unreadable	unreadable
1% HCT	unreadable	2 rings	8	0	12	1 ring	unreadable	unreadable
2% HCT	unreadable	1 ring	1 ring	1 ring	2	0	unreadable	1
3% HCT	unreadable	0	0	1 ring	0	0	unreadable	7
5% HCT	unreadable	0	1 ring	0	1 ring	0	unreadable	4
7% HCT	unreadable	0	1 ring	0	1 ring	0	unreadable	1
11% HCT	unreadable	n/a	n/a	n/a	n/a	n/a	unreadable	0
2-3% HCT	unreadable	n/a	n/a	n/a	n/a	n/a	unreadable	12
5-7% HCT	unreadable	n/a	n/a	n/a	n/a	n/a	unreadable	6
0.5-11% HCT	unreadable	n/a	n/a	n/a	n/a	n/a	unreadable	0

HCT; haematocrit. Two microscopists reviewed thick and thin films from subject 2. Subjects 1 and 3 involved only one microscopist who reviewed both thick and thin films.

*Microscopist did not specifically document the type of parasites in each sample but simply commented there were “rings and trophozoites and a single gametocyte”.

Table summarising microscopy findings from samples taken pre- and during apheresis in subjects 1, 2 and 3.

Table 12. Microscopy findings for subject 4

Sample (HCT)	Reader 1 thick film	Reader 2 thick film	Reader 3 thin film
Pre-apheresis (46%)	7 rings	6 trophozoites 2 female gametocytes	1 trophozoite
Intermediate (64%)	3 trophozoites	6 trophozoites	n/a
Final (3%)	n/a	4 trophozoites	n/a
Spare (5%)	n/a	n/a	n/a
Waste (42%)	3 trophozoites	2 rings 8 trophozoites	1 trophozoite

HCT; haematocrit. Two microscopists reviewed the thick films and one the thin films from subject 4.

Table summarising microscopy findings from samples taken pre- and during apheresis in subject 4.

MOSQUITO TRANSMISSION

Membrane feeding of pre-apheresis or post percoll enrichment samples were undertaken in all 4 subjects, but for logistical reasons membrane feeding on apheresis samples could only be undertaken in subjects 1 and 4 (Table 13).

None of the samples from subject 1 or 2 resulted in successful mosquito transmission (Table 13). In subject 3, the infection rate from the percoll-enriched sample was 5.8-fold higher than the infection rate from pre-apheresis sample (99% vs 17.2% [Table 13]). Likewise, in subject 4, the infection rate from the final bag (3% HCT) was 5.5-fold higher than the infection rate from either the pre-apheresis sample or the intermediate bag (64%HCT) (pre-apheresis: 3.6%, intermediate bag: 4%, final bag: 20% [Table 13]).

The final sample bag (3% HCT) in subject 4 was the only sample obtained using apheresis that demonstrated an increase in transmission over samples obtained pre-apheresis. Due to logistic issues the spare bag (5% HCT), which contained the greatest enrichment for gametocytes compared to pre-apheresis when adjusted for RBC counts in subject 4 (Figure 11B) was not subject to membrane feeding.

Table 13. Mosquito infection rates following membrane feeding assays

Sample	**Subject 1**				**Subject 2**	**Subject 3**		**Subject 4**			
	Pre-apheresis (with-Percoll enrichment)	**Apheresis samples**			**Pre-apheresis (with-Percoll enrichment)**	**Pre-apheresis (without-Percoll enrichment)**	**Pre-apheresis (with-Percoll enrichment)**	***Pre-apheresis (without-Percoll enrichment)**	**Apheresis samples**		
		1% HCT	**2% HCT**	**3% HCT**					**Intermediate (64% HCT)**	**Final (3% HCT)**	**Waste (42% HCT)**
Feeding rate (No. mosquitos fed/No. total mosquitos [%])	127/133 [95.5%]	101/103 [98.1%]	103/104 [99%]	114/116 [98.3%]	75/77 [97.4%]	102/103 [99%]	107/107 [100%]	29/29 [100%]	28/29 [97%]	26/26 [100%]	30/30 [100%]
Mortality rate (No. dead mosquitos/No. total mosquitos [%])	23/133 [17.3%]	3/103 [2.9%]	6/104 [5.8%]	11/116 [9.5%]	7/77 [9.1%]	6/103 [5.8%]	5/107 [4.7%]	1/29 [3.4%]	3/29 [10.3%]	1/26 [3.8%]	1/30 [3.3%]
Infection rate (No. mosquitoes with oocysts/No. mosquitos tested [%])	0/110[0%]	0/105 [0%]	0/98 [0%]	0/105 [0%]	0/77 [0%]	16/93 [17.2%]	99/100 [99%]	1/28 [3.6%]	1/25 [4%]	5/25 [20%]	0/24 [0%]

HCT; haematocrit. Only subjects 1 and 4 involved the testing of samples collected using apheresis. For logistic reasons, eMFA was not carried out in subject 4. Following consideration of gametocyte levels, DMFA was not carried out in subject 2. Reported parameters include feeding rate of mosquitoes, adult mosquito mortality rate after feeding and mosquito infection rate.

*MFA without percoll enrichment to save on blood volume draw for safety reasons.

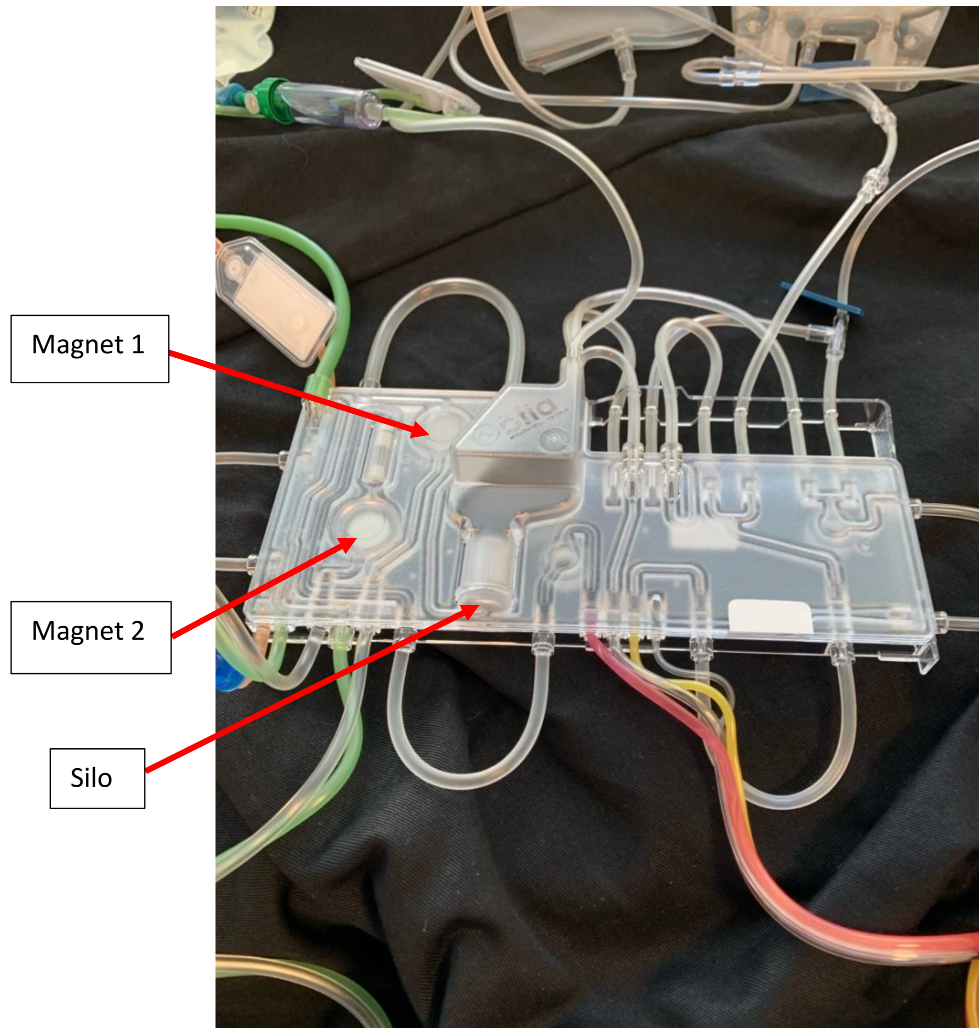
Table summarising membrane feeding assay findings from samples taken pre-apheresis both with and without percoll enrichment and during apheresis across all subjects.

ADDITIONAL 18S QPCR TESTING OUTSIDE OF APHERESIS SAMPLES

To investigate for possible accumulation of parasites in the magnets and tubing structures in the apheresis cassette, qPCR testing was carried out on three blood clots with diameters of up to 1 cm found near two magnets, and a silo like structure that formed part of the single use apheresis tubing and processing cassette from subject 3 (Figure 14). Clots were thoroughly homogenised and tested by 18S qPCR. The greatest enrichment observed relative to pre-apheresis was 1.4-fold in the apheresis cassette magnet 2 sample (Figure 15).

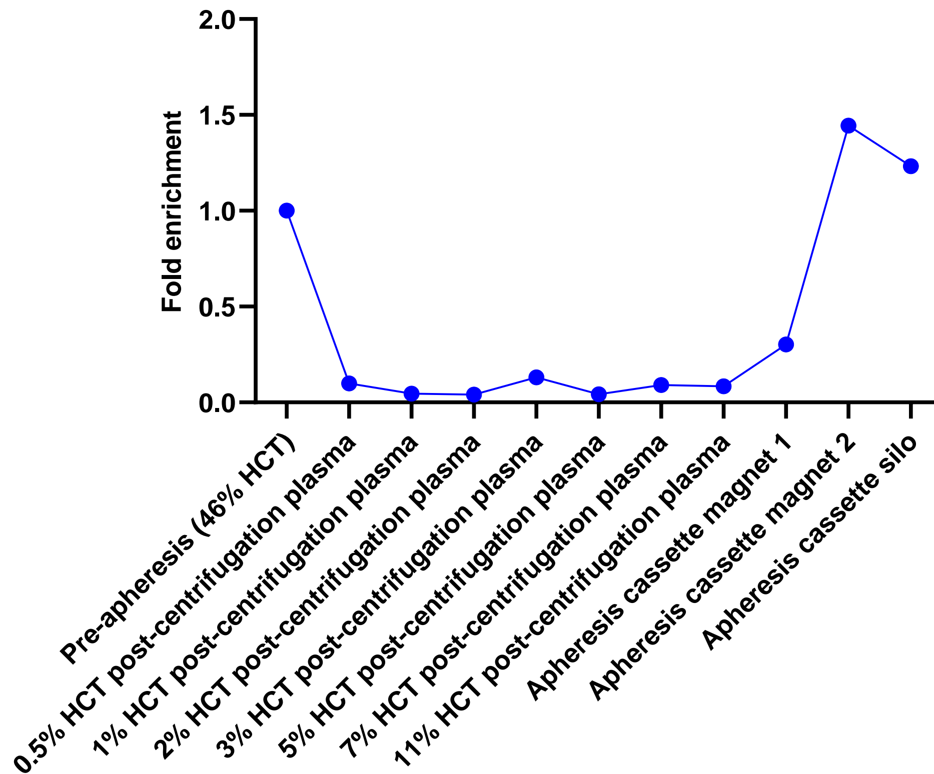
To investigate whether haemolysis may be taking place in the apheresed blood resulting in release of parasite DNA into the extracellular fluid, plasma from apheresis samples in subject 3 was collected by centrifugation of the sample, with the plasma subject to 18S qPCR testing. However, no significant accumulation of parasite DNA was detected in the plasma compared to pre-apheresis (Figure 15).

Figure 14. Apheresis single use cassette



Example of an intermediate density layer apheresis single use casette used during apheresis. The 2 magnets and silo structure, where blood clots were found following subject 3’s apheresis procedure, are identified. Blood clots subsequently underwent 18S qPCR testing.

Figure 15. Subject 3 additional testing for enrichment of parasites/mL determined by 18S qPCR



Fold enrichment of 18S qPCR/mL, compared to pre-apheresis, of blood clots around magnet 1, magnet 2, and silo within the apheresis cassette and of post-centrifugation plasma originating from the collection bag during subject cohort 3.

DISCUSSION

SUMMARY

Using apheresis it was possible to achieve modest concentration of both asexual and gametocyte stages of *P. vivax*. However, the modest level of parasite enrichment (4.9-fold and 1.45-fold per mL of sample for asexual parasites and gametocytes respectively) was insufficient to meet the objectives for the collection of parasites for downstream research.

PRE-CLINICAL EXPERIMENT

Apheresis successfully extracted malaria parasites from venesected blood infected with *P. falciparum*. RBCs in the 1% HCT layer were more likely to be infected with

asexual parasites than in any other HCT layer that was sampled. Analysis of the *P. falciparum* gametocyte markers *pfMGET* qPCR and *pfS25* qPCR suggest male and female gametocytes concentrate greatest in the 1% HCT layer, with an increase of 8 and 2.7-fold per mL of sample respectively when compared to pre-apheresis. Interestingly gametocytes were only visualised in the 1%, 3% and 5% HCT layers, although gametocyte qPCR markers were positive in all samples. This could be explained by different gametocyte stages preferentially depositing at different HCT layers as can be seen in figure 4B. Alternatively this could simply be due to chance as the % parasitemia in all samples were relatively low.

This preliminary experiment provided a guide for the location of asexual parasites and gametocytes for the clinical experiments. However, it was anticipated that RBCs infected with *P. vivax,* being larger than *P. falciparum* infected RBCs, would concentrate at different HCT layers compared to *P. falciparum*[189]. Moreover, blood from an infected subject may behave differently to uninfected whole blood, used during the pre-clinical experiment, as a result of malaria related inflammation and effects on blood coagulation[190]. Hence, during cohort 1 of the clinical experiment samples were again obtained from 1%, 2%, 3%, 5% and 7% HCT layers.

SAFETY FINDINGS

Safety data suggest that apheresis in healthy subjects infected with blood stage *P. vivax* parasites is safe (Table 9). No serious adverse events were encountered, with all adverse events having resolved by the end of study. The majority of adverse events were malaria related, and in line with previous *P. vivax* IBSM studies[60, 72]. Adverse events related to apheresis were largely asymptomatic transient reductions in haematology parameters. Safety data must be interpreted with caution given the low numbers of subjects. Going forward, in the context of *P. vivax* IBSM, it would be advised that this procedure should ideally only be carried out in subjects with normal haemoglobin, lymphocyte and platelet counts at screening to avoid potential complications or the need for blood transfusion.

SAMPLE CONSTITUTION

HAEMATOCRIT AND RED BLOOOD CELL COUNT

Sysmex haemocytometer reports HCT to an accuracy of 0.01 (with 1% HCT = 0.01) thus there is a potential for a rounding error. The effects of any potential rounding error would be magnified at lower HCT layers. However in general RBC count data supported the accuracy of the samples selected during the experiments by the apheresis nurse (Table 10).

RED BLOOD CELL TO WHITE BLOOD CELL RATIO

Figures 7A and 7B demonstrate the RBC/WBC composition of samples collected using apheresis from subjects 1 to 3 and subject 4 respectively. The most striking finding is the difference in RBC/WBC ratio seen in pre-apheresis samples compared to samples obtained via apheresis in subjects 1 to 3 (Figure 7A). This is due to a combination of the reduction in RBC count and increase in WBC count compared to whole blood obtained pre-apheresis. In general as the HCT increases the RBC/WBC ratio increases (Figure 7A).

CONCENTRATION OF ASEXUAL PARASITES

No single HCT layer was seen to concentrate all or even a majority of *P. vivax* asexual parasites. The greatest concentration of parasite/mL of sample was seen in the 7% HCT layer in subject 1. Subject 3 was the only subject where direct apheresis sampling occurred above the 7% HCT layer. In this subject the greatest enrichment was observed in 11% HCT layer which is the highest HCT layer that was sampled, but the increase in enrichment between 7% HCT and 11% HCT layers was relatively moderate from 3.1 to 4.0-fold.

In line with the existing literature, when adjusted for RBC count, asexual parasites were found in higher numbers in the lower HCT level[191]. The greatest enrichment compared to whole blood obtained pre-apheresis was an increase of 138-fold and occurred in the 0.5% HCT sample. Therefore a RBC from the 0.5% HCT sample was 138 times more likely to be parasitised than a RBC from the pre-apheresis sample. The level of enrichment observed in the 0.5% HCT sample should be interpreted with caution as this is based on a single sample. The 1% and 2% HCT layers were sampled from subjects 1 to 3. The level of enrichment between cohorts

varied from 21 to 74-fold and 15 to 74-fold for the 1% and 2% HCT samples respectively. The level of variation calls into question the reproducibility of results in future experiments.

The total number of RBCs in the apheresis samples were much lower than in pre-apheresis samples (Table 10). Hence the total numbers of infected RBCs in any one apheresis sample were only moderately increased compared to whole blood obtained pre-apheresis.

The findings in subject 4 were consistent with subjects 1 to 3 with the greatest concentration of asexual parasites/RBC occurring in the samples with the lowest HCT, namely the final bag (3% HCT) and the spare bag (5% HCT) samples, which experienced enrichments of 20 and 8-fold respectively compared to pre-apheresis (Figure 9B). The level of enrichment for the 3% HCT layer (final bag) from subject 4 was lower but still comparable to the level of enrichment seen in the 3% HCT samples obtained from subjects 1 to 3. Whereas the level of enrichment for the 5% HCT layer (spare bag) from subject 4 was less than the level of enrichment seen in the 5% HCT samples obtained from subjects 1 to 3. Therefore, the addition of a RBC collection stage did not improve the yield of asexual parasites/RBC.

CONCENTRATION OF GAMETOCYTES

Subject 4 was the only subject where there was an increase in gametocytes/mL compared to pre-apheresis (Figure 10B). An increase of 1.45-fold was observed in the intermediate bag. The intermediate bag had a RBC count of 7.18×10^{12}/L compared to 5.22×10^{12}/L for pre-apheresis. $7.18 \times 10^{12} / 5.22 \times 10^{12} = 1.38$. Hence, the 1.45-fold enrichment seen in the intermediate bag may simply represent an overall increase in the number of RBCs in the intermediate bag compared to pre-apheresis whole blood. Percoll enrichment of gametocytes is far greater than enrichment seen during apheresis.

As with asexual parasites, when corrected for RBC count gametocytes were generally higher in the lower HCT samples (Figure 11A and 11B) again in line with the existing literature[191]. From subjects 1 to 3 it was only subject 3 that saw significant enrichment of gametocytes (Figure 11A). This may be explained by the apheresis procedure taking place 11 days post inoculation in subject 3 as opposed to

10 days post inoculation for subjects 1 and 2, thus resulting in more time for gametocyte maturation and a higher pre-apheresis parasitemia relative to subjects 1 and 2. Conversely subject 2 actually had a higher post-apheresis gametocytes/mL count than subject 3. However this could represent a higher proportion of schizonts, containing greater copy numbers of *pvS25* qPCR in subject 2 than subject 3, therefore *pvS25* qPCR/mL results do not represent increased numbers of gametocytes but rather increased copy numbers of *pvS25* qPCR[192]. The enrichment of gametocytes/RBC in subjects 1 to 3 was much more modest than with asexual parasites/RBC (up to 6-fold in 0.5% HCT layer).

In contrast to asexual parasites/RBC, the greatest enrichment of gametocytes/RBC was observed in the spare bag (5% HCT) in subject 4 with an enrichment of 6.1-fold (Figure 11B). This contrasts with the low level or negative enrichment seen in subjects 1 to 3 at the 5% HCT layer. Suggesting that carrying out an initial RBC collection stage does enhance the enrichment of gametocytes/RBC compared to direct sampling from subjects using apheresis.

In all four subjects, whole blood samples taken post-apheresis via venesection contained greater asexual parasite and gametocyte numbers per mL compared to pre-apheresis whole blood samples taken via venesection. If apheresis was successful in capturing and concentrating *P. vivax* parasites, we would have expected to see a decrease. The increase could be explained by parasite maturation with an increased proportion of schizonts[192, 193]. However this explanation seems unlikely as apheresis occurred on different days depending on the subject (day 10; subject 1 and 2, and day 11; subject 3 and 4) and therefore the parasite stage would also be expected to differ. Moreover apheresis procedure only took 1 to 4 hours giving only a small window for parasite maturation.

MICROSCOPY

Samples that were of sufficient quality and composition to be able to be read demonstrated only small numbers of parasitised RBCs. In general there was a spread of parasitised RBCs across the various samples. Apheresis samples did not demonstrate significant parasite concentration compared to pre-apheresis. Microscopy findings mirrored those seen with qPCR. Most of the parasites seen using microscopy were ring forms, with trophozoites and gametocytes observed in

subjects 3 and 4. Both of these cohorts took place on day 11 compared to day 10 for subjects 1 and 2, thus explaining the presence of more mature life cycle stage parasites. Microscopy results suggest any enrichment of parasites from apheresis is, at best, modest which again supports the findings observed with qPCR.

MOSQUITO FEEDING

An enhanced level of transmission to mosquitoes compared to whole blood samples collected pre-apheresis was only observed on one occasion (final sample bag [3% HCT] in subject 4), corresponding to the higher gametocyte concentration in this sample compared to the pre-apheresis whole blood (Figure 11B). A possible explanation of the low success in the transmission studies was the difficulty in maintaining tight temperature control to prevent exflagellation of male gametocytes[194], thereby negatively impacting gametocyte infectivity[195]. Blood was most vulnerable to a temperature drop whilst in the apheresis equipment itself. It was not possible to heat the apheresis equipment, and it was deemed impractical to heat the room where apheresis took place to >35°C. Temperature monitoring was not possible during the experiments. However, the demonstration of transmission success in the final bag (3% HCT) indicates that at least some gametocytes were maintained within a temperature range that did not trigger exflagellation. Regardless of the underlying cause, recent reports of success in improving concentration of gametocytes and enhanced transmission by either percoll[196] or magnetic bead[197] enrichment suggests that such methods are superior to apheresis for concentration of gametocytes for mosquito transmission experiments.

ADDITIONAL 18S QPCR TESTING OUTSIDE OF APHERESIS SAMPLES

It was hypothesised that the magnets within the apheresis equipment (Figure 15) may have concentrated parasitised RBCs containing hemozoin[198]. This greatest level of enrichment observed (1.4-fold in the magnet 2 sample) was insufficient to explain the low levels of parasite concentration in apheresis samples. *pvS25* qPCR was not carried out as gametocytes are detected by the 18S qPCR assay and for reasons of cost[193].

Haemolysis within the apheresis blood collection bags is not the reason behind the poor enrichment of parasites compared to pre-apheresis (Figure 15). There was no way to test for the occurrence of haemolysis within the apheresis equipment.

RETICULOCYTE ANALYSIS

Following analysis of the data collated from subjects 1 to 3, it was hypothesised that the moderate enrichment of parasitised RBCs was due to absence of reticulocyte enrichment (*P. vivax* exclusively invades reticulocytes[36]). Instead of enriching for reticulocytes by selecting the layer in which they are commonly found (2% and 3% HCT[191]), the apheresis procedure resulted in replacement of uninfected RBCs, responsible for diluting reticulocytes (approximately 1% of whole blood[199]) in whole blood, with WBCs. Hence, in order to enrich for reticulocytes the experimental plan for subject 4 was altered. A RBC collection from the infected subject took place in the first instance. This ensured the subsequent product contained very little WBC component. Once this procedure was complete the material extracted via RBC collection underwent apheresis aimed at concentrating reticulocytes and therefore parasitised RBCs. Flow cytometry in subject 4 involved the use of the CD71 marker specific for reticulocytes. Apheresis samples collected post RBC collection demonstrated evidence of reticulocyte enrichment, most marked in the final and spare collection bag (Figure 13). Despite this the addition of the RBC collection did not result in an increase in asexual parasite enrichment by qPCR (Figure 8B).

USE OF APHERESIS IN THE HARVESTING OF PLASMODIUM VIVAX PARASITES

Several hypotheses were formed to explain the relative lack of parasite concentration in samples obtained using apheresis. Firstly, as gametocyte concentrations in apheresis samples were around the level of detection of the *pvS25* qPCR assay any variation in concentration may have been difficult to identify[192]. In response, biological repeats were conducted but did not alter the outcome. Secondly altered RBC/WBC ratio in apheresis samples compared to whole blood affecting the 18S qPCR and *pvS25* qPCR assays. Microscopy did not support this explanation. Thirdly, it is possible that infected RBCs may have attached to ferromagnetic components of the apheresis apparatus, this was not supported by qPCR. Finally, it is

possible that lysis of asexual parasites and gametocyte, occurred during the apheresis procedure. This hypothesis is supported by recent evidence suggesting parasite maturation results in increasing osmotic fragility of *P. vivax* infected RBCs[200]. Although it may have been possible to assess for haemolysis during the procedure, for example by measuring haptoglobins, controlling for a range of other variables would have been difficult.

Regardless of the underlying cause, enrichment suggests that other methods are superior to apheresis for the concentration of gametocytes for mosquito transmission experiments.

Conversely instead of focusing on measuring gametocyte concentration via qPCR and microscopy the focus should be shifted to successful mosquito transmission and harvesting of sporozoites for downstream experimentation. Greater transmission in apheresis samples compared to pre-apheresis samples would suggest apheresis is capable of substantial gametocyte concentration. High levels of transmission can be achieved using percoll. However, apheresis may be used to increase the level of transmission in any given mosquito, potentially increasing the numbers of oocysts in mosquito midguts and subsequently the number of sporozoites in mosquito salivary glands. Given the increase in transmission was relatively modest and only occurred in one sample any future plans should be made with caution.

However, if future apheresis transmission experiments occur and do not demonstrate successful transmission to mosquitos, it would not necessarily be informative. As the absence of transmission may be due to a failure to concentrate gametocytes, or failure to maintain the appropriate temperature required to prevent gametocyte exflagellation[194].

Although these would have been useful avenues to explore given the significant ethical, financial and logistical considerations for IBSM studies, it was decided that this was inappropriate during this study. Furthermore in order to produce a viable outcome which would provide benefit to the wider community, several issues would need to be overcome, of which there is no guarantee. For example currently there is no way to control the temperature of blood within the apheresis apparatus. Blood takes several minutes to go from the subject through the apparatus to the apheresis collection bag during which time the blood may cool to below 37°C, or experience a

drop in temperature of 5°C and thus potentially trigger gametocyte exflagellation[194] and prevent transmission[201]. However the fact that transmission occurred in the final bag (3% HCT) sample from subject 4 would go against this and support the idea that gametocytes were maintained within a temperature range that did not trigger exflagellation.

HUMAN MALARIA PARASITE BANK FORMATION

Theoretically apheresis and percoll may be able to concentrate parasites enough to create HMP bank vials containing 2.51×10^5 parasites (calculations in appendix 12). The HMP013 *P. vivax* parasite bank used to infect the volunteers in the study produces vials each of which contains 2.08×10^6 parasites. In order to produce an equivalent bank to the HMP013 *P. vivax* parasite bank, it would require an 8.3-fold greater pre-apheresis parasitemia to that seen in subject 3 if apheresis and percoll was used.

The HMP013 *P. vivax* bank is not the ideal bank for IBSM studies because of the relatively low parasitemia. The TS *P. falciparum* bank contains 15-fold higher parasites/vial than the HMP013 *P. vivax* bank. In order to produce a parasite bank equivalent to the TS *P. falciparum* bank it would require 120-fold greater enrichment with apheresis and percoll. This equates to subject parasitemias of 1.1×10^7 parasites/mL. Until now the maximum parasite count in *P. vivax* observed across 37 subjects was approximately 310,000 parasites/mL. This particular subject had a high number of AEs and under current standards it would be unethical to allow the parasitemia to increase to the levels needed to make apheresis a viable method for creating a HMP bank. Therefore, unless significant improvements in enrichment can be attained, apheresis should not be used to create HMP banks, and the current practice of collecting blood by venesection is preferable.

Although the level of concentration is not sufficient to produce a viable HMP bank. It may be sufficient to produce material that could be used in other experiments including the assessment of a *P. vivax* stage specific assay by providing parasites for the production of a standard curve.

STRENGTHS AND LIMITATIONS

Strengths of the study include the wide sampling across HCT layers (1%, 2%, 3%, 5% and 7%) and the use of microscopy and qPCR to enumerate parasites.

Limitations included the low number of subjects involved, of which only one underwent a RBC collection. IBSM studies come with significant ethical and funding restrictions, as such low numbers of subjects for studies, particularly exploratory studies, are common. The study was only able to use the female gametocyte marker for *P. vivax* as a reliable male marker is not currently available. It is important to note the limitations in the accuracy of the *pvS25* qPCR assay as a measure for female *P. vivax* gametocytemia. A single *pvS25* genome is not always equivalent to a single gametocyte. For example a schizont may contain more than 20 copies of the *PvS25* genome thus overestimating the true number of gametocytes[192]. Moreover, the absence of in vitro culture for *P. vivax* means the standard curves for the assay are extrapolated from *P. falciparum*, resulting in even greater uncertainty with regards to the true gametocyte density. However for the purposes of quantifying the concentrating effect of apheresis compared to pre-apheresis this is not anticipated to have a large effect.

Several issues, both quality related and operational, compromised the reliability of microscopy data. Selection of the HCT layer for sampling during apheresis occurred by visual inspection of the colour saturation of blood. Although this is a subjective process, it is routinely practiced by the clinical apheresis staff, and HCT and RBC count data supports the accuracy of sampling (Table 10).

Temperature control of pre-apheresis and apheresis samples was attempted during the study to maintain gametocyte infectivity[195]. Blood was most vulnerable to a temperature drop whilst in the apheresis equipment itself. It was not possible to heat apheresis equipment itself and it was deemed impractical to heat the room where apheresis took place to >35°C. Temperature monitoring was not possible during the experiments.

CONCLUSION

Apheresis can be used safely in human volunteers infected with blood stage *P. vivax* and can achieve moderate enrichment of asexual parasites and to a lesser extent

gametocytes. Currently the level of enrichment is not sufficient to produce a HMP bank or significantly improve transmission experiments above venesection and percoll. Given the significant ethical, financial and logistical concerns surrounding *P. vivax* IBSM studies, this work makes is clear that further apheresis studies are not warranted at this point. If apheresis were to be used again in the context of malaria VIS, it should ideally only be carried out in subjects with normal haematological parameters at screening to avoid potential complications or the need for blood transfusion.

CHAPTER 5 LIVER FUNCTION TEST ABNORMALITIES IN INDUCED BLOOD STAGE PLASMODIUM VIVAX MALARIA

INTRODUCTION

Post treatment liver function test (LFT) abnormalities encountered in *P. falciparum*[202] and *P. vivax*[60] malaria volunteer infection studies (VIS), were observed in three of the four subjects involved in the apheresis exploratory study. This emphasised the importance of this safety issue, particularly as it occurs in healthy volunteers purposefully infected with malaria for research purposes. These abnormalities have become an important safety concern and have the potential to threaten the future role of malaria VIS as a tool in antimalarial drug development.

Drug induced liver injury (DILI) remains the single most common cause for safety related drug withdrawal during drug research[203]. DILI usually resolves upon ceasing the offending drug without sequelae[204, 205], but instances of chronic liver impairment[206] and even death[207] have been described. DILI is divided into intrinsic injury which is related to a specific drug and is typically dose dependant, and idiosyncratic injury which occurs in specific susceptible individuals and is typically not dose related and more difficult to predict.

Hy's law is predictive of acute liver failure[208] and hepatocellular damage[204, 209], need for transplantation[209] and death[209] from DILI. Hy's law is met when transaminases are elevated $>3 \times$ ULN together with rises in total bilirubin $>2 \times$ ULN but in the absence of cholestasis (alkaline phosphatase [ALP] $<2 \times$ ULN) and an alternative cause. Hy's law is often utilised as a stopping criteria in the study of new clinical entities (NCE). However, most drugs that cause severe DILI do so infrequently so it typical requires at least a few thousand subjects to demonstrate this abnormality, and therefore severe DILI may not be observed in small drug studies[203]. Hence, close attention must be paid to lesser degrees of DILI during pre-clinical and clinical studies. LFT abnormalities encountered during induced blood stage malaria (IBSM) studies are important safety signals for the safety of healthy volunteers as well as any NCE under investigation. Incorrect assignment of causality of these important adverse events to the NCE has serious implications. Moreover the failure to correctly assign LFT abnormalities to a NCE has even greater implications. Currently there is no reliable biomarker to distinguish DILI from other causes of liver injury. Therefore

comprehensive evaluation of LFT abnormalities observed during human experimental challenge is important.

LFT abnormalities in IBSM were first observed in *P. falciparum* studies. In total 8/197 (4%) non-immune subjects in 4/16 (25%) studies reported severe transaminase elevations >5.1 × ULN, with a further 4 subjects with moderate elevations 2.6-5 × ULN[210]. The 4 studies involved 3 NCE (ferroquine[50], KAE609 [NIH NCT02543086], SJ733 [NIH NCT02867059]) and the registered antimalarial piperaquine[186]. Transaminase elevations were characterised by alanine transaminase (ALT)/aspartate transaminase (AST) ratio >1, ALT peaking 2 to 4 days post treatment and returning to normal 18 to 35 days post treatment. The maximum ALT elevation was 24 × ULN. Elevations in lactate dehydrogenase (LDH) were observed without a rise in bilirubin. Subjects were typically asymptomatic during the time of LFT elevations[210].

Transaminase elevations of >5 × ULN were observed in 4/6 (66%) subjects in a subsequent *P. vivax* IBSM study[60]. The pattern of the elevations mirrored those observed in *P. falciparum* IBSM. It is plausible that transaminase elevations in *P. vivax* and *P. falciparum* IBSM are caused by the same process. Several possible causes have been suggested, including the blood stage malaria infection dose (inoculum), acetaminophen use, and oxidative stress secondary to haemolysis leading to hepatocyte injury[60, 210].

Malaria hepatopathy, defined as bilirubin >2.5 × ULN and rise in transaminase >3 × ULN[211, 212, 213, 214, 215], is a well-recognised phenomenon in both experimentally induced and natural infection across all malaria species[210, 211, 212, 216, 217, 218, 219]. Malaria hepatopathy differs from LFT abnormalities in *P. falciparum*[202] and *P. vivax* VIS[60] in timing and the prominence of bilirubin elevations[50, 220, 221], suggesting that malaria hepatopathy and post treatment LFT changes in VIS may be mediated by different mechanisms.

My analysis aimed to describe in detail and investigate the cause of the LFT abnormalities observed in two *P. vivax* IBSM studies. One of these studies entailed treatment with the investigational antimalarial artefenomel and the other with the licensed drug chloroquine. Neither artefenomel[222] nor chloroquine[223, 224, 225] have

been associated with hepatotoxicity when used in standard dose regimens in healthy subjects. However, LFT changes have previously been reported in clinical studies of artefenomel in malaria endemic settings[226].

I hypothesised that a range of factors may be associated with elevations in liver transaminase levels. These include C-reactive protein (CRP), and temperature, both markers of systemic inflammation, LDH, a potential marker of haemolysis, and the level of parasitemia at the time of treatment. I hypothesised that the rate of parasite clearance and the burden of parasite biomass cleared post treatment were potential contributors to the observed abnormalities. These parameters have not been assessed previously. In addition all of the variables identified above as potential causes of the observed LFT abnormalities were also assessed.

HYPOTHESIS

- Abnormalities in LFTs observed in the *P. vivax* IBSM model are mediated by rapid release of hepatotoxic products by rupture of infected red blood cells that overwhelms the intravascular capacity to detoxify them.
- Abnormalities in LFTs observed in the *P. vivax* IBSM model are mediated by the inflammatory response in non-immune patients.

AIM

- Determine the cause of LFT abnormalities seen in *P. vivax* IBSM.

OBJECTIVES

- Assess the relationship between the rate of malaria parasite clearance and LFT abnormalities following treatment with investigational product.
- Assess the relationship between the burden of parasites cleared following treatment with investigational product and LFT abnormalities.
- Assess the relationship between systemic inflammation and LFT abnormalities.
- Assess the relationship between haemolysis and LFT abnormalities.
- Assess whether LFT abnormalities similar to those observed in *P. vivax* IBSM studies occur in studies from the field.

MATERIAL AND METHODS

CLINICAL METHODS

INDUCED BLOOD STAGE MALARIA STUDIES

Data for the two *P. vivax* IBSM studies were obtained, these included 32 malaria-naive subjects who participated in two clinical trials at QIMR Berghofer between March 2016 and April 2017. Details are reported elsewhere[179] (NCT02573857) and included in Table 14. Subjects were all healthy adults aged between 18 and 55 years. Subjects with a history of, or clinical signs of, liver disease were excluded from the studies. Subjects who tested positive for hepatitis BsAg or core antibody, hepatitis C antibody or HIV, were excluded. All subjects had ALT, AST and total bilirubin levels within the normal range one to three days prior to malaria inoculation. Subjects taking prescription medication, over the counter medication, vitamin, mineral, herbal remedy, or supplements likely to cause abnormal LFTs were excluded. All subjects had consented to the alcohol limitations.

Subjects were inoculated intravenously with the *P. vivax* isolate HMP013. In the artefenomel study 8 subjects were treated with a single 200 mg dose of the drug on day 10. Based on previous studies the 200 mg dose was predicted to be sub-efficacious[51], and as expected 7/8 subjects had recrudescent parasitemia and received a standard course of artemether-lumefantrine on day 21-28. In the chloroquine study, a total of 24 subjects were treated with a standard three-day course of chloroquine, totalling 25 mg/kg, beginning on day 8 (n=8 subjects), day 9 (n=1 subject), or day 10 (n=8 subjects).

Table 14. Clinical trials details

Drug	**Artefenomel**	**Chloroquine**	**Chloroquine**	**Chloroquine**	**Chloroquine**
Cohort (n)	Artefenomel (8)	Chloroquine C1 (8)	Chloroquine C2a (6)**	Chloroquine C2b (2)**	Chloroquine C3 (8)
Inoculation dose size (parasites)	524,000	720,000	722,000	740,000	782,000
Treatment regimen	single dose 200 mg	*3 days			
Day of treatment post inoculation	day 10	day 8	day 10 (one subject treated on day 9)	day 10	day 10
Acetaminophen and/or ibuprofen use	allowed both	allowed both, encouraged ibuprofen only and only reported ibuprofen use	ibuprofen	ibuprofen	acetaminophen

*3-day standard oral curative treatment regimen dosed on a weight basis to a total of ~25 mg/kg. **Cohorts 2a and 2b were combined into Chloroquine cohort C2 for the statistical analysis as the conduct and characteristics of the cohorts were identical apart from the inoculum dose.

Table summarising key differences in study design, inoculation dose and symptom relief across IBSM study cohorts.

ENDEMIC STUDIES

Data were made available from a previously published phase IIa open label study[226] which enrolled patients at the Hospital for Tropical Diseases in Bangkok and Shoklo Malaria Research Unit (SMRU) located on the north western border of Thailand between October 2010 to May 2012. Febrile adult male and non-pregnant female patients aged 18-60 years presenting with symptomatic, microscopically confirmed, *P. falciparum* or *P. vivax* (parasite counts 5000-50,000 /µl) were included. Patients were excluded if they had clinical or laboratory evidence of severe malaria[227], were unable to tolerate oral therapy or had received any other antimalarials within the last 14 days. Individual biochemistry data from 41 patients with microscopically confirmed *P. vivax* mono or mixed infection were analysed. LFTs were measured at screening prior to artefenomel treatment (day 0 pre-treatment), 4 hours post treatment (day 0 post treatment), day 1, day 2 and day 7. Investigators were permitted to conduct additional testing at their discretion.

In the Malaysian study patients were enrolled from July 2013 to November 2015 as part of a randomised controlled trial[228] or a prospective observational study[229], both conducted at Kota Marudu, Kudat, and Pitas District Hospitals. Patients were included if they had PCR-confirmed *P. vivax* monoinfection, were aged >1 year of age, were non-pregnant, had no major comorbidities or concurrent illness, and had not started antimalarial treatment. Parasitemia was calculated by thin blood film microscopy. LFT testing of samples collected from 85 subjects between day 0 and day 7 was undertaken. All had ALT levels tested on days 0 and 7, while 19/85 and 85/85 subjects had AST tested on day 0 and day 7 respectively. Total bilirubin levels were tested in 76/85 subjects on day 0, and 85/85 subjects on day 7.

LABORATORY PARAMETERS

LIVER FUNCTION TEST MEASUREMENTS

LFT parameters measured during the analysis include ALT, AST, bilirubin, gamma-glutamyl transferase (GGT), ALP and albumin. LDH was primarily assessed as a marker of haemolysis as discussed in more detail below. Although serum LDH is a less specific biomarker of haemolysis than haptoglobin, it was routinely measured as

part of the biochemistry panel, and therefore for this analysis it was included as it was the only potential marker of haemolysis[230] available. LFTs were taken at screening, pre-inoculation, pre-treatment with chloroquine or artefenomel, 72 hours post treatment, 5-8 days post treatment and at the end of study (EOS). Additional samples were taken at investigators' discretion. Testing of markers of clotting factor synthesis were not taken during the studies.

Peak ALT level was assessed as the primary LFT biomarker of interest for both the IBSM and endemic studies. ALT is predominantly found in the liver compared to AST which is also found in the brain, kidney, and muscle as well as the liver[231]. Therefore ALT was selected over AST as it is more liver specific[231].

ALT was categorised into ≥2 × ULN and <2 × ULN (ULN=40 IU/L for males and 30 IU/L for females). The cut off of ≥2 times × ULN was used as this was deemed by local liver experts and investigators familiar with phase I studies in healthy volunteers and IBSM studies as significant and unlikely to occur by chance.

Common terminology criteria for adverse events (CTCAE)[232] guidelines use >3 × ULN as significant, but this is specific to investigation of cancer trials. Excluding subjects with ALT rises ≥2 × ULN but <3 × ULN would have reduced the number of subjects considered to have significant ALT changes and thus weaken the ability to identify associations.

Table. 15 Sensitivity analysis for the ALT cut off in IBSM studies

Identifier		**Peak ALT (n%)**		
Drug	**Cohort**	**≥2 × ULN**	**≥2.5 × ULN**	**≥3 × ULN**
Artefenomel	**Artefenomel**	6 (75.0%)	5 (62.5%)	5 (62.5%)
Chloroquine	1	1 (12.5%)	1 (12.5%)	0 (0%)
Chloroquine	2	4 (50.0%)	3 (37.5%)	3 (37.5%)
Chloroquine	3	3 (37.5%)	2 (25.0%)	2 (25.0%)
Overall		14 (43.75%)	11 (34.38%)	10 (31.25%)

ALT; Alanine transaminase, ULN; Upper limit of normal.

Table summarising the effect of altering the cut off for a significant peak ALT measurement from ≥2 × ULN to ≥2.5 × ULN to ≥3 × ULN across IBSM study cohorts and for all subjects treated with artefenomel and chloroquine.

Instead of peak ALT × ULN, peak ALT × baseline was considered as the outcome of interest, but given the significant variations in ALT measurements observed in healthy individuals over time, it was thought to be inferior[233, 234]. Another strategy could be to select patients who were considered to have significant LFT changes based on the timing of peak ALT × ULN and compare this group of subjects against the remaining subjects (those without significant LFT changes). However this was considered too subjective and difficult to replicate in any future analysis.

Alternative measures of liver damage were also considered including MicroRNA-122 (MiR-122). MiR-122 is the single most abundant MiRNA expressed by liver tissue (up to 70% of adult miRNA)[235]. MiR-122 has been identified as a marker of hepatocyte injury in clinical studies involving NCE. MiR-122 elevations occur in mouse studies and clinical cases of acetaminophen overdose[235, 236]. MiR122 usually rises in acetaminophen overdose before ALT and typically by 100 to 10,000-fold. MiRNA is believed to be more specific than ALT for hepatocyte injury versus exercise induced muscular injury where ALT is often raised[237].

Following discussion with a local gastroenterologist familiar with the LFT abnormalities seen in IBSM studies it was decided that MiR122 should not be used during this analysis. This decision was based on a number of considerations; firstly the effects of malaria infection on MiR122 are unknown, secondly the relative lack of experience in interpretation of MiR122 compared to ALT, thirdly greater sensitivity to acetaminophen use may produce a false positive association and finally the logistics, cost of testing and ethical approval for testing were prohibitive.

PARASITE RELATED MEASUREMENTS

Parasitemia was measured prior to treatment and at multiple time-points throughout the study by 18S qPCR[66] and analysed on the log_{10} scale. Overall peak parasitemia was thought to be relevant as a potential causative factor for LFT abnormalities, whereas pre-treatment peak parasitemia was included in the analysis to determine whether parasitemia could be used to predict which subjects develop transaminase elevations. The parasite clearance rate was reported using the parasite clearance half-life ($PCt_{1/2}$) in hours, calculated using the following equation as previously reported[238].

$$PCt_{1/2} = log_{10}(2) / -slope$$

For the artefenomel study, the $PCt_{1/2}$ was censored at parasitemia nadir, ~36 hours post treatment when rescue treatment with artemether-lumefantrine treatment was required. Although chloroquine was administered over three days, the initial parasite $PCt_{1/2}$ was assumed to represent a continuous pharmacodynamic effect[179].

It was hypothesised that there may be a saturable detoxification process for clearance of dead parasites and/or damaged RBCs. Therefore, a surrogate marker capturing the biomass of parasites cleared in the first 24 hours after treatment was calculated. This parasite clearance burden (PCB) was calculated by subtracting the parasitemia, at 24 hours post treatment initiation from the peak parasitemia on the log_{10} scale and reported as a log-fold change. The 24-hour cut off was selected as the majority of parasite clearance occurred over this time interval, although parasitemia remained measurable at a low level beyond this timepoint. Peak parasitemia alone could not be used as it does not necessarily capture the burden of cleared parasites which is a function of peak parasitemia and rate of clearance. Likewise parasitemia at treatment or peak parasitemia determined by extrapolation and y-intercept from the parasite

clearance curve may provide more appropriate measures of parasitemia before the effect of drug induced killing, but neither would incorporate the parasite clearance rate. These measures represent the starting point of drug induced killing, but it is the total reduction of parasites shortly after treatment that is the measure of interest. For example the same starting parasitemia with a very rapid parasite clearance will have a much greater reduction in parasite burden over 24 hours than the same starting parasitemia with a slow rate of parasite clearance.

Another potential measure was the area under the 18S qPCR curve. But again this measure does not capture the total reduction of parasites shortly after treatment. For example a high area under the curve could simply be due to a high starting parasitemia and rapid parasite clearance, or a low starting parasitemia with slow clearance.

The PCB measure does have limitations. It is produced using only two points of measurement increasing the likelihood of producing an unrepresentative value. The 24h post treatment parasitemia measure is especially prone to variation as the value may be around the limit of quantification for the 18S qPCR assay.

The endemic studies did not carry out serial qPCR measures, therefore peak parasitemia and parasite clearance half-life were not reported, as they would not be comparable to the peak parasitemia, parasite clearance half-life or parasite clearance burden in the IBSM studies. Instead median parasitemia levels at study enrolment were reported for the clinical studies.

HAEMATOLOGY PARAMETERS

White cell count (WCC), lymphocyte count, neutrophil count and platelet counts were selected for analysis, as these parameters are commonly altered in IBSM studies. The frequency and timing of WCC, lymphocyte, neutrophil and platelet counts testing in the endemic studies was not considered sufficient for analysis.

MARKERS OF HOST RESPONSE

CRP and temperature were measured as markers of host response.

Retrospective CRP testing was carried out to quantify the level of inflammation experienced by each subject. CRP is a well-recognised measure of inflammation with an established assay and clinicians are familiar with its interpretation.

CRP is produced by hepatocytes predominantly under control of interleukin-6 (IL-6)[239] but can be stimulated by interleukin-1 (IL-1) and Tumour necrosis factor α (TNFα)[240]. Hepatic synthesis occurs rapidly after stimulus and rises above 5 mg/L at about 6 hours after the inflammatory insult before peaking at around 48 hours post stimulus[241]. It has a plasma half-life of 19 hours which is constant under all conditions of health or disease. Thus synthesis rate is the sole determinant of CRP concentration which reflects the intensity of the pathological process[242]. CRP data collected from young healthy adult volunteer blood donors demonstrated a median CRP 0.8 mg/L, 90th centile 3.0 mg/L, 99th centile 10.0 mg/L)[243]. Normal ranges are relatable to study subjects who are young healthy adults.

CRP was selected as published data has indicated that CRP levels are associated with parasitemia[244, 245, 246, 247, 248] and disease severity[246, 249], and generally peaks 1-2 days after initiation of antimalarial treatment[247, 248]. Hence the decision was made to check CRP at baseline (Day of inoculation or 1 to 3 days pre-inoculation), pre-treatment, 24hrs post treatment, 48hrs post treatment, peak ALT (generally 5-8 days post treatment), 24hrs post peak ALT and 48hrs post peak ALT.

Testing was carried out from stored PCR samples at QIMR Berghofer. Some of the desired timepoints did not have corresponding stored PCR samples. Therefore where possible the next available sample was tested instead. This was not an issue for baseline, pre-treatment or 24 hours post treatment samples as PCR samples were taken at this time routinely. However there were several occasions where samples were not present for peak ALT, 24 hours and 48 hours post peak ALT results as subjects were often PCR negative at this point, thus repeat testing was not always necessary as per study protocol.

Both CRP × ULN and × baseline was measured. CRP × ULN was used as an inter-subject measure of inflammation whereas CRP × baseline was considered a superior intra-subject measure of inflammation.

CRP testing did not take place in the endemic studies. Following internal discussions at QIMR Berghofer, for reasons of logistical ease, sample preservation, ethical approval and cost it was decided not to test for CRP.

In IBSM subjects the maximum temperature was recorded within 3 days pre- and post treatment, in order to ensure the measurements reflected malaria infection and treatment. Temperature <37.5°C were considered normal. The frequency and timing of temperature recordings in the endemic studies was not considered sufficient for analysis.

Other markers of inflammation such as TNFα were also considered for analysis. Several studies have attempted to assess the role of TNFα in malaria. TNFα has been shown to be closely related to fever, paroxysms and anaemia in *P. vivax* infected patients[250].TNFα is found in higher levels in acute *P. falciparum* malaria than convalescent malaria and healthy controls[251]. Elevated TNFα has also been shown to have a role in cerebral malaria and fever during *P. falciparum* malaria[252, 253]. TNFα testing did not take place in either IBSM studies under analysis or in the endemic studies. Following internal discussions at QIMR Berghofer, for reasons of logistical ease, sample preservation, ethical approval and cost it was decided not to use TNFα in this analysis. Of note TNFα induces a dose dependant secretion of CRP[254]. Hence it was believed that CRP could potentially act as a proxy measure for TNFα.

CLINICAL REVIEW OF SUBJECTS

The source documents of all 32 subjects involved in the *P. vivax* IBSM studies were reviewed to exclude an alternative diagnosis such as alcohol consumption or concomitant medication use such as acetaminophen. Clinical review of the source documents for patients in the endemic setting was not carried out for logistic reasons.

STATISTICAL ANALYSIS

All data are analysed using Stata v.15, Microsoft Excel® (version 1903) and figures were produced using PRISM v.8. Lachlan Webb statistician at QIMR Berghofer was involved in the production of the statistical analysis plan and data analysis using Stata®. Continuous variables are presented as mean and SD, median and IQR. For each participant maximum and minimum values for all parameters (LFTs,

inflammatory, LDH and parasitemia) were identified from inoculation to drug treatment and from inoculation to EOS.

Logistic regression analysis was used to test for associations between the continuous explanatory parameters and peak ALT × ULN (males=40 IU/L, females=30 IU/L). A fixed cohort effect was added to the regression models to account for any confounding effects due to the inherent differences between cohorts. Correlations between two continuous variables were assessed by Pearson's correlation.

A backwards stepwise regression was used to create two multivariable models for associations to the peak ALT≥2 × ULN outcome. Any variable that was significant ($p<0.05$) in the univariate logistic regression was included in the backwards stepwise regression. In the first multivariable model, parasite clearance rate was forcibly retained into the final model; in the second multivariable model, PCB was included. For the Thailand artefenomel and Sabah dataset, descriptive statistics of frequency and percentage were given for categorical measures. Continuous measures were described using median (IQR) and range. Paired t-tests on the $\log_{10}$ were used to test the differences in LFT results between day 0 (pre-treatment for Thailand artefenomel study) and day 7 as data was skewed and differences non-symmetric.

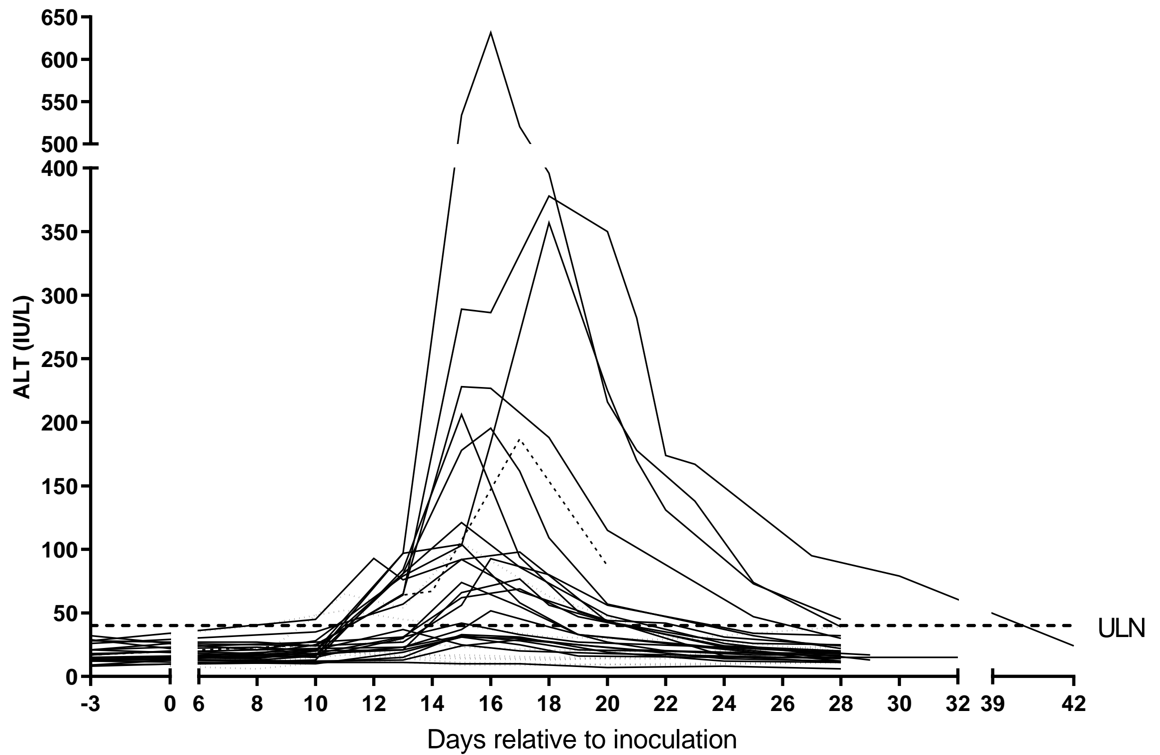
RESULTS

INDUCED BLOOD STAGE MALARIA

DESCRIPTIVE ANALYSIS BY SUBJECT

All 8 subjects recruited to the artefenomel study were male compared to 13/24 (54%) of subjects in the chloroquine group. The median (IQR) age of subjects recruited to the artefenomel and chloroquine studies were 23.5 (22-25.8) years and 24.5 (20-30) years respectively.

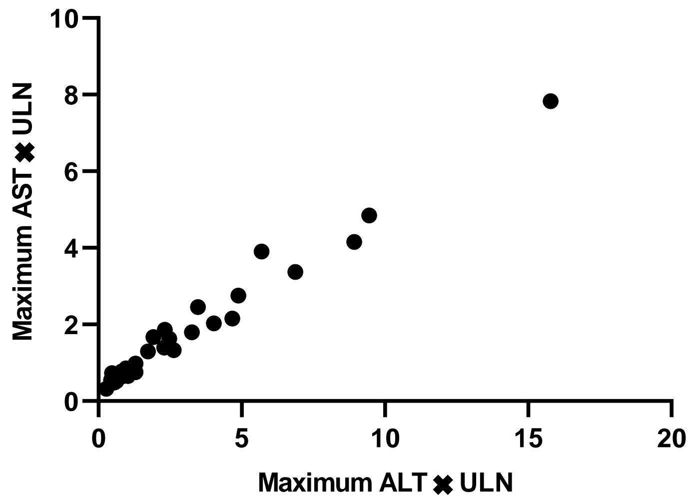
Figure 16. IBSM ALT over time



ALT; alanine transaminase, ULN; upper limit of normal.

Changes in ALT levels (IU/L) over time for all 32 subjects. Each of the 32 subjects are represented by a single trace. Day 0 represents the day of inoculation. Those subjects represented by dotted lines were from chloroquine cohort C1 (n=8), all of which were treated 8 days post inoculation. The dashed line represents subject R013 from chloroquine cohort C2 who was the only subject to be treated on day 9 post inoculation. The ULN for ALT in male subjects (40 IU/L) has been included for reference.

Figure 17. Peak ALT × ULN vs AST × ULN



ALT; alanine transaminase, AST; aspartate transaminase, ULN; upper limit of normal.

Peak ALT × ULN plotted against peak AST × ULN for all 32 study IBSM subjects. Peak ALT × ULN plotted against peak AST × ULN are strongly correlated. In general peak ALT is greater than peak AST.

Following antimalarial treatment 14/32 (44%) subjects had an ALT≥2 × ULN. ALT elevations generally exceeded AST (Figure 17). Levels began to rise 3 days after treatment, peaking between days 5 and 8, and resolved between day 11 and day 32 (Figure 16). The highest ALT observed was 632 IU/L (15.8 × ULN), occurring 6 days post treatment. There was no relationship between age or sex and LFT elevations. Nine (64%) of the 14 subjects with peak ALT≥2 × ULN were male, compared to 12/18 (67%) subjects with peak ALT<2 × ULN. Median age (IQR) of subjects with peak ALT≥2 × ULN and ALT<2 × ULN were 25.5 (22-33) and 22 (20-25) respectively.

No volunteer had significant elevations in ALP or bilirubin. GGT was measured in the artefenomel treated subjects only; all results were within the normal range. Creatinine kinase (CK) was measured in three artefenomel treated subjects and one chloroquine treated subject at, or within 3 days, of the peak ALT; all were within normal range.

There were no features in the past medical history of any of the subjects that suggested impairment of liver function. All subjects had normal examination findings and no suggestion of pre-existing liver pathology. Subject R206 took optimen (a multi-vitamin) but stopped 2 weeks before inoculation and this subject went on to develop moderate transaminase elevation (peak ALT 1.9 × ULN). Otherwise there was no use of prescription medication, over the counter medication, vitamin, mineral, herbal remedy, or supplements likely to cause LFT abnormalities.

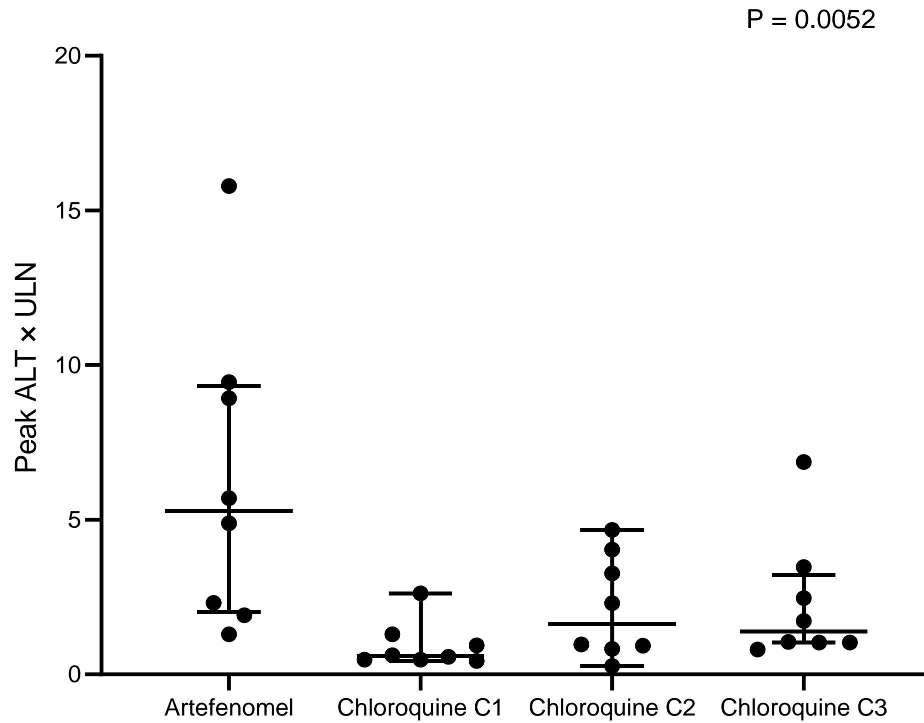
DESCRIPTIVE SUBJECT GRAPHS

Graphical representations for each subject were created to provide an overview of how relevant parameters, thought to be related to the causation of LFT abnormalities, behaved during the IBSM studies and in order to produce a visual representation for each subject.

Subjects with significant ALT abnormalities tended to have a greater number and severity of other malaria related measures and vice versa (appendix 12 Figure 1).

DESCRIPTIVE ANALYSIS BY COHORT

Figure 18. Peak ALT × ULN per IBSM cohort



ALT; alanine transaminase, ULN; upper limit of normal.

Peak ALT of all subjects split by IBSM study cohort with median (IQR) values. Kruskal Wallis P=0.0052.

Median (IQR) peak ALT was greatest in the artefenomel cohort followed by Chloroquine cohort C2, Chloroquine Cohort C3 with subjects in Chloroquine cohort C1 experiencing the smallest elevations.

PARASITEMIA MEASUREMENTS BY COHORT

Table 16. Summary of parasitemia related outcomes per cohort

Drug	Cohort	Peak parasitemia Overall (log parasites/mL) Median (IQR)	Peak parasitemia Pre-treatment (log parasites/mL) Median (IQR)	$PCt_{1/2}$ (hours) Median (IQR)	PCB
***Artefenomel**	***Artefenomel**	5.11 (4.84-5.28)	4.90 (4.58-5.00)	3.60 (3.11-4.31)	2.18 (2.13-2.37)
Chloroquine	**C1**	3.95 (3.78-4.11)	3.65 (3.39-3.91)	5.48 (4.35-5.68)	0.63 (0.44-0.83)
	C2	4.81 (4.35-5.08)	4.65 (4.25-4.91)	4.73 (4.23-5.42)	1.16 (0.83-1.39)
	C3	4.87 (4.70-5.07)	4.62 (4.37-4.84)	5.59 (4.30-6.09)	1.18 (0.95-1.33)
Chloroquine Overall		4.62 (4.08-4.90)	4.30 (3.88-4.83)	5.28 (4.33-5.73)	0.95 (0.71-1.22)
Total		4.76 (4.12-5.07)	4.54 (3.93-4.90)	4.40 (3.86-5.68)	1.16 (0.75-2.09)

$PCt_{1/2}$; parasite clearance half-life, PCB; parasite clearance burden, IQR; interquartile range.

*Artefenomel was only used in one cohort, thus the overall values for artefenomel are the same as for the cohort itself.

Table 16 demonstrates the median (IQR) values for peak parasitemia overall, peak parasitemia pre-treatment, $PCt_{1/2}$ and PCB measures depending on IBSM study cohort.

UNIVARIATE ANALYSIS

Table 17. Univariate analysis

Investigative parameter		**Peak ALT**		**Chi square**	**Logistic regression**		**Logistic regression with fixed cohort effect**	
		<2 × ULN **n=18**	**≥2 × ULN** **n=14**		**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Parasitemia measurements								
$PCt_{1/2}$ (hours)	Median (IQR)	4.57 (4.12-5.69)	4.36 (3.16-5.40)		0.71 (0.36-1.38)	0.31	1.12 (0.472-.66)	0.79
pre-treatment Peak parasitemia (log parasites/mL)	Median (IQR)	4.19 (3.80-4.65)	4.88 (4.54-4.93)		7.15 (1.35-38.02)	**0.021**	4.46 (0.41-48.45)	0.22
Overall Peak parasitemia (log parasites/mL)	Median (IQR)	4.52 (4.09-4.89)	4.94 (4.78-5.25)		5.86 (1.17-29.43)	**0.032**	2.64 (0.25-28.15)	0.42
PCB	Median (IQR)	0.92 (0.70-1.20)	1.81 (1.15-2.20)		5.09 (1.40-18.48)	**0.013**	3.95 (0.31-49.84)	0.29
Markers of host response								
Maximum temperature ≥37.5 (°C)	Median (IQR) n (%)	38.25 (37.30-38.9) 11 (61.1%)	38.85 (38.60-9.50) 14 (100%)	 **0.01^**	3.13 (1.14-8.61)	**0.027**	2.28 (0.65-7.97)	0.20
CRP × ULN To EOS (mg/L)	Median (IQR)	5.20 (2.40-7.60)	11.90 (9.40-14.20)		1.81 (1.21-2.71)	**0.004**	1.98 (1.18-3.33)	**0.010**
CRP × ULN Pre-treatment (mg/L)	Median (IQR)	0.20 (0.20-0.60)	2.60 (0.60-7.00)		2.20 (0.85-5.65)	0.1	bad fit[#]	
CRP Relative to Baseline (mg/L)	Median (IQR)	18.50 (6.00-37.00)	58.00 (36.00-66.00)		1.12 (1.04-1.20)	**0.004**	1.13 (1.04-1.24)	**0.006**
Marker of haemolysis								
LDH × ULN To EOS (U/L)	Median (IQR)	0.92 (0.84-1.08)	1.25 (1.05-1.36)		1.89 (1.21-2.95)	**0.005**	1.78 (1.05-3.02)	**0.034**
LDH × ULN Pre-treatment (U/L)	Median (IQR)	0.68 (0.60-0.75)	0.80 (0.69-0.87)		2.01 (1.06-3.82)	**0.034**	1.51 (0.74-3.06)	0.26
Haematological parameters								
Neutrophils To EOS <1 × LLN (10^9/L)	Median (IQR) n (%)	1.14 (1.07-1.36) 4 (22.2%)	0.97 (0.83-1.25) 7 (50.0%)	 0.14^	0.07 (0.004-1.22)	0.068	0.07 (0.003-1.75)	0.10
Neutrophils Pre-treatment <1 × LLN (10^9/L)	Median (IQR) n (%)	2.17 (2.04-2.56) 0	1.58 (1.3-1.85) 1 (7.1%)		0.09 (0.01-0.59)	**0.012**	0.04 (0.002-0.56)	**0.018**
Platelets To EOS <1 × LLN (10^9/L)	Median (IQR) n (%)	0.98 (0.85-1.17) 10 (55.6%)	0.84 (0.76-0.85) 12 (85.7%)	 0.068	0.0003 (<0.001-0.34)	**0.023**	<0.001 (<0.001-0.33)	**0.029**
Platelets Pre-treatment	Median (IQR)	1.2 (0.99-1.37)	1.01 (0.93-1.23)	0.37	0.03 (0.0008-1.03)	0.052	0.01 (<0.001-2.99)	0.12

<1 × LLN (10^9/L)	n (%)	5 (6.2%)	6 (4.8%)					
Lymphocytes To EOS <1 × LLN (10^9/L)	Median (IQR) n (%)	0.92 (0.61-1.0) 12 (66.7%)	0.59 (0.54-0.71) 14 (100%)	**0.024^**	0.003 (<0.001-0.29)	**0.013**	0.007 (<0.001-2.20)	0.09
Lymphocytes Pre-treatment <1 × LLN (10^9/L)	Median (IQR) n (%)	0.98 (0.61-1.52) 10 (55.6%)	0.59 (0.54-0.71) 13 (92.9%)	**0.02**	0.02 (<0.001-0.60)	**0.023**	0.003 (<0.001-0.86)	**0.044**
White cell count To EOS <1 × LLN (10^9/L)	Median (IQR) n (%)	1.04 (1.00-1.20) 4 (22.2%)	0.87 (0.74-1.09) 10 (71.4%)	**0.005**	0.013 (<0.001-0.73)	**0.035**	<0.001 (<0.001-0.97)	**0.009**
White cell count Pre-treatment <1 × LLN (10^9/L)	Median (IQR) n (%)	1.44 (1.20-1.66) 2 (11.1%)	0.96 (0.83-1.17) 8 (57.1%)	**0.005**	0.01 (<0.001-0.25)	**0.005**	<0.001 (<0.001-0.21)	**0.012**
Antimalarial treatment								
Chloroquine AUC_{96} (µg/mL*h)	Median (IQR)	n=16 60.03 (54.41-71.60)	n=8 53.71 (44.00-63.24)		0.95 (0.89-1.02)	0.17	0.97 (0.90-1.05)	0.48
Chloroquine C_{max} (µg/mL)	Median (IQR)	1.05 (0.90-1.31)	0.93 (0.74-1.13)		0.09 (0.003-3.19)	0.19	0.32 (0.01-20.0)	0.59
Artefenomel AUC_{96} (µg/mL*h)	Median (IQR)	n=2 4.44 (4.01-4.87)	n=6 3.57 (2.72-4.44)		0.60 (0.04-2.64)	0.5		
Artefenomel C_{max} (µg/mL)	Median (IQR)	0.70 (0.66-0.75)	0.51 (0.37-0.69)		0.03 (<0.001-148.0)	0.42		

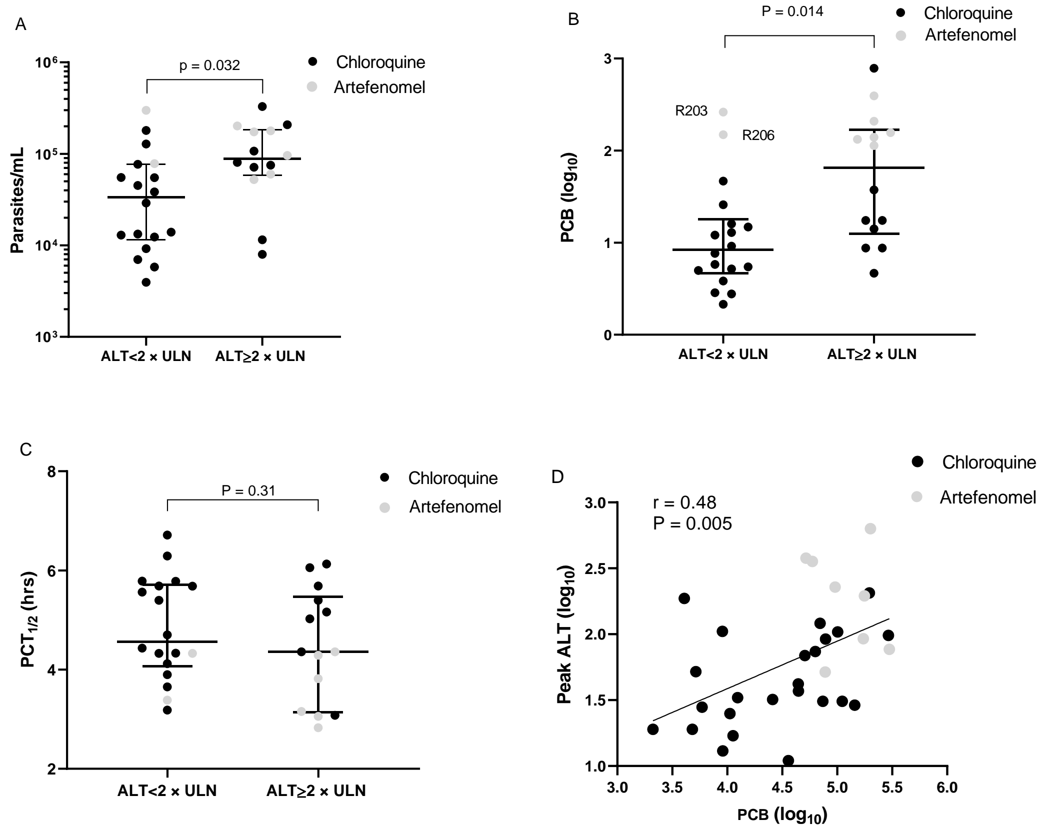
ALT; alanine transaminase, $PCt_{1/2}$; parasite clearance half-life, PCB; parasite clearance burden, CRP; C-reactive protein, LDH; lactate dehydrogenase, ULN; upper limit of normal, LLN; lower limit of normal, OR; odds ratio, AUC_{96}; area under curve 96 hours, C_{max}; maximum concentration of drug, EOS; end of study. OR refer to a 1-unit change in the measure of the explanatory parameter (e.g. 1°C for maximum temperature).

^Fisher's exact test. [#]Insufficient data for good model fit. Imbalance of data by cohort, and singularity in chloroquine cohort C2, make this model a bad fit. Univariate analysis assessing the effect of explanatory variables on peak ALT. Significant effects and associations ($p<0.05$) are in bold. Continuous measures are described with mean (SD), and categorical measures are described with frequency (%). Results from simple binary logistic regression are presented for the continuous measures. That logistic regression is then repeated with a fixed cohort effect included.

The PK antimalarial measures could only be compared separately by drug, as the drug effect that would have to be included would confound with the cohort effect.

PARASITEMIA MEASURES BY SUBJECT

Figure 19. Parasitemia measures



ALT; alanine transaminase, ULN; upper limit of normal, PCB; parasite clearance burden.

Relationship between peak parasitemia overall (19A), PCB (19B) and $PCt_{1/2}$ (19C), and peak ALT. Peak ALT is split into 2 groups; peak ALT≥2 × ULN and peak ALT<2 × ULN. 19D linear regression between peak ALT (log_{10}) and PCB (log_{10}) with Pearson's correlation values. Peak ALT (log_{10}) was used to reduce skew from low level peak ALT measures.

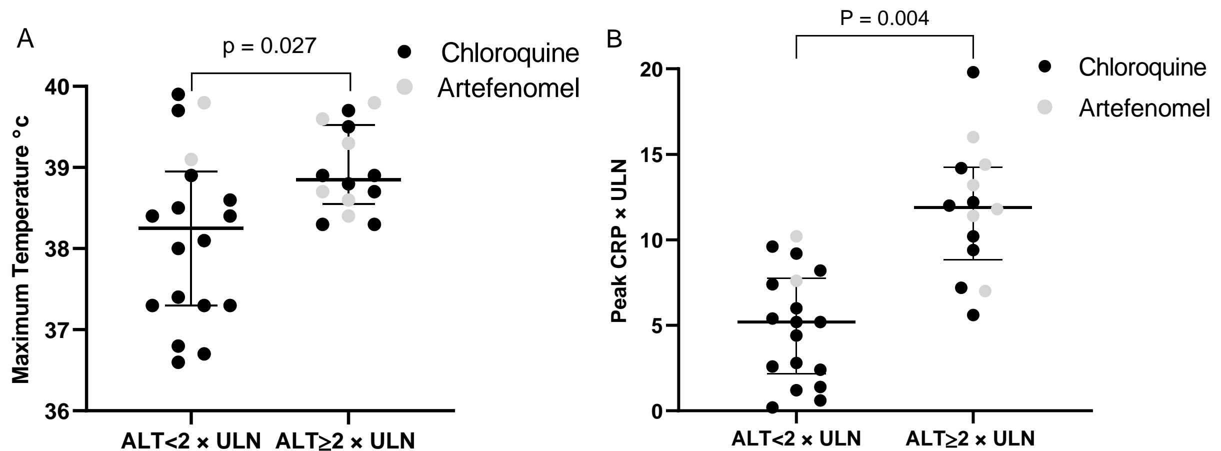
Overall peak parasitemia was significantly associated with peak ALT≥2 × ULN OR=5.86 (95% CI; 1.17-29.43 p=0.032; Figure 19A). The correlation coefficient between peak ALT and peak parasitemia was 0.39 (p=0.027) (appendix 12 Table 3). A one log increase in overall peak parasitemia increased the odds of a subject developing peak ALT≥2 × ULN by 5.86 (log_{10}) fold respectively.

Both the univariate and multivariable analysis for PCB do not show any statistically significant relationship between $PCt_{1/2}$ and ALT abnormalities.

The PCB (Figure 19B) was found to be a significant risk factor for developing a peak ALT≥2 × ULN, with an OR of 5.06 (95% CI 1.40-18.48, p=0.014) for a one log increase in PCB. The relationship between peak ALT log_{10} and PCB is supported by an analysis that shows a significant correlation between peak ALT log_{10} and PCB (0.48; p=0.005; Figure 19D). PCB for the artefenomel treated cohort (mean 2.25 [95% CI; (2.1-2.4)] is greater than for the chloroquine cohorts overall (mean 0.95 [95% CI; 0.71-1.22]) and chloroquine cohorts C2 (mean 1.16 [95% CI; 0.7-1.88]) and C3 (mean 1.18 [95% CI; 0.96-1.41]), the two cohorts where chloroquine treatment occurred on day 10 (Table 16). Multivariable analysis using PCB as the key parameter did not result in it emerging as a statistically significant variable.

MARKERS OF HOST RESPONSE

Figure 20. Markers of host response



ALT; alanine transaminase; ULN; upper limit of normal, CRP; C-reactive protein.

Relationship between maximum temperature °C (20A), and CRP × ULN (20B) with peak ALT. Peak ALT is split into 2 groups; ≥2 × ULN and peak ALT<2 × ULN.

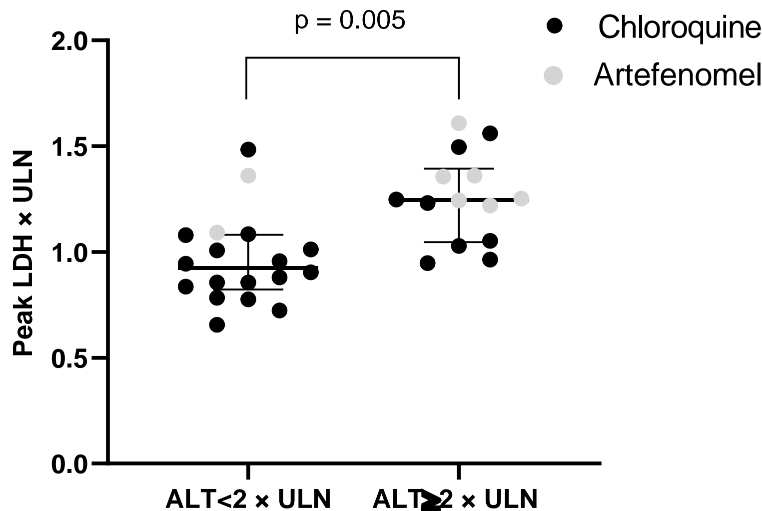
The maximum temperature (Figure 20A) was associated with an increased risk of having a peak ALT≥2 ×ULN, with an OR of 3.13 (95% CI 1.14-8.61, p=0.027) for a one degree increase in temperature. All subjects with a peak ALT>2 × ULN experienced a maximum temperature ≥38.3°C.

An increase in the maximum CRP of 4 mg/L (ULN=4 mg/L) increased the odds of a subject developing peak ALT≥2 × ULN by 1.81-fold (95% CI 1.21-2.71 p=0.004), whereas a 1-fold increase in maximum CRP relative to baseline increases the odds of developing peak ALT≥2 × ULN by 1.12-fold (95% CI 1.04-1.24 p=0.004). CRP levels when tested pre-treatment were typically much lower than peak CRP level occurring post treatment regardless of the day of treatment (8 or 10). On multivariable analysis, the CRP was a statistically significant factor when PCB was the explanatory variable, both before and after cohort effect was accounted for. The correlation coefficients between peak ALT and peak CRP × ULN and CRP relative to baseline were 0.44 (p=0.012) and 0.47 (p=0.007) respectively (appendix 12 Table 3).

Temperature was no longer significant in the logistic regression model when the cohort effect was included, whereas CRP remained significant (p=0.010 for CRP × ULN; p=0.006 for CRP relative to baseline) (Table 17).

MARKER OF HAEMOLYSIS

Figure 21. Maximum LDH × ULN split by ALT outcome



LDH; lactate dehydrogenase, ALT; alanine transaminase, ULN; upper limit of normal.

Relationship between LDH × ULN and peak ALT. Peak ALT is split into 2 groups; peak ALT≥2 × ULN and peak ALT<2 × ULN.

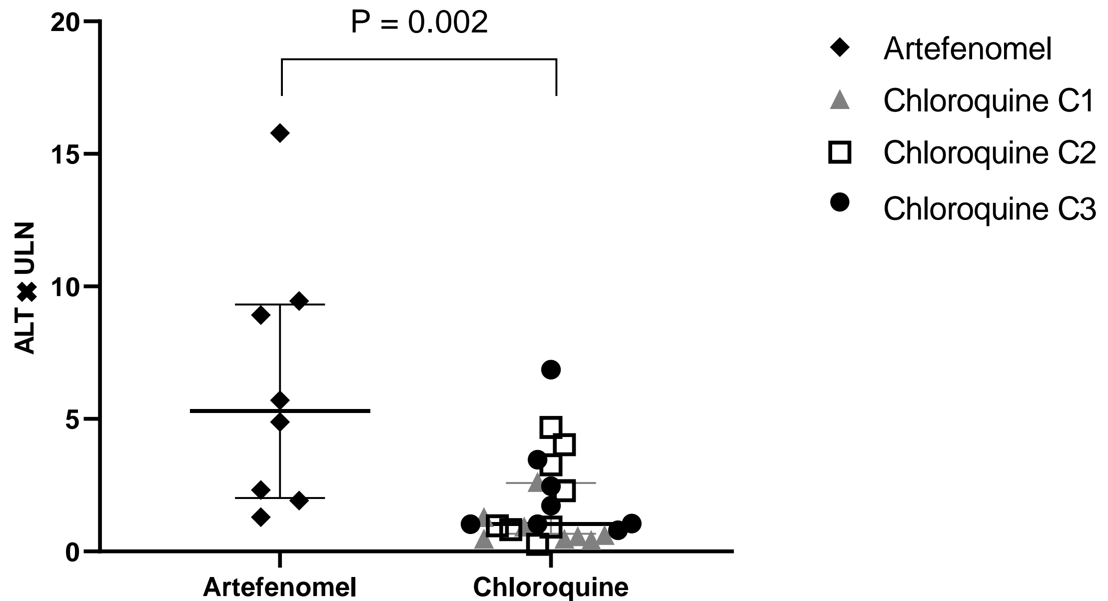
Maximum LDH has a significant association with peak ALT≥2 × ULN OR=1.89 (95% CI 1.21-2.95 p=0.005). An increase in the maximum LDH of 250 U/L (ULN=250 U/L) was associated with an increase in the odds of a subject developing peak ALT ≥2 × ULN by 1.89-fold. Peak LDH retained its significance when cohort effect was included (Table 19) . The correlation coefficient between peak ALT and peak LDH was 0.58 (p<0.001) (appendix 12 Table 3).

HAEMATOLOGICAL PARAMETERS

Most of the changes in laboratory haematology values were significantly associated with ALT elevations ≥2 × ULN (Table 17). These effects remained significant when the cohort effect was included. No pre-treatment cut off for any of these parameters was predictive of the development of significant ALT elevations later in the study. Platelet count was the only significant parameter on multivariable regression with $PCt_{1/2}$ as the fixed explanatory variable. A 0.1 decrease in the pre-treatment platelet count × LLN (e.g. from 0.7 × LLN to 0.6 × LLN) results in an increase in the odds of peak ALT being ≥2 × ULN by 2.79 (95% CI 1.12-6.93, p=0.027) (appendix 12 table 4. The association remained significant when cohort effect was added.

ANTIMALARIAL TREATMENT

Figure 22. Peak ALT × ULN for each IBSM subject per treatment



ALT; alanine transaminase, ULN; upper limit of normal.

Peak ALT × ULN for subjects treated with artefenomel (n=8) and chloroquine (n=24). All subjects treated with artefenomel were from the same cohort. Subjects treated with chloroquine came from three cohorts (chloroquine C1, n=8; chloroquine C2, n=8; chloroquine C3, n=8). The Mann-Whitney test was used to assess for difference in ALT values between the two drug treatments. Median Peak ALT was greater for subjects treated with artefenomel compared to those treated with chloroquine.

Table 18. Logistic regression with antimalarial drug type

Model	Measure	OR (95% CI)	p
Drug (n=32)	**Drug type**		
	Chloroquine	Reference	
	Artefenomel	6.00 (0.98-36.71)	0.053
Drug and Treatment day (n=31)	**Drug type**		
	Chloroquine	Reference	
	Artefenomel	4.50 (0.67-30.23)	0.12
	Treatment Day		
	Treatment day 8	Reference	
	Treatment day 10	4.67 (0.45-48.26)	0.20
Drug and PCB (n=32)	**Drug type**		
	Chloroquine	Reference	
	Artefenomel	0.69 (0.03-17.81)	0.82
	PCB (log_{10} scale)	6.29 (0.64-61.80)	0.12

OR; odds ratio, PCB; parasite clearance burden.

Logistic regression demonstrating the effect of drug and the day of treatment on the likelihood of ALT≥2 × ULN and the effect of drug on ALT≥2 × ULN after being corrected for PCB.

The median peak ALT × ULN level was greater among the 8 subjects administered artefenomel (5.3 IQR; 2.2-9.1), compared to the 24 subjects given chloroquine (1.0 IQR; 0.8-2.5) p=0.002 (figure 22), and a greater proportion of patients in the artefenomel group had a peak ALT≥2 × ULN (6/8 [75%] vs 8/24 [33%]).Thus the OR (95% CI) for developing ALT≥2 × ULN with artefenomel compared to chloroquine was 6.0 (0.98-36.71), but the difference did not quite reach statistical significance (p=0.053). When PCB was incorporated into bivariate model with drug effect this effect disappeared: OR 0.69 (95% CI; 0.03-17.81 p=0.82).

The peak concentration of artefenomel (C_{max}) the bloodstream varied from 319 ng/mL to 981 ng/mL. The bloodstream C_{max} for chloroquine varied from 563 ng/mL to 1606 ng/mL. The active metabolite N-desethylchloroquine (DCQ) bloodstream C_{max} varied from 80 ng/mL to 238 ng/mL. There was no link between peak artefenomel, chloroquine or DCQ concentration (C_{max}) or AUC_{96} and transaminase

elevations (Table 17 and appendix 12 table 6). The time until peak concentration (T_{max}) of artefenomel, chloroquine and DCQ was 3 to 6 hours, 2 to 5.45 hours and 2 to 5.73 hours respectively. All T_{max} time frames were well before the peak of the transaminase increases which typically occur between 120 to 192 hours post dose. Peak ALT occurred before any recrudescence in subjects treated with artefenomel.

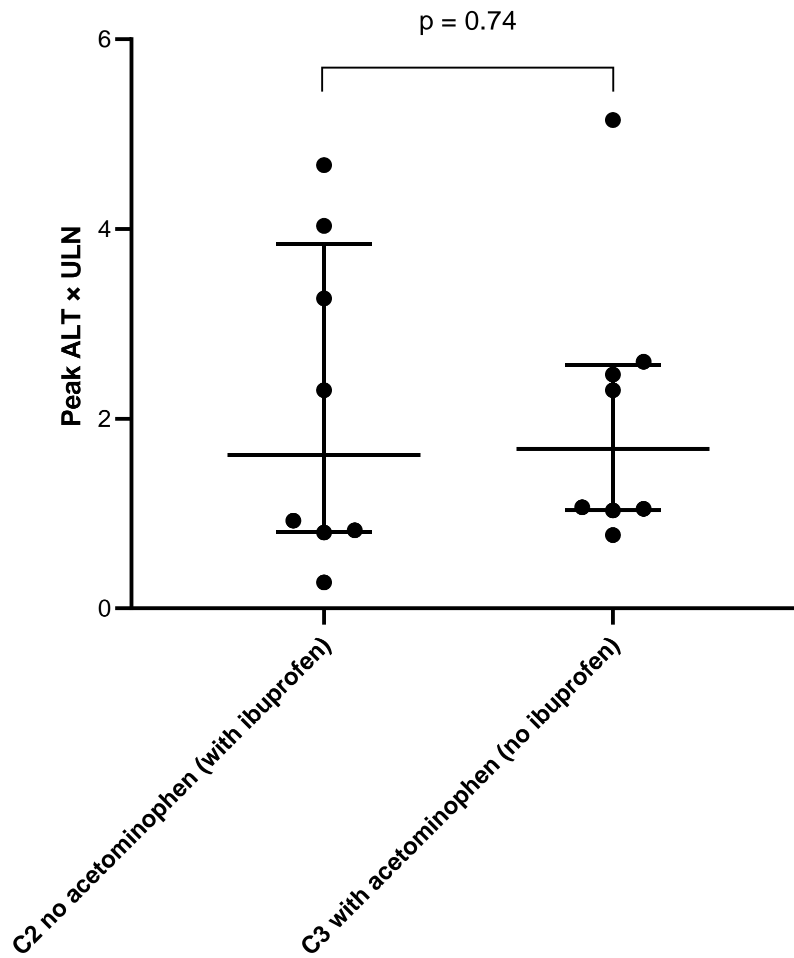
ACETAMINOPHEN AND IBUPROFEN

Table 19. Summary table of pain relief medication per cohort.

Cohort	Statistical measure	Total acetaminophen (g)	Total ibuprofen (g)
Artefenomel	median (IQR) n (%) positive	3.0 (2.5-5.5) 8 (100%)	2.0 (1.8-3.2) 8 (100%)
Chloroquine C1	median (IQR) n (%) positive	- -	1.0 (0-2.0) 5 (62.5%)
Chloroquine C2	median (IQR) n (%) positive	- -	2.4 (1.5-4.6) 8 (100%)
Chloroquine C3	median (IQR) n (%) positive	8.8 (5.0-10.0) 8 (100%)	- -

IQR; interquartile range. Table summarising acetaminophen and ibuprofen use across IBSM cohorts.

Figure 23. Peak ALT chloroquine cohort C2 (no acetaminophen) vs chloroquine cohort C3 (with acetaminophen)



ALT; alanine transaminase, ULN; upper limit of normal.

Peak ALT × ULN of subjects in chloroquine cohort C2 where acetaminophen (no ibuprofen) was used for analgesia/apyretic compared to chloroquine cohort C3 where ibuprofen (no acetaminophen) was used for analgesia/apyretic.

There was no statistically significant difference in median peak ALT between cohort C2 (no acetaminophen use, ibuprofen use [1.6 g IQR; 0.8-3.5]) and cohort C3 (acetaminophen use, no ibuprofen use [1.7 g IQR; 1-2.5]) (p=0.74).

BLOOD STAGE MALARIA DOSE

The total number of parasites inoculated per subject across all 32 subjects ranged from 8.5×10^5 to 1.27×10^6 parasites. The lowest inoculation dose occurred in the

artefenomel cohort, which saw the greatest LFT changes. Chloroquine cohort C2 to C4 had a narrow inoculation dose range (1.17×10^6 to 1.27×10^6 parasites) despite significant differences in LFT abnormalities between the cohorts.

ALTERNATIVE MEDICAL DIAGNOSIS

No alternative medical diagnoses were found that would account for the observed transaminase elevations (e.g. viral hepatitis, alcohol). All subjects had tested negative for hepatitis BsAg and core antibody, hepatitis C antibody and HIV at day 28 following completion of the active malaria infection component of the studies. One subject had a mildly raised bilirubin at day -1 having had a result within the normal range at screening. Study protocol allows for a single repeat of a raised ALT, AST or bilirubin at screening and for all subjects to have normal ALT, AST and bilirubin at day -3 to day -1. All subjects had BMI<30 with acceptable lipid profiles. Thus, in the context of largely normal pre-study LFTs and no significant medical history (e.g. diabetes), undiagnosed non-alcoholic steatohepatitis was thought to be unlikely. The pattern of LFT changes and clinical presentation was not in line with hepatitis A or E.

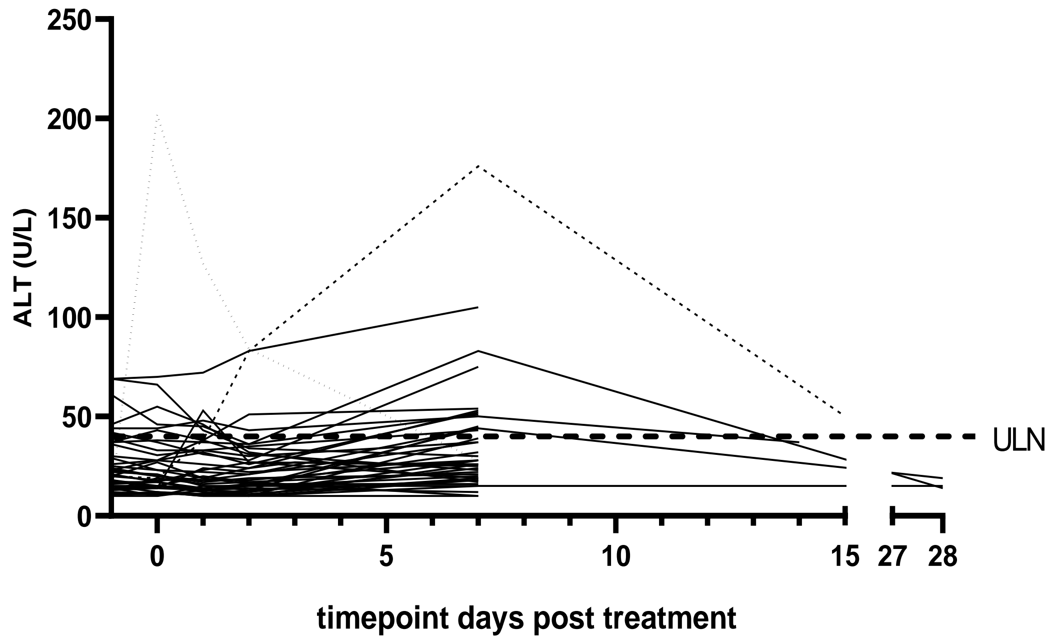
Liver ultrasound was not carried out as it was felt that it would not yield anything significant in the context of asymptomatic transient transaminase elevations that resolved within days. The gold standard investigation to confirm or rule out DILI would be liver biopsy, but this was not carried out for ethical reasons.

ENDEMIC STUDIES

THAI STUDY

41 patients had day 0 and day 7 ALT measures available and were included in the analysis (Figure 24). The median (IQR) age of 41 patients was 26 years (21-30); 32 (78%) were male. Mean (range) parasite count for *P. vivax* infected patients was 15,905/µL (5,010-53,400)[226].

Figure 24. Thai study ALT over time



ALT; alanine transaminase, ULN; upper limit of normal.

Changes in ALT levels (U/L) over time for all 41 subjects. Day -1 represents the day 0 pre-treatment value and day 0 represents the first ALT value post treatment. This was done to improve the graphical representation of the data. For ease of interpretation subjects 02-19 and 01-22 are represented by dotted and dashed lines respectively. The ULN for ALT in male and female subjects (40 U/L) from the assay used in the study has been included for reference.

Table 20. Liver function test measurements in Thai study

Measure (n=41)	Day 0 pre-treatment mean (95% CI)	Day 7 mean (95% CI)	p
ALT (U/L)	21.3 (17.9-25.3)	30.0 (24.7-36.5)	p<0.001
AST (U/L)	21.4 (19.1-23.9)	21.7 (19.2-24.5)	p=0.81
Total bilirubin (µmol/L)	16.6 (13.8-20.2)	7.2 (6.2-8.4)	p<0.001

ALT; alanine transaminase, AST; aspartate transaminase.

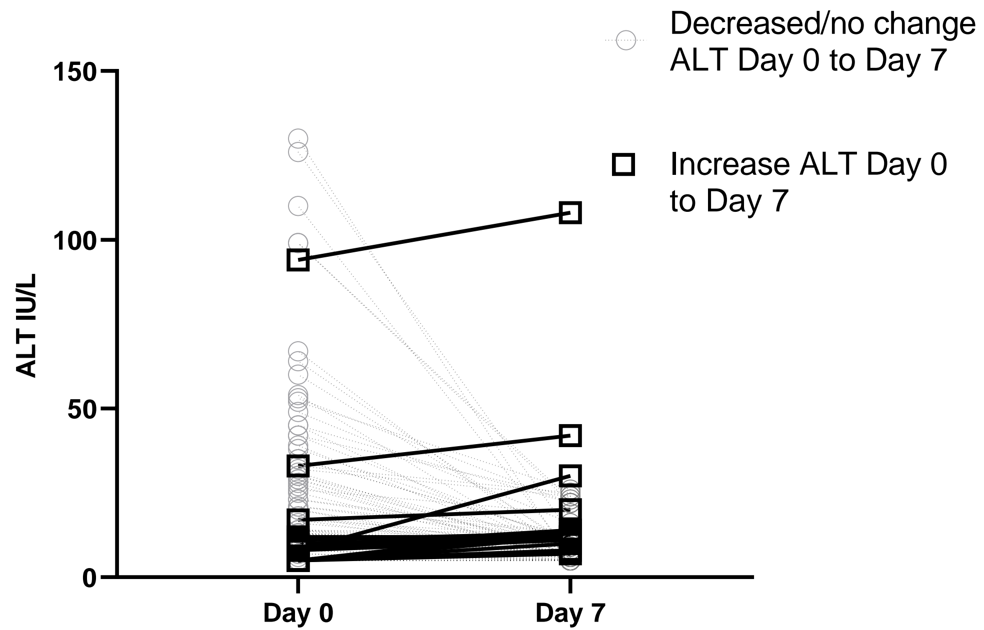
Mean (95% CI) and results of paired t-tests for LFTs between day 0 pre-treatment and day 7 measurements in patients from Thailand artefenomel phase IIa study.

Only one patient had a significant ALT elevation, with a maximum absolute increase of 156 U/L (20 U/L on day 0, 176 U/L on day 7, improving to 50 U/L on day 15) (Figure 24). This patient was treated with a 200 mg dose of artefenomel, the lowest dose used in the study.

MALAYSIAN STUDY

85 patients had day 0 and day 7 ALT measures available and were included in the analysis (Figure 25). The median (IQR) age was 16 years (11-64), and 63/85 (74%) were male. Median (IQR) parasite count was 4048 /µL (1523-8120). Not all subjects had day 0 AST or total bilirubin data (Table 21). 44 (51.8%) of the 85 subjects were treated with artesunate-mefloquine, 32 (37.7%) with chloroquine, 7 (8.2%) with artemether-lumefantrine & primaquine and 2 (2.4%) with chloroquine & primaquine.

Figure 25. Malaysian study ALT over time



ALT; alanine transaminase, ULN; upper limit of normal.

Day 0 and Day 7 ALT results for all subjects split into those who experienced an increase in ALT and those who experienced a decrease or no change in ALT.

Table 21. Liver function test measurements in Malaysian study.

Measure	n	Day 0 mean (95% CI)	Day 7 mean (95% CI)	p
ALT (IU/L)	85	18.1 (15.1-21.7)	9.6 (8.4-10.8)	p<0.001
AST (IU/L)	19	14.9 (11.3-19.8)	16.7 (13.7-20.3)	p=0.25
Total bilirubin (µmol/L)	76	9.8 (7.9-12.2)	5.7 (5.3-6.1)	p<0.001

ALT; alanine transaminase, AST; aspartate transaminase.

Mean (95% CI) and results of paired t-tests for LFTs between day 0 pre-treatment and day 7 measurements in patients from Sabah Malaysia dataset.

No significant changes in ALT (≥2 × ULN) from day 0 to day 7 occurred in any patient in any of the treatment groups .The maximum absolute increase observed was 22 IU/L, in a patient whose ALT increased from 8 IU/L on day 0 to 30 IU/L on day 7. No subjects developed ALT abnormalities like those observed in the *P. vivax* IBSM dataset.

DISCUSSION

INDUCED BLOOD STAGE MALARIA

DESCRIPTIVE SUMMARY

Asymptomatic elevations in transaminases equal to or greater than 2 times the ULN without concomitant increases in bilirubin occurred in 14/32 (44%) subjects involved in the *P. vivax* IBSM studies. Levels generally rose above the ULN 3 days post treatment, peaking 5-8 days post treatment with resolution by 32 days post treatment. Peak ALT was typically greater than peak AST. Transaminase elevations mirroring those seen in the *P. vivax* IBSM studies were rare in the endemic *P. vivax* studies occurring in only 1/41 (2.4%) Thai patients treated with artefenomel, and none of the 85 Malaysian patients.

In this analysis I was unable to identify a single factor that accounted for all the observed transaminase elevations in the *P. vivax* IBSM studies. However, statistically significant associations were observed between significant elevations in transaminase levels and PCB, inflammation and LDH. The abnormalities were not

thought to be related to an alternative medical illness, direct effect of artefenomel or chloroquine, malaria inoculum dose or concomitant medication use including analgesia. No pre-treatment parameter was able to accurately predict which subjects would go on to develop significant transaminase elevations.

ALT elevations were more common and severe in subjects with greater symptoms, and laboratory abnormalities. This may simply reflect that ALT elevations are more common and more severe in sicker patients.

PARASITEMIA RELATED MEASUREMENTS

$PCt_{1/2}$ was not associated with elevations of ALT≥2 × ULN, whereas both peak parasitemia and PCB were significantly associated with ALT≥2 × ULN, with PCB having a mildly stronger association than peak parasitemia (both pre-treatment and overall). PCB is a composite of the rapidity of antimalarial effect and peak parasitemia, and thus encompasses the biomass of parasites cleared immediately post treatment. PCB is the more significant determinant of whether a subject is likely to experience an ALT elevation ALT≥2 × ULN, than $PCt_{1/2}$, as it remained significant on univariate analysis. This could be explained by peak parasitemia being the stronger determinant of PCB, as the variation in peak parasitemia between subjects (3,980 parasites/mL to 331,130 parasites/mL) was greater than the variation in $PCt_{1/2}$ (2.83 to 6.72 hours). Furthermore PCB was more closely correlated to peak ALT than peak parasitemia and $PCt_{1/2}$; correlation coefficient of 0.58 ($p<0.001$), 0.39 ($p=0.027$) and -0.26 ($p=0.16$) respectively.

PCB was significantly higher in subjects treated with artefenomel than in those who received chloroquine. This may explain why ALT elevations occurred more commonly in subjects treated with artefenomel.

MARKERS OF HOST RESPONSE

CRP levels peaked post antimalarial therapy regardless of whether subjects were treated on day 8 or 10, implying that CRP increases are primarily a result of post treatment effects rather than due to malaria infection alone.

Higher maximum CRP and maximum temperature were associated with an increased risk of a peak ALT≥2 × ULN. All subjects with ALT≥2 × ULN had temp >38.3°C.

CRP was the only parameter that remained significant following multivariable analysis with both chosen explanatory variables; $PCt_{1/2}$ and PCB. This might suggest that systemic inflammation contributes to LFT abnormalities. This is in line with findings reported by Reuling et al[220] who described a positive correlation between LFT abnormalities in 16 subjects from a single VIS study and interferon (IFN), IL-6 and interleukin-8 (IL-8) levels 1 day post treatment and a strong correlation between cumulative inflammatory responses (IFNγ, MCP-1, IL-6, IL-8, IL-10, IL12p70, IL-17a, IL-18) and LFT abnormalities ($r=0.65$ $p=0.008$)[255].

MARKER OF HAEMOLYSIS

LDH was the only marker of haemolysis assessed during this analysis. A raised LDH was significantly associated with ALT$\geq$2 $\times$ ULN (OR 1.89 $\times$ ULN [95% CI 1.21-2.95]; $p=0.005$) and, alongside PCB, had the strongest correlation with peak ALT ($r=0.58$; $p<0.001$) aside from AST, suggesting a possible association with haemolysis and LFT abnormalities. However LDH is also elevated in ischaemic liver injury[256], lymphomas[257] and EBV infection[258], none of which were thought to be contributing to the observed LFT abnormalities. This supports the hypothesis that elevated LDH was caused by haemolysis and increases RBC turnover[259]. However it is difficult to separate the effect of haemolysis on LDH from the effect of transient liver injury on LDH. Furthermore the difference in median peak LDH between those subjects with ALT$\geq$2 $\times$ ULN (1.24 $\times$ ULN [IQR 1.05-1.36]) and ALT<2 $\times$ ULN (0.92 $\times$ ULN [IQR 0.84-1.08]) was relatively small suggesting it may not be clinically relevant.

HAEMATOLOGICAL PARAMETERS

Platelet count and neutrophil count were significantly associated with ALT elevation: these effects remained significant when the cohort effect was included. This may be explained by the fact that these parameters are not directly related to TNFα or the effects of haemolysis, but rather intrinsic host regulation of malaria and thus not as strongly affected by cohort as other parameters (PCB). None of the haematology parameters examined turned out to be reliable predictors of future ALT elevation.

ANTIMALARIAL TREATMENT

Subjects in the IBSM studies and treated with artefenomel generally experienced greater elevations in ALT than those treated with chloroquine (Figure 22). Drug related hepatotoxicity alone would not explain why LFT changes occurred in both chloroquine and artefenomel treated subjects, particularly as both possess different mechanisms of action. Chloroquine is a 4-aminoquinolone that acts against large ring form and mature trophozoite stages of malaria[260]. Artefenomel is a novel synthetic trioxolane[226] closely related to artemisinins, it contains the peroxidic pharmacophore believed to be responsible for potent antimalarial activity of artemisinins[261].

Transaminase elevations were not reported in a phase I study with artefenomel undertaken in 26 healthy volunteers without malaria[222]. Moreover the only patient with *P. vivax* infection in the phase IIa Thai study who experienced a transaminase elevation similar to those observed in *P. vivax* IBSM was treated with the lowest dose of artefenomel (200 mg, maximum dose 1200 mg) used in the study, again suggesting a direct drug effect from artefenomel is less likely to be the underlying cause of the LFT abnormalities. Only 1/448 (0.2%) subjects experienced a grade 3 (5.1-10 × ULN) transaminase elevation in a double-blind phase II study of single dose artefenomel (800 mg) in combination with piperaquine (640 mg, 960 mg or 1440 mg) for treatment of *P. falciparum* malaria[262]. The authors did not comment on the dose of piperaquine this subject received or on the presence of lesser transaminase elevations. Although the odds of developing elevations of ALT≥2 × ULN with artefenomel compared to chloroquine were higher, when corrected for PCB, the strength of association with artefenomel disappeared (Table 18).

I focused on PCB because it is a composite of the rapidity of antimalarial effect and peak parasitemia, and thus encompasses the biomass of parasites cleared immediately post treatment. In this way it distinguishes between artefenomel (a fast killing drug) and chloroquine, a drug with a slower effect on parasite clearance. PCB for the artefenomel treated cohort is greater than for the chloroquine cohorts overall and the two cohorts where chloroquine treatment occurred on day 10 (artefenomel subjects were all treated on day 10). Differences in PCB may account for why ALT elevations were greater in magnitude and frequency in subjects treated with artefenomel than with chloroquine.

ACETAMINOPHEN AND IBUPROFEN

The data suggest acetaminophen and ibuprofen are unlikely to have caused the ALT elevations during the *P. vivax* IBSM studies (Figure 23). Acetaminophen when consumed in excessive doses is known to produce ALT elevations in healthy subjects[263]. These elevations are much smaller than the elevations seen in IBSM and typically the total acetaminophen dose received by subjects was much smaller in the *P. vivax* IBSM studies. It is possible that malaria infection may lower the toxic threshold for acetaminophen[264]. However there are a lack of data to support this.

Ibuprofen is not known to cause LFT abnormalities similar to those observed in *P. vivax* IBSM.

CROSS INFECTION

The HMP013 *P. vivax* was used to infect the volunteers. It has been screened extensively for blood borne pathogens and inoculum are produced under sterile conditions and undergo sterility testing after each inoculation. Hence unintentional transmission of infection during malaria inoculation is unlikely to have caused the observed LFT abnormalities. Moreover based on the relatively small variation in *P. vivax* inoculation dose across cohorts and the fact that the greatest number and severity of transaminase elevations occurred in the cohort with the lowest inoculum dose (artefenomel cohort), it appears that the inoculum is not responsible for ALT elevations.

MALARIA PARASITE STRAIN

Whole genome sequencing of HMP013 *P. vivax* HMP bank did not reveal any significant changes compared to the reference strain *P. vivax* Sal 1[265, 266]. Similar LFT abnormalities to those described in the *P. vivax* IBSM studies have been observed in IBSM studies using *P. falciparum 3D7*[50] and *P. falciparum K13 R539T* variant (ANZCTR Trial ID: ACTRN12617000244303 and ACTRN12617001394336), as well as in *P. falciparum* studies where the infection was induced by sporozoites[220]. Therefore, the observed abnormalities are unlikely to be due to a specific effect from any species or strain used during these studies.

COHORT EFFECT

A fixed cohort effect was added to the regression models to account for any confounding effects from different cohort differences (e.g. treatment day, drug, pain relief protocol). A number of parameters went from statistically significance to non-statistically significant once the cohort effect was added (Table 17). These included parasitemia related parameters and temperature. This was expected given the impact factors such as the day of treatment possess over peak parasitemia, or similarly the impact of antimalarial treatment over $PCt_{1/2}$ both of which are determinants of PCB.

ENDEMIC STUDIES

In the Thai study only a single patient (1/41, 2.4%) had LFT changes similar to those observed in *P. vivax* IBSM, which was in line with the rates observed in endemic *P. falciparum* studies[267]. No LFT abnormalities were observed in patients from the Sabah Malaysia study.

COMPARISON WITH OTHER INDUCED BLOOD STAGE MALARIA STUDIES

Although absent from the original *P. vivax* IBSM pilot study[72] transaminase elevations of >5 × ULN were reported in 4/6 (66%) subjects in a *P. vivax* IBSM study that used a different parasite isolate and donor RBCs to infect subjects[60]. The high proportion of subjects with LFT abnormalities in this study, in the context of rapid parasite clearance, and therefore likely high PCB, post artemether-lumefantrine, are consistent with the findings reported here. Furthermore 5/6 (83.3%) subjects in this study experienced temperature >39.0°C, suggesting subjects experienced significant inflammation (authors did not report individual temperature data). CRP was not measured.

Similar transaminase elevations have been observed in *P. falciparum* IBSM[50, 264] suggesting that transaminase elevations in *P. vivax* and *P. falciparum* are caused by the same underlying mechanism. Abnormalities were observed following treatment with ferroquine[50], KAE609 [NIH NCT02543086], SJ733 [NIH NCT02867059]) and piperaquine[186] all of which are considered moderate or fast acting antimalarials and

thus would be expected to have relatively high PCBs[50, 186, 221, 268, 269, 270] in line the *P. vivax* findings.

COMARISON WITH LIVER FUNCTION TEST ABNORMALITIES IN NATURAL MALARIA INFECTION

In this analysis the focus was *P. vivax* related LFT abnormalities. However, studies involving other malaria species were included as the number of studies with *P. vivax* specific data is limited.

Malaria hepatopathy is defined as bilirubin >2.5 × ULN and rise in transaminases >3 × ULN[211, 212, 213, 214, 215]. Rates of malaria hepatopathy varied between 2.6% and 45% across all malaria species[211, 212, 216, 217, 218, 219]. Compared to malaria hepatopathy, LFT changes observed in malaria VIS differ significantly. LFT abnormalities in VIS are characterised by elevations in transaminases but not bilirubin and peak approximately 5-8 days post treatment compared to malaria hepatopathy which typically peaks around the time of treatment[211, 212, 216, 217, 218]. This suggests that malaria hepatopathy and LFT abnormalities in VIS are mediated by different mechanisms.

To date the most comprehensive review of serial LFT testing in naturally acquired infection has been conducted by Woodford et al[271]. 130/861 (15.1%) of returned travellers to Queensland Australia experienced ALT>3 × ULN with moderate (3-5 × ULN), severe (5.1-10 × ULN) and very severe (>10 × ULN) cases totalling 73 (56.2%), 41 (31.5%) and 13 (10%) respectively. 64.6% of these patients experienced their peak within 4 days of admission with the remaining 35.4% experiencing their peak between day 4 and day 11, the latter corresponds to the timing of abnormalities described in the *P. vivax* IBSM study. Woodford et al did not report any data on inflammation, such as temperature or CRP.

In a recent randomised clinical trial in West Africa where four different artemisinin combination therapies were evaluated in 4710 patients with 8640 malaria episodes (7119 *P. falciparum*, 146 *P. malariae,* 31 *P. ovale* and 17 mixed infections), LFT testing occurred on day 0 (pre-dose), 3, 7 and 28. LFT changes mimicking those reported here were observed after treatment with all 4 drug combinations in 0.9% to

3.9% of the 8640 treatment encounters, and were more commonly seen in children <5 years of age[267]. The majority of the significant ALT elevations that were observed, across all four arms, were >3 × ULN (range across four arms: 0.5-3%) with a smaller number of these subjects experiencing ALT>5 × ULN (range across four arms: 0.5-2.3%) and a minority with elevation >10 × ULN (range across four arms: 0-0.5%). The authors were not able to provide a unifying hypothesis for the transaminase elevations that were observed.

Artemisinins, quinine and chloroquine are the most common current and historical treatments for severe malaria. Parasite clearance in *P. vivax* is fastest with artesunate followed by artemether, then chloroquine, with quinine possessing a slower clearance; parasite reduction rate over 24hrs (range) of 844 (110-2613), 508 (57-3,869), 36 (1-1,184) and 4 (1-54) respectively[272]. If drug treatment has no effect on post treatment LFT changes, we would expect to see a similar pattern across all drugs. Alternatively, if PCB is a determinant of transaminase elevations it would be expected that LFT abnormalities occur more commonly in artemisinin-based therapy compared to chloroquine and fewer if any abnormalities in quinine based regimes.

Most studies involving artesunate, chloroquine and quinine that attempted to assess LFT changes did not carry out testing at timepoints equivalent to when abnormalities were observed in IBSM studies[224, 273, 274, 275, 276, 277, 278, 279] or did not comment on LFT findings taken at relevant timepoints[280, 281, 282].

Studies that reported LFT testing at corresponding timepoints to the IBSM abnormalities, persistently identified small numbers of patients with transaminase elevations that mirrored those seen in *P. vivax* IBSM in those treated with artemisinins[267, 283, 284, 285]. LFT abnormalities were much less common, if present at all, in patients treated with chloroquine and quinine compared to those treated with artemisinins[283, 285].

Absence of testing at time points of interest was an issue in many studies assessing the efficacy of antimalarials. Authors would not necessarily have been looking for post treatment LFT abnormalities as artemisinins did not demonstrate LFT abnormalities in phase I studies[223, 286, 287]. Similarly chloroquine and quinine are not

commonly associated with the development of LFT abnormalities that require routine monitoring.

POTENTIAL IMPLICATIONS

Physicians should become familiar with this phenomenon and remain vigilant for evidence of severe liver injury. Although, it is important to recognise that this observation represents a transient and asymptomatic finding. Typically patients would have completed their antimalarial treatment before any significant change in their transaminases. Therefore it should not affect antimalarial treatment. However other drugs, including antibiotics that are commonly prescribed alongside antimalarials for suspected or confirmed bacterial infection (e.g. typhoid), may be incorrectly implicated and stopped or changed unnecessarily. Furthermore it may result in unnecessary investigation, which may prove costly (ultrasound) or even dangerous (liver biopsy). When monitoring for resolution, where possible, is sufficient. Moreover transaminase elevations may be incorrectly assigned as a direct drug effect of antimalarial treatment (e.g. artesunate). In addition, incorrect assignment to a NCE could affect ongoing drug development resulting in delays or even cancellation of the drugs' development. Drugs with rapid parasite clearance are associated with improved prognosis[288]. LFT abnormalities are seemingly linked to PCB, which in turn is partly dependant on parasite clearance rate. Therefore drugs with rapid parasite clearance rates may end up becoming victims of their own success as they are more likely to be associated with transaminase elevations.

This issue is likely to occur in future IBSM studies. IBSM studies are often considered the most appropriate pre-endemic testing medium where such abnormalities can be identified in advance, documented and separated from true drug effect. If encountered in the endemic setting, transaminase elevations would be more difficult to untangle as monitoring, subject review and investigation is much more challenging. Furthermore, if there was to be an incidental rise in bilirubin (e.g. from a concomitant illness or medication) this may result in a technical case of Hy's law. During a large trial this may occur several times, which may result in the trial being stopped. Hence it is important that pharmaceutical sponsors are aware of, and understand, transaminase elevations in malaria, so that the IBSM model is not

avoided for fear of abnormal LFTs affecting drug development. Indeed, the presence of these transaminase elevations could be seen as a "badge of honour" as they are a surrogate marker of rapid parasite clearance.

In light of the findings it could be questioned whether the search for drugs with faster and faster parasite clearance is a necessary and appropriate goal. In theory, if the observed transaminase elevations were to occur in individuals with existing liver impairment it could be problematic. For example it could result in a patient with compensated liver failure becoming decompensated. Reassuringly artesunate has been established in many countries as the 1st line treatment for severe malaria for more than a decade and yet review of the literature did not reveal a single case report of mortality or long-lasting liver impairment associated with transaminase elevations similar to those seen in IBSM studies. However, it is important to note that physicians may not be looking for such abnormalities and thus would not necessarily report them. In retrospect, the absence of LFT testing at timepoints that correspond to transaminase elevations in IBSM studies during the seminal artesunate efficacy trials[130, 131] appears fortunate. As if LFT testing had occurred and led to the identification of transaminase elevations it may conceivably have led to the stalling or even abandonment of artesunate drug development.

POTENTIAL BIOLOGICAL MECHANISM

My analysis suggests PCB and inflammation appear to be factors in determining which subjects develop ALT elevations. A possible explanation may be the response to the demands of rapid haemolysis. Haemolysis is a universal consequence of malaria, affecting both parasitised and unparasitised RBC. Haemolysis leads to the release of free haemoglobin to plasma which is subsequently removed from the circulation by haptoglobin, hemopexin and heme oxygenase 1 (HO-1)[289, 290, 291]. In severe haemolysis haptoglobin, hemopexin and HO-1 capacity is overwhelmed leaving free heme to concentrate in serum. This process is summarised in the below figure.

Figure 26. Haemolysis in malaria

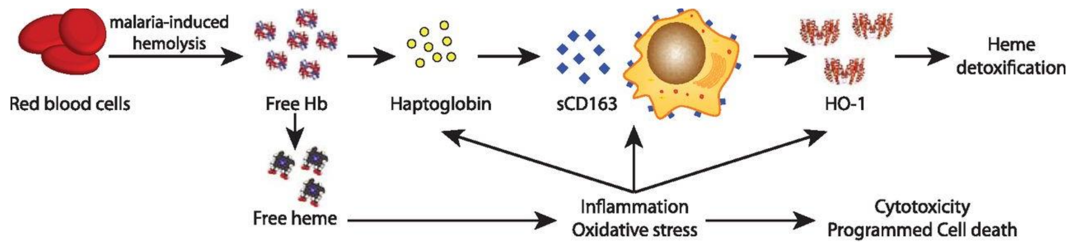


Figure 26 taken from Mendonça et al[292] demonstrating the primary mechanisms underlying haemoglobin metabolism in malaria.

Extracellular free haemoglobin and free heme are both agents that can induce significant oxidative stress[230, 293, 294] resulting in cell death via oxidative stress mediated necrosis and apoptosis[295, 296]. Heme is thought to sensitise cells to TNFα mediated apoptosis[296, 297, 298, 299] manifesting clinically as ALT/AST elevations from hepatocyte leakage[300, 301, 302].

LFT abnormalities similar to those in *P. vivax* IBSM studies have been observed in malaria mouse studies[297, 303]. The addition of zinc protoporphyrin, which inhibits HO-1, resulted in greater LFT abnormalities and hepatocyte apoptosis. Mouse hepatocyte studies demonstrated that at concentrations encountered in vivo during malaria infection, neither free heme nor TNFα were enough to induce cell death independently, but together they produced significant hepatocyte cell death (>25% of total hepatocytes)[296, 297, 298, 299]. Mouse studies suggest that LFT abnormalities are a result of free heme, produced by the saturation of intrinsic mechanisms designed to protect against damage from heme, released through post treatment haemolysis, acting synergistically with malaria induced TNFα.

This may explain why PCB, LDH, temperature and CRP were all associated with an increased risk of developing significant elevations in ALT. TNFα triggers CRP production[240], and has also been shown to be closely related to fever in *P. vivax* infection[250]. CRP and temperature may represent proxy measures of TNFα in this analysis.

Free heme and TNFα mediated hepatocyte apoptosis may explain the LFT abnormalities observed in the West African network study[267] discussed earlier. This

study involved high numbers of subjects all treated with artemisinin-based regimes with fast parasite clearance and thus high PCB. Furthermore there was a relatively large number of children <5 years of age in each arm, who are more likely to mount a vigorous TNFα response than the adult population from the same area[304, 305]. Moreover, LFT testing occurred on day 3 and day 7 increasing the chances that significant elevations in transaminases are identified.

Furthermore this hypothesis could explain the lack of LFT abnormalities in malaria patients treated with chloroquine and quinine compared to artemisinin. The slower onset of action of chloroquine and quinine would be expected to result in less haemolysis and TNFα generation, through hemozoin phagocytosis, and hence more modest transaminase elevations.

This may also explain why significant ALT elevations were not observed in the Sabah Malaysia study. Chloroquine was used in 34/85 (40.1%) of subjects, only one subject was <5 years of age and LFTs occurred on day 0 and 7 only, resulting in lower PCB, greater levels of malaria immunity[304] and fewer datapoints to detect transaminase elevations respectively. It should be noted that in general immunity to *P. vivax* malaria is significantly lower in Sabah than malaria endemic areas in West Africa[28, 304], and thus patients are likely to produce higher amounts of TNFα which may play a key role in the development of transaminase elevations. Such characteristics may reduce the likelihood of detecting transaminase elevations, but the absence of any elevations suggests an alternate explanation. One possibility is that patients in Sabah could possess a genetic polymorphism that limit the formation of toxic free heme[306] a potential factor in the causality of the LFT abnormalities. However genetic predisposition alone does not explain why transaminase elevations occur across several VIS sites around the world, are more common and severe in artefenomel treated subjects compared to chloroquine treated subjects, nor the relatively recent recognition of this phenomenon, or the association with PCB. Alternatively the high prevalence of iron deficiency[307, 308, 309] in the population may protect against the formation of free heme[306]. Iron deficiency anaemia is common in Sabah with levels varying from 20% in adolescents[307], 40% in pregnant females[308, 309] and up to 55% children[310]. Data on the prevalence of iron deficiency specifically in Bangkok and north west[311] Thailand is limited.

Although there is evidence to support the hypothesis that post treatment LFT abnormalities in malaria are secondary to free heme and TNFα, this remains unproven and requires further investigation.

UNIFYING HYPOTHESIS ADDRESSING PLASMODIUM VIVAX INDUCED BLOOD STAGE MALARIA

I hypothesise that transaminase elevations in IBSM *P. vivax* studies are thought to be due to a combination of; the use of drugs with fast parasite clearance overwhelming pathways designed to protect against haemolysis resulting in free heme. Free heme together with TNFα, produced in greater amounts by non-immune human volunteers compared to individuals infected in the endemic setting[253, 312], result in hepatocyte apoptosis manifesting as transaminase elevations. Furthermore, ascertainment bias meant LFT testing in IBSM studies occurred at timepoints corresponding to peak transaminase elevations in contrast to studies in the endemic setting.

STRENGTHS AND LIMITATIONS

Rich data sampling in IBSM studies allowed for the investigation of parasite clearance rate, PCB, haemolysis and inflammation as potential explanations of LFT abnormalities. Despite being the largest dataset of LFT abnormalities in *P. vivax* IBSM, the relatively small number of subjects mean any conclusions should be made with caution, and a larger dataset would be required for a more robust multivariable analysis.

Differences between cohorts, meant comparisons across cohorts was difficult and could have the outcome of removing the power of certain associations e.g. in the case of PCB which is strongly affected by both day of treatment and the antimalarial used.

Additional testing for markers of haemolysis, free heme, and TNFα was not undertaken. Instead PCB, LDH and markers of inflammation were considered as potential proxy measures for free heme and TNFα[246, 247, 249, 250]. PCB has not been described before, as such there is no existing literature regarding its association with haemolysis. An advantage of PCB is that it can easily be applied to other studies as it uses existing data and does not require any additional sampling. Conversely, as it is

calculated solely from two data points it may be susceptible to bias. Moreover during this analysis LDH was interpreted primarily as a marker of haemolysis. However it is difficult to exclude a direct effect from the liver.

RECOMMENDATIONS

Physicians must be made aware of transaminase elevations post antimalarial treatment and remain vigilant for evidence of severe liver injury. Although, it is important to recognize that these observations represent a transient and asymptomatic finding and monitoring for resolution is all that is required currently. Researchers and pharmaceutical companies should become familiar with this phenomenon so that alternate explanations for the causation of LFT changes can be considered in the course of clinical development of antimalarials.

Prospective testing for TNFα and free heme during the time of malaria infection and subsequent transaminase elevation in a *P. vivax* IBSM study would provide definite evidence for the proposed hypothesis. Furthermore if possible, samples should be taken during the study that allow for retrospective testing of haptoglobin, hemopexin, ferritin and HO-1. In addition routine testing for CRP and calculation of PCB is being considered in future IBSM study designs.

Retrospective testing of existing patient samples from IBSM studies, other VISs or endemic studies for markers of haemolysis and inflammation would provide a more rapid source of data to corroborate the findings observed during this analysis. Testing for inflammatory markers other than CRP and TNFα, as have occurred in other studies, would be useful[220].

Extending the analysis to the remaining *P. vivax*, and larger, *P. falciparum* IBSM datasets may provide additional information. Specifically maximum temperature, laboratory markers for inflammation and haemolysis, and parasitemia related factors including peak parasitemia, $PCt_{1/2}$ and PCB should be compared against peak ALT. Ideally this should be implemented across a range of IBSM studies involving NCEs and existing antimalarial treatments, including any that have been implicated as possible causes of transaminase elevations. Studies lacking transaminase elevations should be included as relevant controls. In order to better understand the effects of

$PCt_{1/2}$ and PCB on post-treatment transaminase elevations, it would be desirable to include studies assessing antimalarials with a range of parasite clearance rates.

Going forward it may be prudent to re-evaluate the conventional study cut-off of ALT≥2.5 × ULN, originating from cancer treatment trials[313], to a cut off that may more accurately capture the observed phenomenon in healthy volunteers thus allowing for a more accurate assessment of causality and risk factors, namely ALT≥2 × ULN. A reduction in the cut off could only be implemented following assessment of individualised data to ensure ALT elevations peak post treatment in asymptomatic subjects with elevations in ALT being greater than AST and occurring without significant elevations in bilirubin and with no alternative cause.

It would be valuable to retrospectively review existing data from natural malaria infection studies for evidence of transaminase elevations, beginning with studies involving returned travellers with malaria treated with artemisinin-based therapies, as such patients are more likely to yield positive findings.

Prospective LFT testing during a large randomised control trial comparing a fast and slow acting antimalarial in the treatment of *P. vivax* malaria in the endemic setting and returned travellers would provide the ideal way of assessing for this phenomenon in natural infection. Conversely routinely incorporating LFT testing on days 3, 5 and 7 post treatment into antimalarial trial study design would be easier to implement.

CONCLUSION

Post treatment LFT changes are transient, asymptomatic and appear to be more common in *P. vivax* IBSM than in natural infection. Evidence indicates that PCB, haemolysis and systemic inflammation post antimalarial treatment may be drivers for the transaminase elevations observed in *P. vivax* IBSM studies. However, the mechanisms underlying these abnormalities remain unclear and require further investigation. Going forward it would be appropriate to conduct a similar analysis on LFT abnormalities seen in *P. falciparum* VIS studies and incorporate LFT testing on days 3, 5 and 7 post treatment initiation, where possible, into the study design of antimalarial clinical trials. Researchers and pharmaceutical companies should

become familiar with these findings so that this phenomenon can be better understood, and alternate explanations of the causation of LFT changes can be considered in the development of antimalarials.

CHAPTER 6 DISCUSSION

Volunteer infection studies (VIS) have become valuable tools for basic research and the development of new interventions against a number of infectious diseases[39, 40, 41, 42, 43]. Safety is critical to the future success of VIS. A single serious adverse event could risk the future of VIS models worldwide. Moreover the media and political fallout in the event of a serious breach of safety could have wide ramifications.

In the absence of long-term culture and the resulting limitations on assessments of drug efficacy, *P. vivax* IBSM offers the potential to link pre-clinical research and expensive field studies. The aim of this thesis was to explore safety concerns around the *P. vivax* IBSM model, by centring around an exploratory study using apheresis as a method to extract and concentrate all stages of *P. vivax* parasites from healthy volunteers infected with blood stage *P. vivax*. Specific safety concerns for *P. vivax* IBSM include transfusion related infection, transfusion reactions, alloimmunisation, exposure to infectious materials and onward malaria transmission. These concerns were addressed prior to initiation of the *P. vivax* apheresis exploratory study. Mitigation strategies against existing safety concerns in malaria VIS have been well established. To prepare for the apheresis study, I conducted a review of the safety of apheresis in the treatment of *P. falciparum* infection. To provide a comprehensive analysis the review was extended to include the use of apheresis in babesiosis, as babesiosis shares many similarities with malaria[121, 122]. Furthermore to maximise the benefit to the scientific and clinical community the scope of the review was increased to include an analysis of apheresis in the treatment of loiasis and severe pertussis infections.

The quality of the published data retrievable for the review was low, consisting of case studies, case series and cohort studies with no randomised control trials. With the exception of apheresis in severe pertussis, no serious safety concerns were identified. The aim of apheresis treatment in severe pertussis (reduction of leukocyte burden), differ considerably from malaria and thus the safety concerns were considered sufficiently distinct to be excluded. Efficacy data supported the use of apheresis as adjuvant treatment in patients hospitalised for babesia, and prior to chemotherapy in loiasis with microfilarial count >8000/mL.

The data did not support the routine use of apheresis in severe *P. falciparum* malaria. However adjunctive treatment with apheresis tended to be initiated in the sickest patients with severe *P. falciparum* (higher parasitemias and APACHE-2 scores)[132, 172, 173] which may account for the lack of demonstratable benefit when compared to chemotherapy alone[157, 161]. The available data support the safety of apheresis in severe *P. falciparum*. Thus, apheresis could be utilised in the treatment of severe *P. falciparum* with poor prognosis, particularly when there is no access to intravenous artesunate[314], the patient is allergic to artemisinin[167, 168] or at risk of infection with an artemisinin resistant *P. falciparum*[315]. If used, apheresis should not delay initiation of antimalarial treatment, it should take place at least 4 hours post artesunate dosing, and should be discontinued if the patient develops hypotension during the procedure, to avoid any adverse events from the use of intravenous fluids[169]. Although the focus should remain on improving access to intravenous artesunate and the development of new clinical entities (NCE) active against artemisinin resistant parasites, in the meantime apheresis may be considered on a case by case basis.

A mismatch exists between the location of most loiasis[316, 317] and severe *P. falciparum*[28] cases and the location of most apheresis units. This together with the low numbers of severe pertussis[140, 318] and babesia[133, 172, 173] cases mean that randomised control trials assessing the use of apheresis in any of these conditions are unlikely to occur. Therefore the data compiled during the review is the best currently available and unlikely to be greatly improved in the near future.

The phase 1 exploratory study, aimed at assessing the use of apheresis in healthy volunteers infected with blood stage *P. vivax* in order to harvest all life cycle stages of *P. vivax*, did not identify any serious safety concerns. However it failed to produce sufficient concentration of *P. vivax* parasites to meet its study objectives. At the end of each cohort an analysis was conducted, and hypotheses were formed as to why the desired outcomes were not met. The study plan was subsequently adjusted in light of these hypotheses but still failed to produce results that supported the use of apheresis as a method for harvesting parasites for downstream experimentation. The data accrued were in agreement with previous data that suggested that *P. vivax* and *P. falciparum* gametocytes and asexual parasites preferentially concentrate around the Buffy coat, and that concentrating for reticulocytes does not necessarily

improve the yield of *P. vivax* parasitised RBCs[191]. This information may be used in future attempts to concentrate and preserve *P. vivax* parasites outside of the setting of apheresis, or in the future use of adjuvant apheresis for the treatment of *P. falciparum*. However, with the exception of cohort 4 the procedure employed during the apheresis exploratory study is likely to differ substantially to that used for treatment.

Increased transmission to mosquitos in membrane feeding using apheresis samples compared to pre-apheresis was only identified on one occasion. Although an interesting finding, the magnitude of improvement was insufficient to support the use of apheresis in this context. As percoll enrichment results in a higher rate of transmission[73] this is preferred. Given the expense and safety concerns in IBSM studies, conducting such a study based on a single result would be inappropriate.

The level of parasite concentration seen was not sufficient to warrant further investigation, given the significant ethical and resource concerns in IBSM studies. It is important that funding and political support for *P. vivax* research continues. Without this support it is unlikely that an effective, reliable and logistically acceptable source of *P. vivax* parasites necessary for basic and translational research will be discovered. This limitation remains a major impediment to the discovery of new hypnozoiticidal therapies.

No new safety issues were identified during the apheresis exploratory study. However, an existing safety concern, namely transaminase elevations, were seen in three out of the four subjects who took part in the study.

LFT abnormalities observed in *P. vivax* IBSM are thought to result from antimalarials inducing rapid parasite clearance overwhelming pathways designed to protect against haemolysis resulting in the release of free heme. I hypothesise that free heme together with TNFα, produced in greater amounts by non-immune human volunteers compared to individuals infected in the endemic setting[253, 312], induce hepatocyte apoptosis resulting in transaminase elevation.

The associations between peak ALT and parasite clearance burden (PCB), LDH, CRP and temperature are in line with this hypothesis. In my study I described a new metric, namely PCB. PCB was designed to represent the level of saturation of

intrinsic pathways designed to protect against the potentially damaging by-products of haemolysis post antimalarial treatment[230, 289, 293, 294, 319, 320]. PCB was calculated without the need for further sampling and can be readily applied to other malaria VIS where qPCR has been used to monitor parasite clearance post treatment. Furthermore the abnormalities seen in *P. vivax* IBSM have been identified in field studies[267]. Understandably these abnormalities have been met with concern from clinicians, researchers, ethics committees and pharmaceutical collaborators.

Several hypotheses have attempted to explain transaminase elevations[220], none of which fully explain why the observed abnormalities occurred across different VIS methodologies, different parasite species and strains, multiple antimalarial candidates and were not prominent in the history of clinical research in malaria. Free heme and TNFα induced hepatocyte apoptosis is a possible explanation for the observed abnormalities. Clinicians who manage naturally acquired malaria should become familiar with these observations to avoid unnecessary investigation or treatment.

In retrospect adverse events caused by rapid clearance of large malarial parasite loads would be in line with post treatment findings in other blood borne parasitic infections, namely loiasis[321], lymphatic filariasis[322] and onchocerciasis[323]. It could be considered fortuitous that such problems were not anticipated during the era of artemisinin development. Had they been anticipated, additional LFT testing during investigational studies may have been the result. The subsequent identification of LFT abnormalities may have led to the discontinuation of artemisinin drug development.

Available data on the use of apheresis in the treatment of severe *P. falciparum* infection do not support its implementation for this indication. While the conduct of an appropriately powered randomised control trial would provide a definite answer, the logistic challenges of undertaking such a study represent important barriers. Establishment of apheresis in resource limited settings for the treatment of other conditions, such as sickle cell disease[324, 325, 326], could allow for future randomised control trials comparing standard therapy and adjunctive apheresis to standard therapy alone. Until the necessary infrastructure and financial support are in place for a randomised control trial, it would be sensible to establish a clinical registry,

with standardised reporting of apheresis procedures carried out in the treatment of severe *P. falciparum.* Important measures would include recording of pre and post apheresis parasitemia, safety data and clinical outcomes.

The apheresis exploratory study was terminated when it became apparent that the procedure was not sufficiently concentrating parasites. It is possible that technical advances or improvement in processes may facilitate future experiments for example increasing the level of gametocytes to permit more efficient transmission in mosquito feeding assays, and potentially increasing the number of sporozoites harvested from mosquito salivary glands. Such experiments may also define the buoyancy of *P. vivax* parasites across haematocrit (HCT) layers, potentially informing any future apheresis studies.

Going forward research should include extending the LFT analysis to *P. vivax*, and larger, *P. falciparum* IBSM datasets. It would be prudent to use ALT≥2 × ULN cut off to allow for a more accurate assessment of causality and risk factors for post treatment LFT abnormalities. Moreover, prospective testing for free heme and TNFα during an IBSM study would provide definitive evidence of free heme and TNFα induced hepatocyte apoptosis. Furthermore, mechanistic studies in pre-clinical models, such as the establishment of a malaria mouse model with testing of LFTs, free heme, markers of haemolysis and TNFα post antimalarial treatment, will allow for greater understanding of the processes underlying the observed LFT abnormalities.

A large multi-arm randomised control trial comparing fast and slow acting antimalarials, including those associated with transaminase elevations in IBSM, for the treatment of *P. vivax* infection, both in endemic settings and returned travellers, with prospective LFT testing would advance understanding of post treatment LFT abnormalities in natural infection. Given the expense and resources required for such a study, incorporating LFT testing on days 3, 5 and 7 post treatment into the design of antimalarial drug trials would be more readily implemented. Finally, it would be prudent to conduct a more comprehensive review of existing data from natural malaria infection studies for evidence of transaminase elevations.

P. vivax IBSM studies are a safe and acceptable model for the study of *P. vivax* parasite biology, immunology and transmission, and the development of new interventions against this parasite. However, the precise execution of existing safety controls, risk assessment of new safety concerns and the thorough and prompt assessment of genuine safety signals are necessary to maintain the acceptability of *P. vivax* IBSM.

SUMMARY OF KEY FINDINGS

Apheresis may be a useful adjunct to chemotherapy in the treatment of patients hospitalised for babesia, and prior to chemotherapy in loiasis with microfilarial count >8000/mL. The data did not support the use of apheresis in critical pertussis infection or for patients with severe *P. falciparum* malaria.

Apheresis can be carried out safely in healthy volunteers infected with *P. vivax* and is able to achieve a modest level of parasite enrichment compared to whole blood sampling (4.9-fold and 1.45-fold per mL of sample for asexual parasites and gametocytes respectively) but the level of enrichment was not sufficient to recommend apheresis as a method for harvesting *P. vivax* parasites.

Post treatment transaminase elevations are transient, asymptomatic and appear to be more common in *P. vivax* IBSM than in natural infection. Evidence indicates that parasite clearance burden, haemolysis and systemic inflammation post anti-malarial treatment may be drivers for the observed transaminase elevations.

APPENDICES

APPENDIX 1. SAFETY AND EFFECTIVENESS OF APHERESIS IN THE TREATMENT OF INFECTIOUS DISEASES: A SYSTEMATIC REVIEW PROTOCOL.

Keywords

apheresis; erythrocytapheresis; falciparum, leukocytapheresis; red cell exchange; malaria; babesia; babesiosis; loa; loiasis; pertussis, cytapheresis, plasmapheresis

Inclusion criteria

Types of participants

This review will consider studies that include:

- Male or Female patients of any age with severe *falciparum* malaria treated using apheresis
- Male or Female patients of any age with loiasis infection treated using apheresis
- Male or Female patients of any age with babesiosis infection treated using apheresis
- Male or Female patients of any age with severe pertussis infection treated using apheresis

Types of intervention(s)/phenomena of interest

This review will consider studies that evaluate automated apheresis Types of automated apheresis to be evaluated are removal of:

- red cells (erythrocytapheresis)
- white cells (leukocytapheresis)
- plasma (plasmapheresis)

In addition to type, the following characteristics of apheresis will be abstracted from papers (where documented):

- apheresis protocol (e.g. number of cycles)

- apheresis equipment (e.g., Hemonetics model, COBE Spectra, etc)
- continuous or discontinuous removal and replacement of blood cells

This review will not consider studies that evaluate whole blood exchange transfusion.

Types of outcomes

This review will consider studies that include the following outcomes relating to effectiveness and safety of apheresis. In case of any uncertainty of inclusion/exclusion of a study other clinical authors will be asked to review and clarify.

Outcomes relating to effectiveness are:

Malaria

- Reduction in percentage parasitaemia
- Clinical outcome (survival vs death)

Loiasis

- Percentage reduction microfilariae
- Successful reduction of microfilariae <8000/mL
- Resolution of symptoms

Babesiosis

- Percentage reduction parasitaemia
- Clinical outcome (survival vs death)

Pertussis

- Percentage reduction in leukocyte count
- Clinical outcome (survival vs death)

Outcomes relating to safety are:

Malaria

- Number of adverse events due to apheresis
- Type of adverse events due to apheresis
- Number of complications
- Type of complications

Loiasis

- Number of adverse events due to apheresis
- Type of adverse events due to apheresis
- Number of complications
- Type of complications

Babesiosis

- Number of adverse events due to apheresis
- Type of adverse events due to apheresis
- Number of complications
- Type of complications

Pertussis

- Number of adverse events due to apheresis
- Type of adverse events due to apheresis
- Number of complications
- Type of complications

Types of studies

Literature searches will not be restricted to a type of study; however, it is anticipated that most (or all) of the relevant studies will be case reports.

Search strategy

The search strategy aims to find both published and unpublished studies. A two-step search strategy will be utilized in this review. An initial limited search (see below) of MEDLINE, PUBMED, EMBASE and CINAHL will be undertaken followed by an analysis of the text words contained in the title and abstract, and of the index terms used to describe article. Secondly, the reference list of all identified reports and articles will be searched for additional studies. Studies published in all languages will be considered for inclusion in this review. Papers not written in English and identified as potentially relevant (based on their title and/or abstract) will be translated as required. Because apheresis was introduced in the 1970s, searches will be restricted to studies published from 1 January 1969 to current. Animal studies will be excluded.

The indexes of the following journals will be hand searched: *Journal of Clinical Apheresis*, *Transfusion and Apheresis Science, Therapeutic Apheresis and Dialysis,*

The databases to be searched include: MEDLINE, EMBASE, PUBMED, COCHRANE and CINAHL.

The search for unpublished studies will include American society for apheresis.

Data extraction

Data from papers will be extracted into four Excel spreadsheets (1 spreadsheet per condition). The data extracted will include specific details about the apheresis intervention, populations, study methods and outcomes of relevance to the review questions and objectives.

Data synthesis

Quantitative data will, where possible be pooled in statistical meta-analysis using JBI-MAStARI. All results will be subject to double data entry. Effect sizes expressed as odds ratio (for categorical data) and weighted mean differences (for continuous data) and their 95% confidence intervals will be calculated for analysis #modify text as appropriate#. Heterogeneity will be assessed statistically using the standard Chi-square and explored using subgroup analyses based on the different study designs included in this review. Where statistical pooling is not possible the findings will be presented in narrative form including tables and figures to aid in data presentation where appropriate. However, going into the review, we expect that the data will only allow for descriptive analysis.

Severe Malaria

Manifestation	Features
Initial World Health Organization criteria from 1990	
Cerebral malaria	Unarousable coma not attributable to any other cause, with a Glasgow Coma Scale score ≤ 9. Coma should persist for at least 30 min after a generalized convulsion
Severe anemia	Haematocrit <15% or haemoglobin < 50 g/L in the presence of parasite count >10 000/μL
Renal failure	Urine output <400 ml/24 hours in adults (<12 mL/kg/24 hours in children) and a serum creatinine>265 μmol/L (>3.0 mg/dl) despite adequate volume repletion
Pulmonary edema and acute respiratory distress syndrome	The acute lung injury score is calculated on the basis of radiographic densities, severity of hypoxemia, and positive end-expiratory pressure
Hypoglycemia	Whole blood glucose concentration <2.2 mmol/l (<40 mg/dL)
Circulatory collapse (algid malaria)	Systolic blood pressure <70 mmHg in patients >5 years of age (<50 mmHg in children aged 1–5 years), with cold clammy skin or a core-skin temperature difference >10°C
Abnormal bleeding and/or disseminated intravascular coagulation	Spontaneous bleeding from gums, nose, gastrointestinal tract, or laboratory evidence of disseminated intravascular coagulation
Repeated generalized convulsions	≥3 convulsions observed within 24 hours

Manifestation	Features
Acidemia/acidosis	Arterial pH <7.25 or acidosis (plasma bicarbonate <15 mmol/L)
Macroscopic haemoglobinuria	Haemolysis not secondary to glucose-6-phosphate dehydrogenase deficiency
Added World Health Organization criteria from 2000	
Impaired consciousness	Rousable mental condition
Prostration or weakness	
Hyperparasitaemia	>5% parasitized erythrocytes or >250000 parasites/µL (in nonimmune individuals)
Hyperpyrexia	Core body temperature >40°C
Hyperbilirubinemia	Total bilirubin >43 µmol/L (>2.5 mg/dL)

APPENDIX 2. APHERESIS OF SUBJECTS WITH INDUCED BLOOD STAGE P. VIVAX PROTOCOL.

Apheresis of subjects with induced blood stage *Plasmodium vivax*

Protocol Identifying Number: QP17C14/P2355

Principal Investigator: Prof. James McCarthy

Sponsor: QIMR Berghofer Medical Research Institute (QIMR Berghofer),

300 Herston Road, Herston QLD 4006

Version 4.1

15 Nov 2018

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ACTH	Adrenocorticotrophic Hormone
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BSPC	Blood Stage *Plasmodium* Challenge
CHMI	Controlled Human Malaria Infection
CMNC	Continuous Mononuclear Cell
CMV	Cytomegalovirus
CRF	Case Report Form
CRU	Clinical Research Unit
CTCAE	Common Terminology Criteria for Adverse Events
DFA	Direct feeding assay
DRE	Disease-Related Events
EBV	Epstein-Barr Virus
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EOS	End of Study
FSH	Follicle Stimulating Hormone
G6PD	Glucose-6 phosphate dehydrogenase
GCP	Good Clinical Practice
GMP	Good Manufacturing Practices
HMP	Human Malaria Parasite
HREC	Human Research Ethics Committee
IB	Investigator’s Brochure
IBSM	Induced Blood Stage Malaria
ICH	International Conference on Harmonisation
IMM	Local Independent Medical Monitor
IUD	Intrauterine
LFT	Liver function test
MCB	Master Cell Bank
MedDRA	Medical Dictionary for Regulatory Activities
MFA	Membrane feeding assay
PBMC	Peripheral Blood Mononuclear Cells
PI	Principal Investigator
PRN	Pro Re Nata (as needed)
PT	Preferred Term
QIMR Berghofer	Queensland Institute of Medical Research Berghofer

qPCR	Quantitative polymerase chain reaction
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
Rh	Rhesus
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCID	Severe Combined Immunodeficiency
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
SRT	Safety Review Team
SUSAR	Suspected Unexpected Serious Adverse event
TEAE	Treatment-Emergent Adverse Event
WHO	World Health Organization
WOCBP	Women of Childbearing Potential

Investigator declaration

I have read the protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct this protocol as outlined herein and will make a reasonable effort to complete the study within the time designated.

I agree to personally conduct or supervise the described study.

The study will be conducted in accordance with the following:

- World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (Fortaleza, Brazil 2013).
- NHMRC National Statement on Ethical Conduct in Human Research (2007, updated May 2015).
- Notes for Guidance on Good Clinical Practice – Annotated with TGA Comments (CPMP/ICH/135/95), as adopted by the Australian Therapeutic Goods Administration (July 2000).
- Current ethics approved Clinical Trial Protocol.

I agree to inform all subjects that the study drug is being used for investigational purposes and I will ensure that the requirements related to obtaining informed consent are in accordance with ICH Guidelines for Good Clinical Practice (GCP) section 4.8 and local requirements.

I agree to report adverse events that occur in the course of the study to the Sponsor in accordance with ICH Guidelines for GCP section 4.11 and local requirements.

I have read and understand the information in the Investigator's Brochure, including the potential risks and side effects of the study drug.

I agree to promptly report to the Human Research Ethics Committee (HREC) all changes in the research activity and all unanticipated problems involving risk to subjects. I will not make any changes to the conduct of the study without HREC and Sponsor approval, except when necessary to eliminate apparent immediate harm to subjects.

I agree to maintain adequate and accurate records and make those records available in accordance with ICH Guidelines for GCP section 4.11 and local requirements.

I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.

I understand that the study may be terminated or enrolment suspended at any time by the Sponsor, with or without cause, or by me if it becomes necessary to protect the best interest of the subjects.

_______________________________ Date: ______________________________

Prof. James McCarthy, Principal Investigator

Signatories

The undersigned agrees that the protocol was written in accordance with the World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human

Subjects (Fortaleza, Brazil 2013), the National Statement on Ethical Conduct in Human Research

(NHMRC, 2007, updated May 2015) and the Notes for Guidance on Good Clinical Practice – Annotated with TGA Comments (CPMP/ICH/135/95), as adopted by the Australian Therapeutic Goods Administration (July 2000).

Name	Signature	Date
Protocol Writer: **Dr Anand Odedra, MBBS** Q-Pharm Pty Ltd (Visiting Medical Officer) and QIMR Berghofer Medical Research Institute		

This clinical trial protocol has been reviewed and approved by the Sponsor.

Name	Signature	Date
Sponsor Representative: **Prof. David Whiteman, MBBS, PhD, FAFPHM** Deputy Director QIMR Berghofer Medical Research Institute		

Title: Apheresis of subjects with induced blood stage *Plasmodium vivax*

Précis:

This is a Phase 1 exploratory study designed to determine the safety and feasibility of using apheresis as a method for extracting all lifecycle stages of malaria parasites from the blood of healthy subjects experimentally infected with blood stage *P. vivax*. This study will be conducted in up to 8 subjects (8 cohorts of 1 subject each).

Subjects will be inoculated intravenously on Day 0 with approximately 1100 viable *P. vivax* HMPBS02-*Pv* parasite-infected erythrocytes. On an outpatient basis, subjects will be monitored

daily via phone and then will attend the clinical unit daily from 4 days post-inoculation for blood sampling to measure parasitaemia via qPCR targeting the *P. vivax* 18S rRNA gene (referred to as malaria 18S qPCR), to monitor symptoms and signs of malaria, and to record AEs.

The threshold for the commencement of apheresis and subsequent antimalarial rescue treatment with artemether/lumefantrine will occur when parasitaemia is >20,000 parasites/mL or the Malaria Clinical Score is >6 (within 24 hours of notification) or at the Investigator's discretion. On the day that this threshold is reached (expected to occur on Day 10, 11 or 12), the subject will be admitted to the clinical unit for initial safety assessments before undergoing apheresis whilst being supervised by the apheresis specialist nurse.

The subject will then be administered the first dose of artemether/lumefantrine and will remain confined within the clinical unit for 72 hours (or less -48h minimum- at the discretion of the investigator if the subject is deemed clinically well and the subject is happy for early release) to monitor for safety and tolerability of apheresis and rescue therapy, and to ensure adequate clinical and parasitological response to treatment. In the unlikely event that artemether/lumefantrine fails to clear parasitaemia, subjects will be treated with chloroquine. If oral administration of either artemether/lumefantrine or chloroquine is not possible (e.g. the subject is vomiting), the subject will receive intravenous treatment with artesunate. After discharge from the clinical unit, subjects will be followed up on an out-patient basis for monitoring of safety and parasite clearance. Follow-up for safety assessments will be performed on Day 28±3, Day 56±7 (phone call only), and Day 90±7 (End of Study).

Subjects will also be evaluated for the presence of sexual parasite stages (gametocytes) and other parasite lifecycle stages in the blood during the study using reverse transcriptase qPCR (qRT-PCR). Parasite lifecycle stage qRT-PCR may also be used at the

Investigator's discretion to determine the timing of direct feeding assays (DFAs) and/or membrane feeding assays (MFAs) which may occur at up to 2 time-points prior to apheresis and rescue treatment initiation.

Objectives:

Primary objectives

- To further assess the safety of the *P. vivax* induced blood stage malaria (IBSM) model following inoculation of healthy subjects with *P. vivax* isolate HMPBS02-*Pv*.
- To assess the safety of apheresis in the *P. vivax* IBSM model following inoculation of healthy subjects with *P. vivax* isolate HMPBS02-*Pv*.

Secondary objectives

- To assess the feasibility of apheresis as a method of extracting and concentrating all stages of malaria parasites following inoculation of healthy subjects with *P. vivax* isolate HMPBS02-*Pv*.
- To evaluate the success of cryopreservation of all stages of *P. vivax* parasites from blood products extracted via apheresis from healthy subjects inoculated with. *P. vivax* isolate HMPBS02-*Pv*.

Exploratory

- To explore the potential for apheresis to be used as a method for producing a *P. vivax* Human Malaria Parasite (HMP) bank to be used for future IBSM studies.
- To evaluate the transmission of *P. vivax* gametocytes to mosquitoes.

Exploratory optional

- To collect and store plasma and peripheral blood mononuclear cells (PBMCs) harvested using apheresis for future research on the immunology and pathophysiology of *P. vivax*.
- To assess development and/or functionality of antibodies to asexual stage parasites and/or gametocytes.

Endpoints:

Primary endpoint

The primary objectives will be assessed by adverse events (AE) recording, safety laboratory parameters, vital signs recording, electrocardiograms (ECGs), physical examination findings and Malaria Clinical Score recording.

Secondary endpoints

The secondary objectives will be assessed as follows:

- The feasibility of apheresis as a method of extracting and concentrating all stages of malaria parasites will be determined by qPCR, qRT-PCR, microscopy, and flow cytometry on blood products extracted using apheresis, and expressed as a percentage of baseline parasitaemia (pre-apheresis).
- The success of cryopreservation of gametocytes will be determined by measuring their infectivity to mosquitoes using a membrane feeding assay, reported as prevalence of infection (percentage of oocyst positive mosquitoes). The success of cryopreservation of asexual parasites will be determined based on their viability tested using *in vitro* re-invasion assay.

Exploratory endpoints

The exploratory objectives will be assessed as follows:

- The potential for apheresis to be used as a method for producing a *P. vivax* HMP bank will be determined based on the efficiency of extraction and concentration of parasites, the viability of extracted parasites, the successful leukodepletion of the sample, and the

confirmation that the donor subject is free from blood-borne infections.

- Transmission of *P. vivax* gametocytes to mosquitoes will be determined using a direct feeding assay and/or membrane feeding assay, and reported as prevalence of infection (percentage of oocyst positive mosquitoes).

Optional exploratory endpoints

The optional exploratory objectives will be assessed as follows:

- The successful collection of PBMCs will be determined, if considered appropriate, by cell counts and cell viability tested using trypan blue staining.
- Detection and evaluation of anti-blood-stage or anti-gametocyte specific antibodies using immunological assays (including but not limited to enzyme-linked immunosorbent assays and indirect immunofluorescence assays).

Population: A maximum of 8 subjects will be enrolled in this study (8 cohorts of 1 subject each). Subjects will be malaria naïve healthy adults (male), aged between 18-55 years old, who meet all of the inclusion criteria and none of the exclusion criteria.

Phase: Phase 1 (exploratory study)

Number of Sites enrolling subjects: Single Centre
Q-Pharm Pty Ltd
Level 5, 300C Herston Rd and
Level 6, Block 8, Royal Brisbane and Women's Hospital Herston, QLD 4006, Australia

Description of Study Agent(s):

Malaria challenge agent:
The *P .vivax* HMPBS02-*Pv* Master Cell Bank (MCB) was produced from blood donated from a returned traveller from India who presented with clinical manifestations of malaria. Each inoculum dose will be prepared aseptically from an aliquot of the *P. vivax* HMPBS02-*Pv* MCB. Each subject will be inoculated intravenously with a dose of approximately 1100 viable parasite-infected erythrocytes in 2 mL saline for injection.

Antimalarial rescue medication:

Artemether/lumefantrine

Riamet® (20 mg Artemether and 120 mg Lumefantrine) will be administered to all subjects post apheresis or earlier if apheresis cannot performed for safety reasons. A course of treatment comprises 6 doses of 4 tablets administered orally over a period of 60 hours (total course of 24 tablets). Each dose of tablets should be taken with food or drinks rich in fat (e.g., milk).

Chloroquine (if required)

Subjects will be administered Chloroquine if artemether/lumefantrine fails to clear the malaria parasites. Chloroquine tablets each containing 250 mg chloroquine phosphate (equivalent to 155 mg chloroquine base) will be administered orally. An initial dose of chloroquine will be administered as 4 tablets, followed by a dose of 2 tablets each at 6, 24 and 48 hours (i.e. a total dose of 2.5 g chloroquine phosphate [1.550 g base]).

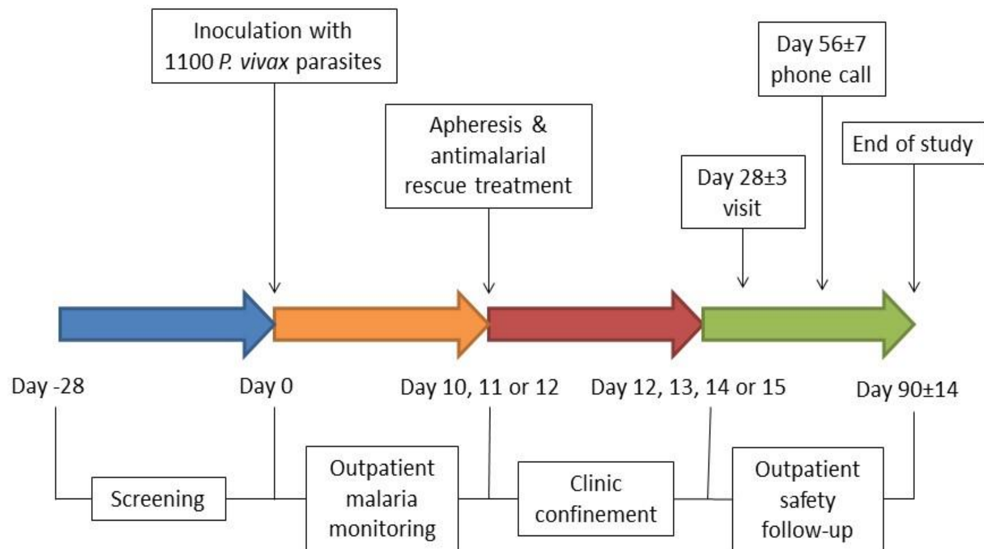
Artesunate (if required)

Treatment of subjects with intravenous artesunate will only occur in the event that subjects are unable to complete oral treatment with either artemether/lumefantrine or chloroquine (e.g. the subject is vomiting). This would be done at the recommended dose regime of 2.4 mg/kg at approximately 0, 12, 24 hours and then daily for up to 7 days or until able to take oral drugs.

Study Duration: Approximately 12 months.

Subject Duration: Approximately 4 months.

SCHEMATIC OF STUDY DESIGN



1 KEY ROLES

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

Despite the decrease in malaria incidence achieved in the last 17 years, this parasitic disease still threatens almost half of the world's population. In 2015, there were 214 million cases of malaria and 438,000 deaths (1). Most malaria cases occurred in sub-Saharan Africa. However, Asia, Latin America, the Middle East and parts of Europe are also at risk (1).

Whilst *Plasmodium falciparum* (*P. falciparum*) is the most prevalent malaria parasite in Africa, *Plasmodium vivax* (*P. vivax*) has a wider geographical distribution. In 2015, 41% of the malaria cases that occurred outside the African continent were caused by *P. vivax* resulting in an estimated 3,100 deaths (1). Moreover, 70 to 80 million cases per year of relapsing malaria occur due to infection with *P. vivax*.

The World Health Organization (WHO) has declared that the response to malaria is a global development priority and has changed their recommendation from control to eradication

programs. A robust development pipeline of potential drug candidates is required in order to meet this target and the screening process for determining safety and clinical efficacy of potential antimalarials requires fast, efficient test systems.

Controlled human malaria infection (CHMI) is increasingly being used to evaluate antimalarial drug candidates (2-4, 14-24). CHMI studies with *P. falciparum* and *P. vivax* use the induced blood stage malaria (IBSM) model, whereby subjects are infected with blood stage malaria parasites. The availability of the IBSM model offers a pathway to test the efficacy of *P. falciparum* and/or *P. vivax* vaccines and drugs in non-immune subjects, in a rapid and cost effective manner. One of the advantages of the blood stage challenge model is its ability to allow analysis of antimalarial efficacy, by providing opportunities to monitor parasite growth after challenge. This serves as a robust surrogate for the activity of a test compound/vaccine. Validation studies have shown a high correlation between natural and experimental infections, which further justifies the use of CHMI for testing new vaccines or drugs (2, 4). The safety and utility of this approach for assessment of antimalarial efficacy has been augmented by the implementation of a rapid, sensitive and robust real-time PCR assay for quantification of parasitaemia (5). A significant benefit of the IBSM model for *P. vivax* studies is the exclusion of the liver stage of the parasite that is the source of relapses, thus removing risk of recurring *P. vivax* infection from occult hypnozoites.

In the absence of a method of *in vitro* culture of *P. vivax*, the only way to source parasites is *ex vivo*. This hampers all aspects of development of tools to eliminate this parasite. For example, to test and develop candidate hypnozoiticidal drugs, a reliable source of *P. vivax* sporozoites is required. Currently this entails an expensive, logistically complex and unreliable process of sourcing *P. vivax*-infected mosquitoes from endemic areas of Asia. In addition to the logistic issues, the parasites are not genetically homogenous.

Recently we have been successful in transmitting *P. vivax* to mosquitoes and harvesting sporozoites. In a recent study we pooled 75 mL blood from 6 subjects, concentrated parasites by density gradient centrifugation and fed the concentrate to 6 pots of 100 *Anopheles Stephensi* mosquitoes (ANZCTR reference ID: ACTRN12616000174482). Approximately 95% of fed mosquitoes were infected; an average yield of approximately 4,000 sporozoites per salivary gland was obtained when mosquitoes were dissected 14 days later. Although this system offers great potential to study transmission biology, ethical and logistic issues will preclude it from becoming a source of sporozoites for downstream work.

Apheresis is the removal of a specific component of an individual's blood with the remainder of the blood being returned to the individual. Apheresis may involve the removal of red cells (erythrocytapheresis), white cells (leukocytapheresis), plasma (plasmapheresis) or platelets (thrombocytapheresis). Currently centrifugal apheresis is the preferred method whereby blood components can be separated based on buoyancy. Centrifugal machines use citrate to prevent clotting of extracorporeal blood, thereby minimising the bleeding risk; they pack red cells to a haematocrit of ≥80%, therefore requiring smaller blood volumes to achieve extraction and enable peripheral veins to be used (6).

Since its introduction in the 1970s, apheresis has been used to treat a wide array of conditions, including essential thrombocythaemia, familial hypercholesterolaemia, graft versus host disease and babesiosis. Parasite apheresis has also been used to reduce Loa parasitemia, to prevent post-treatment encephalopathy (7-9). Until now apheresis in malaria has been limited to the context of exchange blood transfusion and red cell exchange as adjuncts to intravenous quinine therapy for severe *P. falciparum* malaria (parasitemia >5%), as per WHO recommendations prior to the availability of IV artesunate (10). The rapid parasite clearance resulting from artesunate therapy has resulted in exchange transfusion and red cell exchange falling out of favour as a treatment for malaria.

2.2 RATIONALE

We propose to carry out a exploratory study to assess the use of apheresis as a means to harvest and concentrate all stages of *P. vivax* including gametocytes from up to 8 human subjects infected with blood stage *P. vivax* malaria. The harvested parasites would then be cryopreserved; the asexual parasites could then be used to produce a human malaria parasite (HMP) bank for use in future IBSM model studies, while the gametocytes could be used to infect *Anopheles* mosquitoes. Previous work has established that cryopreserved *P. vivax* gametocytes are infectious to mosquitoes (11). These infected mosquitoes, or sporozoites derived from them, could be used to infect healthy subjects in radical cure challenge models. Additionally, these sporozoites could be used in *in vitro* microfluidic devices that sustain human hepatocyte culture (12), as well as in humanised mouse models such as the severe combined immunodeficiency (SCID) mouse model which has shown the potential of acting as a model for the hepatic stage of malaria infection (13). Furthermore, it would transform *P. vivax* mosquito membrane feeding assays, obviating the need for a gametocytaemic test subject. We estimate that in collecting the entire parasite population from up to 5L of a subject's blood by apheresis there would be sufficient gametocytes to feed ~400 pots of 100 mosquitoes. From a total of ~40,000 mosquitoes the theoretical yield would be ~3.2 x10^7 sporozoites.

This explorative study has been designed to establish for the first time apheresis as a method of acquiring purified *P. vivax* parasites from healthy human subjects infected with IBSM. Venesection (whole blood removal using large bore cannula) cannot be used to select a specific haematocrit and therefore purified parasites cannot be sourced as efficiently as through apheresis, hence our decision to use apheresis.

Hypotheses:

- The apheresis procedure is safe in healthy subjects experimentally infected with blood stage *P. vivax*.
- Apheresis can be used to extract and concentrate all stages of *P. vivax* parasites at numbers greater than can be attained by simple blood draws.
- Parasites harvested via apheresis can be successfully cryopreserved.
- Cryopreserved gametocytes can successfully be used to infect *Anopheles* mosquitoes.

- Asexual parasites extracted by apheresis can be used to produce a HMP bank for future IBSM model studies.
- Peripheral blood mononuclear cells (PBMCs) can be collected using apheresis.

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 KNOWN POTENTIAL RISKS

Apheresis

Apheresis is generally considered to be a safe and well tolerated method of treatment as well as a method for collecting blood component donations from healthy volunteers. Apheresis in the form of automated red blood cell exchange has been used as an adjunct to anti-malarial treatment, particularly during the quinine era to remove *P. falciparum* infected red cells. During review of the literature, we identified 42 patients treated with red cell exchange as adjunct therapy, the only significant adverse effect experienced as a result of automated red cell exchange was transient hypotension (blood pressure 70/40) which resulted in halting of apheresis for 10 minutes before starting again once the hypotension had resolved (25-39). In the current study, subjects will have far lower parasitaemia than that seen in severe malaria. Furthermore, subjects will be infected with blood stage *P. vivax* which is generally considered one of the benign malarias. There is no documented use of apheresis in *P. vivax* infection, but the good safety profile evident in subjects with severe *P. falciparum* infection suggests that this procedure should be well tolerated by healthy subjects with subclinical *P. vivax* infection. However, it is important to note that we will be carrying out either a Continuous Mononuclear Cell (CMNC) procedure on our subjects (this is the procedure typically performed to collect stem cells and lymphocytes) or a double red cell collection, although both procedures fall under the umbrella of apheresis. Double red cell collection involves removal of haemoglobin containing red bloods cells thus the haemoglobin count could drop. The haemoglobin count will recover with time but may result in a mild transient iron deficiency anaemia. As participants will have normal haemoglobin counts going into the study, the expected drop in haemoglobin is not expected to be high enough to result in more than mild anaemia. In addition, as a result of the drop in haemoglobin, the subjects may experience a period of relative fatigue while the haemoglobin count recovers over a few weeks. The level of fatigue is not anticipated to affect the participants daily activities or work.

Leukapheresis has been used successfully to acquire PBMCs from subjects immunised with radiation-attenuated *P. falciparum* sporozoites via mosquito bite prior to malaria challenge (40). PBMCs have been extracted via leukapheresis from healthy volunteers in order to study differences in immune responses to *P. vivax* between Duffy positive and Duffy negative individuals and were subsequently cryopreserved (41).

The potential for minor transient complications is documented below.

The Australian haemovigilance report published in 2016 states that during 2013-2014 they recorded over 518,000 apheresis donations (plasmapheresis and plateletpheresis), the total rate of adverse events was 212 per 10,000 apheresis procedures (42). There were no associated deaths. The most common adverse events associated with plasmapheresis were vasovagal (65/10,000), haematoma and bruising (13/10,000). The vast majority of these adverse events were mild in severity. Adverse events requiring hospitalisation were 2/10,000 and 4/10,000 for plasmapheresis and plateletpheresis respectively. Plateletpheresis is generally considered to have greater complication rates due to the greater need for anti-coagulation and longer duration of the procedure. The vast majority of these hospital attendances were brief and uneventful requiring no treatment. With the exception of citrate reactions which are known to be more common in CMNC procedures, other adverse events are thought to be similar to other forms of apheresis.

Risk management of citrate reactions

Citrate reactions are probably the most likely adverse events that may occur during the apheresis procedure. They are a result of low blood calcium levels caused by the anticoagulant citrate commonly used instead of heparin for apheresis due to its lower tendency to cause bleeding and its short half-life. Citrate reactions can occur in around 48% (43) of CMNC produces, the vast majority of which present as mild tingling around the mouth, nose, ears, fingers and toes (44). Other symptoms of mild citrate reactions include sneezing, headache, shivers, and mild drops in blood pressure. Very occasionally citrate reactions can cause anxiety, irritability, abdominal discomfort, nausea and vomiting, spasms of muscles of the hands and feet and more significant drops in blood pressure. Very rarely citrate reactions may be severe and can manifest with seizures and abnormal heart rhythms. Subjects will be supervised during the apheresis procedure by nursing staff experienced in the delivery of apheresis, and if any of the above symptoms occur the subject will be given calcium replacement which usually results in a rapid resolution of symptoms and halting of further progression. To reduce the chances of citrate reactions we will only include subjects in the trial who have no history of low blood calcium levels and with normal calcium levels on screening blood tests. If the subject develops signs or symptoms of low calcium levels during apheresis they may be advised verbally to commence a high calcium diet for 24 hours post apheresis. This is in line with routine management of citrate reactions within the apheresis unit. The decision to commence a high calcium diet will be made by the apheresis specialist staff and the exact nature of that high calcium diet will also be determined by the apheresis specialist staff in conjunction with the subject (e.g. avoid milk if lactose intolerant).

Risk management of fainting and pre-fainting

Apheresis is an isovolaemic procedure hence there is thought to be little if any physiological challenge to the donor as a result of the procedure (45) hence syncope and presyncope is less common. Evidence from the United States suggests that double red cell and platelet donation have reduced instances of syncope compared with whole blood donation (46). Vasovagal reactions causing fainting can occur during or after donation sometimes up to 8 hours after

donation. Events that occur in the donation center are termed immediate whereas those that occur after donation are termed delayed. A specialist apheresis nurse will be present throughout the procedure and in the event of pre-faint or faint they will act according to their standard protocols (see Appendix 3) e.g. by halting the apheresis procedure temporarily or permanently. Patients most commonly experience fainting and pre-fainting during venous cannulation often because of difficult venous access. We will reduce the likelihood of this by only including subjects with no previous history of syncope as a result of venous cannulation or blood taking and good venous access. Furthermore, we will avoid blood draws from veins located in the ante-cubital fossa in the lead up to apheresis in order to preserve the veins. Venous cannulation will be carried out by experienced staff. Delayed events are much less common than immediate reactions. In the unlikely event of a delayed event, the subject will be under medical supervision in confinement and thus will be managed appropriately. We will be initiating apheresis 8-12 days after inoculation of *P. vivax* at which point the subject is likely to have symptoms similar to a mild/moderate “flu like” illness. The symptoms tend to be well controlled by simple analgesia such as acetaminophen (the use of ibuprofen immediately prior to apheresis is not recommended due to potential for altered platelet function). In general these subjects will be far more healthy than those with severe *P. falciparum* malaria requiring therapeutic apheresis, which evidence suggests is generally well tolerated.

Risk management of bruising and haematoma during venous cannulation

The risk of bruising and haematoma is approximately 13/10,000 procedures, the vast majority of these are mild in nature and require no intervention. Low platelet counts commonly occur in malaria and may increase the risk of bleeding and haematoma. However, apheresis procedures have been done successfully with very low platelet counts. We will be using peripheral venous access as it is less invasive thus safer and the more commonly used method of access. We will reduce the chances of bruising and haematoma by only including subjects with good venous access and we plan to avoid blood draws from the veins in the ante-cubital fossa in the lead up to apheresis in order to preserve the veins. Venous cannulation will be carried out by experienced staff. The subject will not be permitted to take ibuprofen 48 hours before apheresis.

Risk management of haemoglobin drop

Statistical analysis of the trends in haemoglobin during *P. vivax* IBSM studies at QIMR Berghofer have shown on average the haemoglobin drop by 10 days post inoculation is 3 g/L and the average maximum fall in haemoglobin at any point during the study is 13 g/L with a standard deviation of 7 g/L. We estimate a maximum drop of 20% in haemoglobin from the double red cell collection apheresis, this approximates to 30 g/L. Thus, the maximum anticipated fall of around 43 g/L will leave a haemoglobin of 92 g/L, a level well above the cut-off for transfusion (70 g/L).

General risk management procedures

There have been cases of air emboli (air bubbles in the blood) occurring as a result of apheresis. However, modern machines have a built in alarm that can detect air emboli. There is also the possibility of machine malfunction although this is rare, but if it occurred it may mean that some of the blood that was removed may not be returned. This is unlikely to cause any significant problems to the subject.

The apheresis procedure will be supervised by experienced and trained staff in the delivery of apheresis. A specialist apheresis nurse will be present throughout the procedure. In addition one of our trial doctors experienced in malaria management will be present during the procedure or contactable by phone and available at the site of apheresis within 10 minutes in case of any concerns. Furthermore, the apheresis unit will have a haematology doctor/consultant within easy access in the event of any other issues. The haematology staff will retain the right to stop the apheresis procedure at any time as per their discretion. Time on the apheresis machine will be approximately 2-4 hours. The subject may have to remain in the apheresis unit slightly longer to allow connection to the apheresis apparatus.

Induced blood stage malaria

In this study, a cryopreserved inoculum containing *P. vivax* isolate HMPBS02-*Pv* will be used.

The *P. vivax* parasite HMPBS02-*Pv* bank has been previously used to inoculate 34 malaria

study subjects using the IBSM model (24). No SAEs related to the challenge inoculum were reported in the subjects exposed to date.

Risk management of blood borne infections

Overall, the risk of infection from a possible blood borne virus from the blood transfused in this study would be expected to be very low for a number of reasons. First, the donor was screened and tested negative for presence of acute blood borne infections. Furthermore, the volume of blood used in the IBSM model for transmitting malaria is significantly lower than in a transfused unit, thus reducing the risk for transfusion reaction. The white cells are removed from the master cell bank during the production process, which lowers the risk of Epstein-Barr virus (EBV) and Cytomegalovirus (CMV) infection due to transfusion. The bank tested PCR negative for both CMV and EBV viruses even though the donor of the bank was antibody positive for both CMV and EBV.

As part of the safety monitoring, all study subjects will have serum stored for testing of bloodborne virus infections before entry and after the completion of the study.

Risk management of reaction to the blood sample

The malaria inoculum contains a small number of red cells from the original donor. The risk for development of red cell antibodies in this study is considered extremely low. The donor of the malaria cell bank used in this study was confirmed to be blood group O, Rh (D) positive; people with blood group O are generally considered “universal donors”, as recipients of their blood have minimal risk of developing red cell alloantibodies when given much larger volumes of blood than is envisaged for the process routinely used in the IBSM model. However, it is possible that subjects could suffer a transfusion reaction after they receive the inoculum, or develop antibodies to the donor red blood cells that may make blood transfusion more difficult in the future. Subjects will also be monitored for signs and symptoms in the period immediately after the administration of the malaria parasite dose and screened at the end of the study for occurrence of red cell alloantibodies as part of the safety monitoring.

Risk management of malaria infection

The number of blood stage parasites used to infect the study subjects in this study is much lower than that which reaches the blood after the bite of a single malaria-infected mosquito, where approximately 30,000 parasites are released into the blood when they break out of a single infected liver cell. For this study, following administration of the challenge inoculum, the growth of the parasites as well as any symptoms in the subjects will be closely monitored. The threshold for commencement of treatment will be when parasitaemia is ≥ 20,000 parasites/mL or the clinical symptom score is >6 (then treatment will begin within a 24 h period from notification) or at the Investigator’s discretion. This treatment threshold has been selected because it is below the point at which advanced and severe clinical symptoms of malaria infection are likely to occur. Furthermore, treatment may be started before the threshold is met by the Investigator at their discretion.

The parasites used in the challenge inoculum for this study are known to be sensitive to the standard anti-malarial drugs artemether/lumefantrine and Chloroquine. As such, there is no serious risk of clinical malaria providing that the inoculated subjects comply with the curative anti-malarial regimen as directed by the Investigator.

Risk management of liver function derangements

Transient, asymptomatic liver function test (LFT) derangements have been reported in several subjects in IBSM studies (19, 23, 47,48). These LFT derangements which consisted of ALT/AST elevation with no change in bilirubin did not require treatment, and resolved by the end of the studies. Following an independent review involving drug-induced liver injury experts, it was found that these LFT elevations, are most likely a direct consequence of the malaria infection rather than reflecting a direct drug-induced liver injury caused by an investigational antimalarial drug. As a precaution all subjects in this study will undergo regular safety

monitoring to assess for asymptomatic LFT abnormalities. Subjects are required to reduce intake of possibly hepatotoxic substances during the course of the study including alcohol and acetaminophen (acetaminophen can be used as second line symptom control after ibuprofen and will not exceed 4g/day in the study).

Antimalarial rescue medications

Artemether/lumefantrine, chloroquine, and artesunate risks are detailed in their respective approved manufacturer's prescribing information (Appendix 4).

Mosquito feeding

Subjects will be continuously monitored during the direct feeding of mosquitoes. If a participant develops severe skin sensitivity or experiences discomfort through the mosquito feed process, they will be given the option to withdraw from this process. Treatment for the skin irritation will be provided.

In summary, the risk to subjects in this study will be minimised as follows:

- Adherence to the inclusion/exclusion criteria, specific contraception rules for subjects.
- Close clinical and laboratory monitoring to ensure the safety and wellbeing of the healthy subjects.
- Monitoring and management by specialist staff trained in the delivery of apheresis, haematology team can stop apheresis at any point at their discretion.
- Only subjects without a history of low blood calcium and with normal calcium levels on screening blood tests will be included.
- Subjects will be advised to have a high calcium diet in the 24 hours prior to apheresis.
- Subjects will not be allowed to utilise ibuprofen in the 48 hours prior to apheresis.
- Acetaminophen ≤ 4g/day
- Subjects with good venous access will be selected and ante cubital fossa veins will not be used for blood sampling prior to apheresis.
- Admission to clinical unit for at least 48 h following the apheresis procedure and initiation of anti-malarial treatment.
- If subject requires hospitalisation this will be done at the Infectious Diseases Unit, Royal Brisbane and Women's Hospital.
- A Safety Review Team will meet approximately 4 weeks after the inoculation of each subject to review safety data before proceeding with the inoculation of a subsequent subject.

2.3.2 KNOWN POTENTIAL BENEFITS

There are no known direct benefits to the subjects in this study. There may be a benefit to the subjects from the results of the screening tests and procedures (blood tests, physical examination and electrocardiogram).

3 OBJECTIVES AND PURPOSE

Primary objectives

- To further assess the safety of the *P. vivax* induced blood stage malaria (IBSM) model following inoculation of healthy subjects with *P. vivax* isolate HMPBS02-*Pv*.
- To assess the safety of apheresis in the *P. vivax* IBSM model following inoculation of healthy subjects with *P. vivax* isolate HMPBS02-*Pv*.

Secondary objectives

- To assess the feasibility of apheresis as a method of extracting and concentrating all stages of malaria parasites following inoculation of healthy subjects with *P. vivax* isolate
  HMPBS02-*Pv*.
- To evaluate the success of cryopreservation of all stages of *P. vivax* parasites from blood products extracted via apheresis from healthy subjects inoculated with. *P. vivax* isolate
  HMPBS02-*Pv*.

Exploratory

- To explore the potential for apheresis to be used as a method for producing a *P. vivax* Human Malaria Parasite (HMP) bank to be used for future IBSM studies.
- To evaluate the transmission of *P. vivax* gametocytes to mosquitoes.

Exploratory optional

- To collect and store plasma and peripheral blood mononuclear cells (PBMCs) harvested using apheresis for future research on the immunology and pathophysiology of *P. vivax*.
- To assess development and/or functionality of antibodies to asexual stage parasites and/or gametocytes.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

This is a Phase 1 exploratory study designed to determine the safety and feasibility of using apheresis as a method for extracting all lifecycle stages of malaria parasites from the blood of healthy subjects experimentally infected with blood stage *P. vivax*. This study will be conducted in up to 8 subjects (8 cohorts of 1 subject each). Approximately 4 weeks after the inoculation of each subject, the safety review team (SRT) will meet to review the progress of the subject before going forward with the inoculation of a subsequent subject.

Subjects will be consenting and eligible healthy adults males, aged between 18-55 years old. Subjects will be inoculated intravenously on Day 0 with approximately 1100 viable *P. vivax* HMPBS02-*Pv* parasite-infected erythrocytes.

On an outpatient basis, subjects will be monitored daily via phone and then will attend the clinical unit daily from 4 days post-inoculation for blood sampling to measure parasitaemia via qPCR targeting the *P. vivax* 18S rRNA gene (referred to as malaria 18S qPCR), to monitor symptoms and signs of malaria, and to record AEs.

The threshold for the commencement of apheresis and subsequent antimalarial rescue treatment with artemether/lumefantrine will occur when parasitaemia is >20,000 parasites/mL or the Malaria Clinical Score is >6 (within 24 hours of notification) or at the Investigator's discretion. Within 24h of the day that this threshold is reached (expected to occur on Day 9, 10 or 11), subjects will be admitted to the clinical unit (expected on day 10, 11 or 12) for initial safety assessments before being escorted to the apheresis unit in the Cancer Care Services by Q-pharm staff. The subject will then undergo the apheresis procedure as per the Standard Operating Procedure (SOP) (see Appendix 3) whilst being supervised by the apheresis specialist nurse (the apheresis procedure is expected to take 2-4 hours). The subject will then be escorted back to the clinical site and administered the first dose of artemether/lumefantrine. The subject will remain confined within the clinical unit for 72 hours (or less - 48h minimum- at the discretion of the investigator if the subject is deemed clinically well and the subject is happy for early release) to monitor for safety and tolerability of apheresis and rescue therapy, and to ensure adequate clinical and parasitological response to treatment. In the unlikely event that artemether/lumefantrine fails to clear parasitaemia, subjects will be treated with chloroquine. If oral administration of either artemether/lumefantrine or chloroquine is not possible (e.g. the subject is vomiting), the subject will receive intravenous treatment with artesunate. After discharge from the clinical unit, subjects will be followed up on an out-patient basis for monitoring of safety and parasite clearance. Follow-up for safety assessments will be performed on Day 28±3, Day 56±7 (phone call only), and Day 90±7(End of Study).

Subjects will also be evaluated for the presence of sexual parasite stages (gametocytes) and other parasite lifecycle stages in the blood during the study using reverse transcriptase qPCR (qRTPCR). Parasite lifecycle stage qRT-PCR will also be used at the Investigator's discretion to determine the timing of malaria transmission experiments; direct feeding assays (DFAs) and/or membrane feeding assays (MFAs) which may occur at up to 2 time-points prior to apheresis and rescue treatment initiation.

4.2 STUDY ENDPOINTS

4.2.1 PRIMARY ENDPOINT

The primary objectives will be assessed by adverse events (AE) recording, safety laboratory parameters, vital signs recording, electrocardiograms (ECGs), physical examination findings and Malaria Clinical Score recording.

4.2.2 SECONDARY ENDPOINTS

The secondary objectives will be assessed as follows:

- The feasibility of apheresis as a method of extracting and concentrating all stages of malaria parasites will be determined by qPCR, qRT-PCR, microscopy and flow cytometry on blood products extracted using apheresis, and expressed as a percentage of baseline parasitaemia (pre-apheresis).
- The success of cryopreservation of gametocytes will be determined by measuring their infectivity to mosquitoes using a membrane feeding assay, reported as prevalence of infection (percentage of oocyst positive mosquitoes). The success of cryopreservation of asexual parasites will be determined based on their viability tested using *in vitro* reinvasion assay.

4.2.3 EXPLORATORY ENDPOINTS

The exploratory objectives will be assessed as follows:

- The potential for apheresis to be used as a method for producing a *P. vivax* HMP bank will be determined based on the efficiency of extraction and concentration of parasites, the viability of extracted parasites, the successful leukodepletion of the sample, and the confirmation that the donor subject is free from blood-borne viruses.
- Transmission of *P. vivax* gametocytes to mosquitoes will be determined using a direct feeding assay and/or membrane feeding assay, and reported as prevalence of infection (percentage of oocyst positive mosquitoes).

4.2.4 OPTIONAL EXPLORATORY ENDPOINTS

The optional exploratory objectives will be assessed as follows:

- The successful collection of PBMCs will be determined by cell counts and cell viability tested using trypan blue staining.

- Detection and evaluation of anti-blood-stage or anti-gametocyte specific antibodies using immunological assays (including but not limited to enzyme-linked immunosorbent assays and indirect immunofluorescence assays).

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 SUBJECT INCLUSION CRITERIA

Subjects eligible for inclusion in this study must fulfil **all** of the following criteria:

Demography

I01. Adult male subjects between 18 and 55 years of age, inclusive who do not live alone (from Day 0 until at least the end of the anti-malarial drug treatment) and will be contactable and available for the duration of the trial and up to 2 weeks following end of study visit.

I02. Body mass index between 18.0 and 32.0 kg/m^2, inclusive and a minimum body weight of 50 kg.

Health status

I03. Certified as healthy by a comprehensive clinical assessment (detailed medical history and complete physical examination).

I04. Vital signs after 5 minutes resting in supine position:

- 90 mmHg ≤ systolic blood pressure (SBP) ≤ 140 mmHg,
- 50 mmHg ≤ diastolic blood pressure (DBP) ≤ 90 mmHg,
- 50 bpm ≤ heart rate (HR) ≤ 100 bpm.

I05. Normal standard 12-lead electrocardiogram (ECG) after 5 minutes resting in supine position, QTcF≤450 ms with absence of second or third degree atrioventricular block or abnormal T wave morphology.

I06. Laboratory parameters within the normal range, unless the Investigator considers an abnormality to be clinically irrelevant for healthy subjects enrolled in this clinical investigation in accordance with approved clinically acceptable laboratory ranges documented prior to study start. More specifically for serum corrected calcium, creatinine, hepatic transaminase enzymes (aspartate aminotransferase, alanine aminotransferase), and total bilirubin (unless the participant has documented Gilbert syndrome) should not exceed the approved acceptable ranges and haemoglobin must be equal or higher than the lower limit of normal.

I07. As there is the risk of adverse effects with artemether/lumefantrine and chloroquine in pregnancy, it is important that any subjects involved in this study do not get pregnant.

I08. All subjects must be Duffy Blood group positive and have blood type O.

Contraception

Male subjects must agree to use a double method of contraception including condom plus diaphragm or condom plus stable oral/transdermal/injectable hormonal contraceptive by female partner during the study.

Abstinent heterosexual male subjects must agree to start a double method if they start a sexual relationship during the study.

Adequate contraception does not apply to male subjects with same sex partners, provided they agree to start a double barrier method if they start a sexual relationship with a female during the study.

Adequate contraception rules do not apply to males who have undergone a vasectomy and had testing to confirm the success of the vasectomy.

Adequate contraception rules do not apply to males with female partners who are not of child bearing potential (e.g. due to total hysterectomy) and this female is the sole partner for that participant.

Adequate contraception does not apply to subjects of childbearing potential with same sex partners (abstinence from penile-vaginal intercourse), when this is their preferred and usual lifestyle.

Regulations

I010. Having given written informed consent prior to undertaking any study-related procedure.

5.2 SUBJECT EXCLUSION CRITERIA

Subjects fulfilling **any** of the following criteria will not be eligible for inclusion in this study:

Medical history and clinical status

E01. Any history of malaria or participation in a previous malaria challenge study.

E02. Any history of retinal abnormalities, disease of the retina or macula of the eye, visual field defects, hearing disorders (e.g. reduced hearing, tinnitus).

E03. Must not have travelled to or lived (>2 weeks) in a malaria-endemic region during the past 12 months or planned travel to a malaria-endemic region during the course of the study (for endemic regions see http://www.map.ox.ac.uk/browse-resources/).

E04. Has evidence of increased cardiovascular disease risk (defined as >10%, 5 year risk for those greater than 35 years of age, as determined by the Australian Absolute Cardiovascular Disease Risk Calculator (*http://www.cvdcheck.org.au/*). Risk factors include

sex, age, systolic blood pressure (mm/Hg), smoking status, total and HDL cholesterol (mmol/L), and reported diabetes status.

E05. History of splenectomy.

E06. Presence or history of drug hypersensitivity, or allergic disease diagnosed by an allergist/immunologist and/or treated by a physician for allergy or history of a severe allergic reaction, anaphylaxis or convulsions following any vaccination or infusion.

E07. Presence of current or suspected serious chronic diseases such as cardiac or autoimmune disease (HIV or other immuno-deficiencies), insulin-dependent and non-insulin dependent diabetes, progressive neurological disease, severe malnutrition, acute or progressive hepatic disease, acute or progressive renal disease, porphyria, psoriasis, rheumatoid arthritis, asthma, epilepsy, or obsessive compulsive disorder.

E08. History of malignancy of any organ system (other than localised basal cell carcinoma of the skin or *in situ* cervical cancer), treated or untreated, within 5 years of screening, regardless of whether there is evidence of local recurrence or metastases.

E09. Subjects with history of schizophrenia, bi-polar disease, or other severe (disabling) chronic psychiatric diagnosis including depression or receiving psychiatric drugs or who has been hospitalised within the past 5 years prior to enrolment for psychiatric illness, history of suicide attempt, or confinement for danger to self or others.

E10. History of serious psychiatric condition that may affect participation in the study or preclude compliance with the protocol, including but not limited to past or present psychoses, disorders requiring lithium, a history of attempted or planned suicide, more than one previous episode of major depression, any previous single episode of major depression lasting for or requiring treatment for more than 6 months, or any episode of major depression during the 5 years preceding screening.

The Beck Depression Inventory (Appendix 5) will be used as an objective tool for the assessment of depression at screening. In addition to the conditions listed above, subjects with a score of 20 or more on the Beck Depression Inventory and/or a response of 1, 2 or 3 for item 9 of this inventory (related to suicidal ideation) will not be eligible for participation. These subjects will be referred to a general practitioner or medical specialist as appropriate. Subjects with a Beck score of 17 to 19 may be enrolled at the discretion of the Investigator if they do not have a history of the psychiatric conditions mentioned in this criterion and their mental state is not considered to pose additional risk to the health of the volunteer or to the execution of the study and interpretation of the data gathered.

E11. Frequent headaches and/or migraines, recurrent nausea, and/or vomiting (more than twice a month).

E12. Presence of acute infectious disease or fever (e.g., sub-lingual temperature ≥38.5°C) within the 5 days prior to inoculation with malaria parasites.

E13. Evidence of acute illness within the 4 weeks prior to screening that the Investigator deems may compromise participant safety.

E14. Significant inter-current disease of any type, in particular liver, renal, cardiac, pulmonary, neurologic, rheumatologic, or autoimmune disease by history, physical examination, and/or laboratory studies including urinalysis.

E15. Participant has a clinically significant disease or any condition or disease that might affect drug absorption, distribution or excretion (e.g. gastrectomy, diarrhoea).

E16. Participation in any investigational product study within the 12 weeks preceding the study.

E17. Blood donation, any volume, within 1 month before inclusion, or participation in any research study involving blood sampling (more than 450 mL/unit of blood), or blood donation to the Australian Red Cross Blood Service (Blood Service) or other blood bank during the 8 weeks preceding the treatment drug dose in the study.

E18. Participant unwilling to defer blood donations to the Blood Service for at least 6 months.

E19. Medical requirement for intravenous immunoglobulin or blood transfusions.

E20. Participant who has ever received a blood transfusion.

E21. Symptomatic postural hypotension at screening, irrespective of the decrease in blood pressure, or asymptomatic postural hypotension defined as a decrease in systolic blood pressure ≥20 mmHg within 2-3 minutes when changing from supine to standing position. In the event of a single asymptomatic postural hypotension reading the Investigator may repeat the reading once only at their discretion.

E22. Previous history of syncope or presyncope during blood donation or blood taking.

E23. History or presence of alcohol abuse (alcohol consumption more than 4 units per day) or drug habituation, or any prior intravenous usage of an illicit substance.

E24. Smoking more than 5 cigarettes or equivalent per day and unable to stop smoking for the duration of confinement. Subjects may smoke up to 5 cigarettes or equivalent per day for the rest of the study.

E25. Ingestion of any poppy seeds within the 24 hours prior to the screening blood test (subjects will be advised by phone not to consume any poppy seeds in this time period).

E26. Excessive consumption of beverages or food containing xanthine bases, including Red Bull, chocolate etc., more than 400 mg caffeine per day (equivalent to more than 4 cups per day).

Interfering substance

E27. Any vaccination within the last 28 days.

E28. Any corticosteroids, anti-inflammatory drugs, immunomodulators or anticoagulants. Any participant currently receiving or having previously received immunosuppressive therapy, including systemic steroids including adrenocorticotrophic hormone (ACTH) or inhaled steroids in dosages which are associated with hypothalamic-pituitary-adrenal axis suppression such as 1 mg/kg/day of prednisone or its equivalent or chronic use of inhaled high potency corticosteroids (budesonide 800 µg per day or fluticasone 750 µg).

E29. Any recent (<6 weeks) or current systemic therapy with an antibiotic or drug with potential anti-malarial activity (i.e. chloroquine, piperaquine, benzodiazepine, flunarizine, fluoxetine, tetracycline, azithromycin, clindamycin, doxycycline etc.).

General conditions

E30. Any participant who, in the judgment of the Investigator, is likely to be noncompliant during the study, or is unable to cooperate because of a language or mental deficit.

E31. Any participant in the exclusion period of a previous study according to applicable regulations.

E32. Any participant who is the Investigator or any sub-investigator, research assistant, pharmacist, study coordinator, or other staff thereof, directly involved in conducting the study.

E33. Any participant without a good peripheral venous access.

Biological status

E34. Positive result on any of the following tests: hepatitis B surface (HBs Ag) antigen, antihepatitis B core antibodies (anti-HBc Ab), anti-hepatitis C virus (anti-HCV) antibodies, antihuman immunodeficiency virus 1 and 2 antibodies (anti-HIV1 and anti HIV2 Ab), syphilis (EIA).

E35. Positive urine drug test for any drug listed in Section 7.2.1 (Drug Screening) unless there is an explanation acceptable to the Investigator (e.g., the participant has stated in advance that they consumed a prescription or over-the-counter product which contained the detected drug) and/or the participant has a negative urine drug screen on retest by the pathology laboratory. Any participant testing positive for acetaminophen (acetaminophen) at screening may still be eligible for study participation, at the Investigator's discretion.

E36. Positive alcohol breath test.

Specific to the study

E37. Cardiac/QT risk:

- Family history of sudden death or of congenital prolongation of the QTc interval or known congenital prolongation of the QTc interval or any clinical condition known to prolong the QTc interval.
- History of symptomatic cardiac arrhythmias or with clinically relevant bradycardia.
- History of electrolyte disturbances, particularly hypokalaemia, hypocalcaemia, or hypomagnesaemia.
- Electrocardiogram (ECG) abnormalities in the standard 12-lead ECG (at screening) which in the opinion of the Investigator is clinically relevant or will interfere with the ECG analyses on study.

E38. Known hypersensitivity to artemether/lumefantrine or chloroquine or any of their excipients, or 4-aminoquinolines, artemether or other artemisinin derivatives, lumefantrine, piperaquine.

E39. Known severe reaction to mosquito bites other than local itching and redness.

E40. Unwillingness to abstain from consumption of grapefruit or Seville orange from initiation of the study (Day 0 until completion of antimalarial treatment).

E41. Unwillingness to abstain from consumption of quinine containing foods/beverages such as tonic water, lemon bitter, from inoculation (Day 0) to the end of the antimalarial treatment.

E42. Use of prescription drugs or non-prescription drugs and herbal supplements (such as St

John's Wort), within 14 days or 5 half-lives (whichever is longer) prior to the inoculation administration. (Note: diazepam interferes with the analysis of blood levels of chloroquine and thus should not have been used for at least 8 weeks prior to administration of the study drug). If needed (i.e. an incidental and limited need) ibuprofen up to 1.2 g/day or acetaminophen is acceptable up to 4 g/day. The subject must inform the investigator of any ibuprofen or acetaminophen use at the next convenient time. Limited use of other non-prescription medications or dietary supplements not believed to affect subject safety or the overall results of the study, may be permitted on a case-by-case basis following approval by the Sponsor in consultation with the Investigator. Subjects are requested to refrain from taking non-approved concomitant medications from recruitment until the conclusion of the study.

E43. History of coagulopathy or bleeding diasthesis.

Subjects who are excluded from participation on study days for any of the above reasons may be eligible to participate on a postponed schedule if the Investigator considers this appropriate.

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

A maximum of 8 subjects are planned to be enrolled. It is estimated that up to 20 subjects may need to be screened to complete enrolment.

No restrictions will apply for ethnic or racial categories. The expected population may include all Australian racial categories. Subjects will be recruited from the QIMR Berghofer Human Research Ethics Committee (QIMR Berghofer HREC) approved database of healthy subjects maintained by Q-Pharm, or by a general or study specific advertisement via print, radio or poster media to students of Queensland universities or to the general community, as approved by the QIMR Berghofer HREC.

Subjects who complete the study up to Day 90±7/EOS will be paid $2750 compensation for their participation. Subjects who withdraw or are withdrawn from the study will be compensated on a fractional basis for their involvement unless they are withdrawn as a consequence of their misconduct. Reserve subjects who do not participate in the study will be paid $150 compensation for the inconvenience associated with their attendance for screening and for their attendance on the inoculation day, in case they are required to participate. Subjects who are screened but are ineligible for the study will be paid up to $75 compensation for the inconvenience associated with their attendance for screening (unless there is evidence that the subject knowingly omitted information about their eligibility before screening e.g. positive urine drug screen or positive alcohol breath test etc.).

5.4 SUBJECT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

Subjects are free to withdraw from the study at any time. A subject may be considered withdrawn if he/she states an intention to withdraw, fails to return for scheduled protocol visits for any reason, or becomes lost to follow-up. Subjects may also be withdrawn by the Investigator. Possible reasons for withdrawal by the Investigator include the occurrence of a serious adverse event (SAE), failure by the subject to comply with the requirements of the protocol, or for any other reason at the Investigator's discretion.

5.4.2 HANDLING OF SUBJECT WITHDRAWALS OR TERMINATION

The Investigator will make every effort to determine the primary reason for a subject's withdrawal from the study and record this information in the Case Report Form (CRF). For subjects who are lost to follow-up, the Investigator will demonstrate "due diligence" by documenting all steps taken to contact the subject (e.g. dates of telephone calls, home visit, etc.) in the source documents.

If the subject is withdrawn from the study procedures or follow-up for any reason, with the subject's permission, medical care will be provided for any SAEs that occurred during participation in the study until the symptoms of any SAEs are resolved and the subject's condition becomes stable. Follow-up for AEs is described in Section 8.3.

If earlier withdrawal from further study procedures occurs, the subject will be asked to complete the antimalarial treatment. The subjects will also be asked to complete the early termination evaluation as described in Section 7.3.5.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

The Sponsor, Principal Investigator, HREC and Regulatory Authorities independently reserve the right to discontinue the study at any time for safety or other reasons. This will be done in consultation with the Sponsor where practical. In the event of premature trial termination or suspension, the above-mentioned parties will be notified in writing by the terminator/suspender stating the reasons for early termination or suspension (with the exception of the Sponsor's responsibility for notifying the Regulatory Authorities). After such a decision, the Sponsor and the

Investigator will ensure that adequate consideration is given to the protection of the subjects' interest and safety. The Investigator must review all subjects as soon as practical and complete all required records.

In addition to the classic assessment of SAEs and the occurrence/severity of other AEs by the Sponsor and the Investigator, after exploring potential confounding factors, the following criteria should be considered as guidance for the decision to stop inoculation of further subjects:

- A subject experiences an SAE that is related to the inoculum.
- The haematology team or apheresis specialist may decide to stop apheresis based on safety, at their discretion.
- The Investigator and Sponsor may decide to stop inoculation based on other safety signals not described in the above criteria.

6 STUDY AGENT

6.1 STUDY AGENT(S) AND CONTROL DESCRIPTION

6.1.1 ACQUISITION

P. vivax HMPBS02-_Pv_ challenge agent

The *P. vivax* HMPBS02-*Pv* MCB was derived from blood group O rhesus positive blood donated from a returned traveller from India who presented with clinical manifestations of malaria. The *P. vivax* HMPBS02-*Pv* MCB was cryopreserved, aliquoted into cryovials and stored under liquid

nitrogen under controlled conditions. Refer to the *P. vivax* HMPBS02-*Pv* Investigator's Brochure for more details. A *P. vivax* HMPBS02-*Pv* MCB cryovial will be retrieved from storage, thawed, and used to aseptically prepare the inoculum at Q-Gen.

Rescue medications

Artemether/lumefantrine (Riamet®) distributed by Novartis Pharmaceuticals Australia Pty Ltd, and chloroquine distributed by Alliance Pharmaceuticals Ltd will be acquired by Q-Pharm. Artesunate is the recommended parenteral treatment for malaria in Australia. Currently, it is a

Special Access Scheme drug, and has been sourced from Guilin Pharmaceutical (Shanghai) Co., Ltd. Import was facilitated by Medicines for Malaria Venture. The manufacture of intravenous artesunate is undertaken in a WHO Pre-Qualified GMP facility

(*http://www.mmv.org/access/access-portfolio/artesun-injectable-artesunate*).

6.1.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

P. vivax HMPBS02-*Pv* challenge agent

Each inoculum dose will contain parasitised and unparasitised RBCs, resuspended in 0.9% Sodium Chloride Intravenous Infusion, in a total volume of 2 mL in syringes. The syringes will be double contained following preparation and labelled in accordance with GCP guidelines, the Australian Clinical Trial Handbook (Guidance in conducting clinical trials in Australia using "unapproved" therapeutic goods), v2.0, March 2018 and PIC/S Guide to Good Manufacturing Practice for Medicinal Products Annex 13 (January 2017).

Rescue medications

Artemether/lumefantrine tablets are pale yellow, flat, round, uncoated tablets with bevelled edges, marked with N/C and a score line on one side and CG on the other, supplied in blister packs containing 16, 24, or 400 tablets. Chloroquine tablets are round, flat, white, uncoated tablets with a break line on one side, supplied in a bottle containing 100 tablets. Artesunate for i.v administration is presented as a powder for reconstitution (60 mg artesunic acid) in a vial.

All rescue drugs will be labelled according to identity, brand or source, and batch number. The contents of the label for drug to be administered to the subjects will be in accordance with all applicable regulatory requirements.

6.1.3 PRODUCT STORAGE AND STABILITY

P. vivax HMPBS02-*Pv* challenge agent

The malaria challenge agent is prepared at Q-Gen on inoculation day (Day 0). The time between preparation of the inoculum and administration to each subject will be a maximum of

1.5 hours, during which time the syringes will be kept at the required temperature as recorded on the label.

Rescue medications

- Artemether/lumefantrine: store below 30°C protected from moisture.
- Chloroquine: store below 30°C.
- Artesunate: store in tightly closed containers, protected from light.

All drugs will be held in appropriate locked storage conditions at the clinical unit until required.

6.1.4 PREPARATION

P. vivax HMPBS02-*Pv* challenge agent

The inocula will be prepared aseptically at Q-Gen (QIMR Berghofer) from a frozen cryovial of the *P. vivax* HMPBS02-*Pv* MCB by nominated QIMR Berghofer staff under the guidance of the Investigator. The infected erythrocytes will be thawed, washed, re-suspended in normal saline, diluted in a final volume of 2 mL of clinical grade saline, and dispensed into syringes. Any remaining unused infected RBCs will be discarded as per approved standard operating procedures.

Rescue medications

Artemether/lumefantrine, chloroquine are available as tablets and no preparation is required.

Artesunate powder for reconstitution will be dissolved in 1 mL sodium bicarbonate (5%), then 5 mL sodium chloride (0.9%) will be added to create a 10 mg/mL solution (total volume 6 mL).

6.1.5 DOSING AND ADMINISTRATION

P. vivax HMPBS02-*Pv* challenge agent

An inoculum dose, containing an estimated ~1100 viable *P. vivax*-infected erythrocytes in a volume of 2 mL, will be administered intravenously to each participant on the morning of Day 0. The actual number of parasites inoculated will take into account the loss of viability resulting from cryopreservation, storage and thawing. On inoculation day, subjects may have food until at least half an hour prior to inoculation. Subjects will undergo intravenous cannulation with an appropriate gauge cannula. Placement and patency will be checked by flushing the vein with 510 mL of clinical grade saline. The inoculum will be injected, and the cannula again flushed with 5-10 mL of clinical grade saline. The cannula will then be removed, and haemostasis ensured by use of an appropriate dressing. Following inoculation of the subjects, the parasite count of the inocula will be quantified by malaria 18S qPCR.

Rescue medications

Artemether/lumefantrine

All subjects will receive compulsory treatment with artemether/lumefantrine following apheresis directly after returning to Q-Pharm. The threshold for treatment will occur when parasitaemia is

>20,000 parasites/mL or the Malaria Clinical Score is >6 or at the Investigator's discretion. Artemether/lumefantrine tablets containing 20 mg artemether and 120 mg lumefantrine will be administered as 6 doses of 4 tablets (total course of 24 tablets) given over a period of 60 hours (total dose of 480 mg artemether and 2.88 g lumefantrine). Each dose of tablets administered orally should be taken with food or drinks rich in fat (e.g., milk). Subjects will be reminded of the potential side effects of artemether/lumefantrine (Riamet®) and given the Consumer Medicine Information for Riamet® (Appendix 4).

Chloroquine (only if required)

Subjects will only be administered chloroquine if artemether/lumefantrine fails to clear the malaria parasites. For example, if qPCR results indicate unsatisfactory clearance of the parasitaemia, defined as 2 consecutive qPCR time-points showing less than 20% of baseline (i.e. the parasitaemia before drug treatment) by 72 hours, subjects may be administered chloroquine. The decision to institute early curative treatment will be made in consultation with the Local Independent Medical Monitor (IMM), who is an external malaria expert, to advise on the safety of continuing observation without rescue versus administration of curative treatment. Chloroquine tablets containing 250 mg chloroquine phosphate (equivalent to 155 mg chloroquine base) will be administered as an initial oral dose of 4 tablets, followed by 2 tablets at 6, 24, and 48 hours (total dose of 2.5 g chloroquine phosphate [1.550 g base]).

Artesunate (only if required)

Treatment of subjects with i.v. artesunate will only occur in the event that subjects are unable to complete oral treatment with either artemether/lumefantrine or chloroquine (e.g. the subject is vomiting). This would be done at the recommended dose regime of 2.4 mg/kg at approximately 0, 12, 24, hours and then daily for up to 7 days or until able to take oral drugs.

6.1.6 ROUTE OF ADMINISTRATION

Inoculum

Intravenous.

Rescue medications

- Artemether/lumefantrine: oral.
- Chloroquine (if required): oral.
- Artesunate (if required): intravenous.

6.1.7 STARTING DOSE AND DOSE ESCALATION SCHEDULE

Dosing with the malaria challenge agent and rescue drugs is presented in Section 6.1.5. No dose escalation will be performed.

6.1.8 DOSE ADJUSTMENTS/MODIFICATIONS/DELAYS

Not applicable.

6.1.9 DURATION OF THERAPY

- Inoculum: single dose.
- Artemether/lumefantrine: 3 days.
- Chloroquine (if required): 3 days.
- Artesunate (if required): maximum of 7 days.

6.1.10 TRACKING OF DOSE

The *P. vivax* challenge inocula, artemether/lumefantrine, chloroquine (if required) and artesunate (if required) will be administered at the clinical research Unit (CRU) in the presence of clinic staff.

6.2 STUDY AGENT ACCOUNTABILITY PROCEDURES

The Q-Pharm pharmacist or designee, as nominated by the Investigator, is responsible for maintaining accurate study agent accountability records throughout the study. Study agents include the malaria challenge agent and the rescue medication. Dispensing, accountability and documentation will be in accordance with Q-Pharm standard procedures. All products will be inventoried upon receipt by the Q-Pharm pharmacist. The condition of the products at the time of receipt by the pharmacist will be documented, as will the time restrictions of use for the syringes containing the malaria challenge agent. The lot numbers and expiry dates of the inoculum and antimalarial drugs will be documented. The Q-Pharm pharmacist or delegate will ensure that the received products are the specified formulation.

The storage, handling and the disposal of the challenge agents will be in accordance with approved procedures. All dosages prescribed and dispensed to the subjects and all dose changes during the study must be recorded in the CRFs. All drug supplies are to be used only in accordance with this protocol, and not for any other purpose. All used medications will be fully documented. Used and unused drug containers must be destroyed at the site once drug accountability is final and has been checked by the Sponsor or its delegate, and written permission for destruction has been obtained from the Sponsor.

Study products and study accountability logs will be available to the Sponsor or their representative as part of the study monitoring procedures. Upon completion of the study, copies of all study drug management records will be provided to the Sponsor. Original records will be maintained at the clinical site with the rest of the study records.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY SPECIFIC PROCEDURES

Medical history

Past Medical/Surgical History Includes:
History of all known allergies
Current medications, including over-the-counter and herbal preparations
History of substance abuse and recreational drug use
History of depression, anxiety, mental illness, emotional problems, use of psychiatric medications and
Surgical procedures and results

Physical examination

Complete Physical Examination Includes:

Weight **(Screening only)**
Height **(Screening only)**
Review of systems excluding genitourinary examination and including the following:
Head, neck (including thyroid), ears, eyes, nose and throat
Heart/circulation
Chest
Lungs
Abdomen
Skin
Neurological exam

Symptom-Directed Physical Examination: physical examinations will be symptom-directed at specified time-points (i.e. systems will be reviewed only if clinically indicated at the discretion of the Investigator).

Beck Depression Inventory

All subjects will be required to complete the Beck Depression Inventory at screening. This is a validated questionnaire used as an objective tool for the assessment of depression (See Appendix

5).

Vital signs

Vital signs (temperature, heart rate, respiratory rate and blood pressure) will be measured at screening after the subject has rested in the supine position for at least 5 minutes and in the standing position within 2-3 minutes when changing from the supine to standing position (blood pressure and heart rate only). At all other time-points, vital signs will be measured after the subject has rested in the seated position for at least 5 minutes. Tympanic temperature will be taken at the clinical unit, and sublingual temperature will be taken by subjects at home for practical reasons.

Electrocardiograms

A single 12-lead ECG will be recorded after resting supine for at least 5 minutes.

Apheresis

Apheresis will be performed according to the SOP included in Appendix 3. This procedure is similar to the double red blood cell donation procedure used by the Council of Europe and the American Red Cross (49). 386 mL of red blood cells can be collected safely in subjects with normal haemoglobin and haematocrit levels according to this well-recognised procedure.

Blood sampling

Blood will be collected for clinical laboratory evaluations including haematology, clinical chemistry, serology and pregnancy testing (see section 7.2.1). Blood samples will also be collected to monitor malaria parasitaemia and for immunology/pathophysiology research (see section 7.2.2). The estimated blood volume required for the these tests is listed in Appendix 1. The total volume of whole blood drawn from each adult male subject in addition to the red blood cells collected during the apheresis procedure will not exceed 200 mL in any given 30-day period. This volume includes allowance for unscheduled safety laboratory assessments that may be required at the discretion of the Principal Investigator or the Sponsor to ensure subject safety. The subject haemoglobin count will be checked regularly during the study. Subjects with haemoglobin levels below 120 g/L will not undergo apheresis. Subjects with haemoglobin count below 135 g/L will be excluded from the study at screening.

Urine sample collection

Urine will be collected for urinalysis and drug screening (see section 7.2.1).

Adverse event recording

Adverse events will be recorded as described in section 8 and follow the CTCAE V4.03 gradings.

Malaria Clinical Score

The following 14 signs/symptoms frequently associated with malaria will be graded using a 4point scale (absent: 0; mild: 1; moderate: 2; severe: 3) and summed to generate a total

Malaria Clinical Score (maximum score possible is 42). Individual scores for each symptom as well as the total score will be recorded.

Headache	Anorexia
Myalgia (muscle ache)	Nausea
Arthralgia (joint ache)	Vomiting
Fatigue/lethargy	Abdominal discomfort
Malaise (general discomfort/uneasiness)	Fever
Chills/Shivering/Rigors	Tachycardia
Sweating/hot spells	Hypotension

7.1.2 STANDARD OF CARE STUDY PROCEDURES

Not applicable.

7.2 LABORATORY PROCEDURES/EVALUATIONS

7.2.1 CLINICAL LABORATORY EVALUATIONS

Haematology

Full blood count (FBC) with differential
White blood cell count (WBC)
WBC differential (diff)
A manual blood smear should be reviewed if there are immature/abnormal cells detected on the automated differential or if an automated differential was not able to be performed.
neutrophils (NEUT)
lymphocytes (LYM)
monocytes (MON)
eosinophils (EOS)
basophils (BAS)
Red blood cell count (RBC)
Haemoglobin (HGB)
Haematocrit (HCT)
Platelet count (PLAT)
Reticulocyte count (RETI) (Day -3 to -1 eligibility confirmation visit or at screening if between Day -3 to -1, and Day 28±3 or early termination visit only)
Blood Group and Rh(D) (Screening only)
Duffy antigen (Screening only)

Biochemistry

Sodium (SODIUM)	Alkaline phosphatase (ALP)

Potassium (K)	Alanine aminotransferase (ALT, SGPT)
Chloride (CL)	Aspartate aminotransferase (AST, SGOT)
Bicarbonate (BICARB)	Corrected calcium (CA) ***(Screening and 1-3 days Pre apheresis and immediately post apheresis)***
Glucose (GLUC)	Phosphate (PHOS)
Urea	Lactate dehydrogenase (LDH)
Creatinine (CREAT)	Magnesium ***(Screening and 1-3 days Pre apheresis and immediately post apheresis)***
Estimated glomerular filtration rate (eGFR)	Cholesterol ***(Screening only)***
Albumin (ALB)	Triglycerides ***(Screening only)***
Globulin	HDL ***(Screening only)***
Total protein	Urate
Total bilirubin (BILI)	
Direct bilirubin (BILDIR)	

Urinalysis

Urine will be tested by dipstick at the clinical unit. If there are any abnormalities considered clinically significant in blood, leucocytes or protein, the urine will be sent for formal laboratory urinalysis per the clinical unit standard procedure.

Glucose (GLUC)
Bilirubin (BILI)
Ketone (KETONES)
Specific gravity (SPGRAV)
Blood
pH
Protein (PROT)
Urobilinogen (UROBIL)
Nitrite
Leukocytes (WBC)
Formal laboratory analysis (if required)

Urine drug screens and alcohol breath tests

If the results of the urine drug screens or alcohol breath tests are positive, subjects may be allowed to continue, or may be delayed or withdrawn according to site-specific instructions.

All subjects will be questioned about concomitant medications and use of recreational drugs. The urine drug screen may be repeated if the potential subject denies usage of any of these agents and the test result is believed to be a false positive.

Subjects testing positive for acetaminophen at screening and/or inoculation day may still be eligible for study participation, at the Investigator's discretion. Subjects requiring acetaminophen on a daily basis would not be eligible to enrol in the study, as the use of any over-the-counter medication during the study is restricted and potential subjects should not discontinue their usual medications in order to participate in the study.

Urine Testing:	
Amphetamines	Opiates
Methamphetamines	Phencyclidine
Barbiturates	Tetrahydrocannabinol (cannabis)
Benzodiazepines	Tricyclic antidepressants
Cocaine	Paracetamol (acetaminophen)
Methadone	
Alcohol breath test	

Serology

HIV-1/HIV-2 antigen/antibody
Hepatitis B (HBsAg, anti-HBc (IgG + IgM if IgG is positive))
Hepatitis C (anti-HCV)
Syphilis EIA
Hepatitis A (anti-HAV) (IgM) - performed off stored sample for testing, at Investigator's discretion
Hepatitis E (anti-HEV) (IgM) - performed off stored sample for testing, at Investigator's discretion
EBV - performed off stored sample for testing, at Investigator's discretion
CMV - performed off stored sample for testing, at Investigator's discretion

Assessment of blood for HMP bank ***(at screening or 1-3 days pre inoculation and again at Day 28 or Day 90; all timepoints at discretion of Investigator)***

The HMP bank questionnaire may be presented to the subject at the Investigator's discretion at screening or the Day -3 to Day -1 visit, in order to assess eligibility to act as a donor for creation of a HMP bank (Appendix 6). If the subject is deemed suitable as a donor, additional blood (55 mL, Appendix 7) may be collected at screening or Day -3 to Day -1 and again at either Day 28±7 or Day 90±7 at the Investigator's discretion for the tests listed below and if the whole blood volume does not exceed the 200 mL per 30 day period maximum.

Barmah Forest Virus IgG	Flavivirus IgM
Barmah Forest Virus IgM	Dengue PCR
River Ross Virus IgG	EBV PCR

River Ross Virus IgM	CMV PCR
Human T cell Lymphotropic Virus 1/2	Parvo B19 PCR
Human Herpes Virus 6	Human Herpes Virus 7

If the flavivirus IgM test is positive, tests may be performed at the Investigator's discretion for confirmation and identification of the specific flavivirus. This may include tests for Japanese encephalitis virus, West Nile virus, Dengue virus (may include typing of 4 Dengue serotypes), and Murray Valley encephalitis virus.

Other laboratory tests

- RBC alloantibodies (Screening and Day 28±3 or Day 90±7 or early termination visit only – see 7.3.3 Follow-up).
- Glucose-6-phosphate dehydrogenase (G6PD) (Screening only).

Safety serum storage

Blood for serum storage as safety retention samples will be collected at Day 0 and at the final study visit.

7.2.2 OTHER ASSAYS OR PROCEDURES

Malaria monitoring

Blood will be collected to monitor malaria parasite numbers using qPCR targeting the 18S rRNA gene. Additional blood (up to approximately 2 mL per time-point) may be collected for parasite lifecycle stage qRT-PCR at the Investigator's discretion. This is to evaluate for the presence of sexual parasite stages (gametocytes) and other parasite lifecycle stages in the blood. This blood may also be used for research into various aspects of parasite biology e.g. gametocytes, parasite lifecycle stages, recrudescence, commitment etc. The qRT-PCR may target genes including but not limited to: the female gametocyte-specific transcript *pvs25*, the male gametocyte-specific transcript *pfMGET*, and the ring-stage transcript *pfSBP-1* as appropriate. This testing will occur between inoculation and day 28.

Microscopic examination for evidence of parasitaemia or gametocytaemia may be conducted at the Investigator's discretion. Thick films may be prepared from blood collected at time-points coinciding with MFA.

Malaria transmission and mosquito infectivity assays

Transmission of gametocytes to *Anopheles* mosquitoes may be determined using direct skin feeding assays (DFA) and/or membrane feeding assays (MFA). Mosquitoes will be maintained in a controlled environment in the PC3 QIMR Berghofer insectary.

DFA: Subjects will be escorted to the insectary and asked to allow mosquitoes to feed on them at up to 2 time-points. Approximately 30 mosquitoes will be distributed into containers with gauze lids and starved prior to feeding assays. Mosquitoes will be allowed to bite on the volar surface of the forearms, thighs or calves of subjects to directly feed for approximately 15±5 minutes until fully engorged.

MFA: Up to approximately 80 mL blood (total) may be collected if maximum whole blood volume is not exceeded over either 1 or 2 time-points and transported at ~37°C. Mosquitoes (approximately 100) will be distributed into containers with gauze lids and starved prior to feeding assays. Mosquitoes will be allowed to feed for approximately 30 minutes on the blood through bovine caecum or parafilm membranes on water jacketed glass feeders attached to a 37°C water bath.

For both the DFA and MFA, after feeding, the number of non-engorged mosquitoes will be recorded. Following the feeds, the mosquitoes will be maintained in a controlled environment and will be provided with a sugar solution supplemented with 0.05% para-amino benzoic acid to promote the sporogonic cycle. Seven to ten days after blood feeding, mosquitoes will be dissected to quantify oocysts in midgut preparations using either malaria 18S qPCR, microscopy (oocysts will be stained with 0.1% mercurochrome) or CS ELISA.

A subset of fed mosquitoes may be kept for up to 24 days in order to investigate for development of salivary gland sporozoites. The prevalence and/or intensity of oocyst infection will be determined in the blood fed mosquitoes. The number of mosquitoes dying prior to dissection will be recorded.

Detection and evaluation of anti-blood-stage or anti-gametocyte specific antibodies

Plasma from blood samples taken for other purposes, e.g. membrane feeding assays, immunology samples etc., may be used at the Investigator's discretion for the detection and evaluation of anti-blood stage or anti-gametocyte specific antibodies. The plasma may be used for immunological assays including, but not limited to, enzyme-linked immunosorbent assays and indirect immunofluorescence assays.

Plasma samples and peripheral blood mononuclear cells for research on the immunology and pathophysiology of *P. vivax*

Plasma and PBMCs harvested using apheresis may be collected and stored for research on the immunology and pathophysiology of *P. vivax*, at the Investigator's discretion. A baseline sample (20 mL) for plasma and PBMCs may also be collected on Day 0 pre-inoculation, at the Investigator's discretion if the maximum whole blood volume is not exceeded.

7.2.3 SPECIMEN PREPARATION, HANDLING, AND STORAGE

Biological samples will be retained for the time required for assessment for analysis, and may then be discarded. Safety serum samples will be stored indefinitely with the permission of the subjects for any retrospective safety assessments.

7.2.4 SPECIMEN SHIPMENT

Samples collected will be shipped to nominated local or international laboratories for assessment. The site staff will be responsible for shipment of samples to analytical laboratories for testing. Samples must be packed securely together with completed shipment forms in shipping containers together with sufficient dry ice as per Shipper procedures.

7.3 STUDY SCHEDULE

7.3.1 SCREENING

A screening visit will be scheduled after an initial telephone interview conducted by clinical unit staff has occurred to review background information. For the screening visit, potential subjects will be told to come to the clinical unit after an overnight fast of ≥8 hours. During this initial screening visit, the potential subject will read the Participant Information Sheet and be encouraged to ask questions. Individuals willing to be considered for inclusion may sign the screening consent form during the screening visit, or return to the clinical unit after further consideration. The subject will be given a copy of the Participant Information Sheet and signed consent form for their records. The signed and dated originals will be held on file by the CRU. Participation consent must be obtained from all eligible subjects prior to screening tests.

After providing written consent to participate, the subject will be examined by a medical officer and physical examinations, vital signs and ECG testing will be done together with collection of blood and urine samples for safety assessment. The subjects will be fully informed of the nature of the study at this time, and advised of the requirement to repeat some screening tests during the
Day -3 to Day -1 safety visit (if required) and/or on the day of malaria challenge inoculum administration to determine their continuing eligibility. Subjects must confirm that they will not be living alone from Day 0 until completion of antimalarial treatment.

The pre-study screening will be conducted within 4 weeks prior to the Day 0 malaria challenge day and will include:

1. Provide the Main Participation Information Sheet and Informed Consent form and apheresis specific Participation Information Sheet and Informed Consent form and give the subject sufficient time to review the contents.
2. Explain the study via the main Participation Information Sheet and gain Informed Consent from the subject.

3. Ensure the subject has signed the main Participant Information Sheet and Informed Consent and received a signed copy.
4. The subject will then be escorted to the apheresis unit in the Cancer Care Services department at RBWH by a Q-pharm staff. A specialist in apheresis will explain the apheresis component of the study via the apheresis specific Participation Information Sheet and gain Informed Consent from the subject. The subject will also be provided with a leaflet explaining the apheresis procedure (lymphocyte collection leaflet).
5. A specialist in apheresis will ensure the subject has signed the apheresis specific Participant Information Sheet and Informed Consent and received a signed copy.
6. The subject will then be escorted back to the apheresis unit at the RBWH by Q-pharm staff.
7. A screening number will be assigned to each subject.
8. Elicit a complete medical history and use of medications.
9. Elicit a social history including alcohol and tobacco use.
10. Undertake a full physical examination.
11. Ask subject to complete the Beck Depression Inventory.
12. Ask subject to complete the HMP bank questionnaire and collect blood samples for HMP bank assessment (**at Investigator's discretion** if the maximum whole blood volume is not exceeded) (Section 7.2.1).
13. Assessment of the cardiovascular disease risk (defined as >10%, 5 year risk when greater than 35 years of age) as determined by the Australian Absolute Cardiovascular Disease Risk Calculator (*http://www.cvdcheck.org.au/*). Risk factors include sex, age, systolic blood pressure (mm/Hg), smoking status, total and HDL cholesterol (mmol/L), and reported diabetes status.
14. Perform alcohol breath test.
15. Record vital signs.
16. Obtain a single 12-lead ECG.
17. Collect urine for urinalysis and urine drug screen.
18. Collect blood samples for haematology, biochemistry, RBC alloantibodies, G6PD testing, Duffy antigen, and serology (syphilis, viral hepatitis B and C and HIV).
    Blood samples must not be taken from veins located in the ante-cubital fossa.
19. Verify subject meets inclusion/exclusion criteria.

Subjects who complete all screening procedures and satisfy all entry criteria will be considered eligible to participate in this study. To be eligible for study entry, laboratory values at screening must not be outside the range of the normal values at a level deemed to be clinically significant. For eligibility parameters a repeat may be requested to exclude laboratory error.

If screening laboratory results are abnormal, e.g. HIV testing, the volunteer will be referred for appropriate counselling. If any clinically significant abnormalities are detected during screening, the subject will be referred for follow-up tests to a general practitioner or medical specialist as appropriate.

7.3.2 ENROLLMENT/BASELINE

Day -3 to Day -1 eligibility confirmation visit

Subjects (including reserve subjects) will report to the CRU between Day -3 to Day -1 for the following baseline assessments, unless screening laboratory assessments were conducted within this period, in which case repeat sampling will not be required.

1. Ask subject to complete the HMP bank questionnaire and collect blood samples for HMP bank assessment (**at Investigator's discretion** if the maximum whole blood volume is not exceeded).
2. Collect blood samples for haematology and biochemistry analysis.

Blood samples must not be taken from veins located in the ante-cubital fossa.

3. Collect urine for urinalysis.

The timing of these assessments is to ensure that results are available for review by the Investigator prior to inoculation on Day 0. Subjects with clinically significant laboratory findings at this stage will not be eligible for malaria parasite inoculation.

Administration of Malaria Challenge Inoculum (Day 0)

Each subject (and up to 3 reserve subjects) will report to the CRU on the morning of Day 0. The Investigator will review the subjects' screening results prior to their enrolment into the study. The Investigator will emphasise the requirement to return for malaria drug treatment after the malaria inoculation. Subjects will be reviewed by the Investigator to confirm their continued eligibility for the study, including confirmation that they will not be living alone from Day 0 until the end of antimalarial treatment by checking housemates contact details recorded at screening visit.

On admission to the study centre, subjects will be required to undertake further screening procedures to determine whether they remain eligible to be enrolled. Subjects may have food until at least half an hour prior to inoculation. A reserve subject may be asked to replace a subject who does not continue to meet eligibility. These reserves will be compensated for the study visit even if not inoculated, as described in the Participant Information Sheet and Consent Form.

The procedures that will be undertaken prior to inoculation include:

1. Verify that all applicable eligibility criteria have been met.
2. Elicit information regarding any new medical conditions or illnesses since screening.
3. Perform alcohol breath test and urine drug screen.
4. Conduct symptom-directed physical examination.
5. Record vital signs.
6. Obtain a single 12-lead ECG.

7. Cannulate subjects with an indwelling intravenous cannula for the malaria inoculum, and record which arm is utilised.
   Cannulation must not be performed in veins located in the ante-cubital fossa.
8. Collect blood samples for malaria 18S qPCR (parasitaemia baseline sample), parasite lifecycle stage qRT-PCR (parasite lifecycle stage baseline sample), immunology/ pathophysiology (baseline sample), and safety serum storage.
   Blood samples must not be taken from veins located in the ante-cubital fossa.

Administration of the malaria inoculum:

1. Administer the malaria inoculum of ~1100 viable *P. vivax* infected human RBCs intravenously.
2. Observe for a minimum of 60 minutes after administration of the inoculum to evaluate for immediate adverse reactions.
3. Educate subjects on signs and symptoms of malaria (Appendix 2).
4. Emphasise to subjects the importance of returning on the nominated day for antimalarial treatment.
5. Provide subjects with diary cards and thermometers to record any temperature readings during the study in the event of symptoms of fever. Subjects will also record symptoms and concomitant medications on the diary cards during the study.
6. Record adverse events and concomitant medications.
7. Record vital signs prior to leaving the clinic (approximately 60 minutes after inoculation).
8. Record malaria clinical score prior to leaving the clinical unit (malaria clinical score baseline sample; see Section 7.1.1).

7.3.3 FOLLOW-UP

Malaria monitoring via phone (Day 1 to Day 3)

During this period, subjects are expected to be asymptomatic. A daily phone call or text message will be made to the subjects by clinic staff to monitor subject well-being and to solicit any adverse events.

Daily visits for malaria monitoring (Day 4 until treatment day)

Follow-up from Day 4 until the day of apheresis and rescue treatment initiation will be undertaken through daily visits (approximately 8:00 AM) to the clinical site. It may be required to increase the frequency of the visits to 3 times a day from day 9 until treatment to allow collection of blood samples for parasitaemia determination. The study investigator reserves the right to confine subjects earlier than planned for symptoms relief and observation prior to apheresis and treatment.

The following procedures will occur during these visits:

1. Perform symptom-directed physical examination when signs and symptoms of malaria are identified and it is clinically indicated at the Investigator's discretion.
2. Record vital signs.
3. Collect blood samples for malaria 18S qPCR and parasite lifecycle stage qRT-PCR (if required). Sample may be collected up to 3 times per day from Day 9 until treatment day (at the discretion of the investigator and if maximum whole blood volume is not exceeded). **Blood samples must not be taken from veins located in the ante-cubital fossa.**
4. Record malaria clinical score.
5. Record AEs and use of concomitant medications.
6. Advise subject that ibuprofen cannot be taken within 48 hours of apheresis.
7. Haematology and biochemistry (including calcium and magnesium) will be performed on Day 9.

Study days for malaria transmission assays

The transmission of parasites to mosquitoes may be measured using DFAs and/or MFAs at up to 2 time-points prior to apheresis and rescue treatment. Parasite lifecycle stage qRT-PCR will be used at the Investigator's discretion to determine the timing of DFAs and/or MFAs. At these transmission assay time-points, vital signs will be recorded and a symptom-directed physical examination will be performed if symptoms or signs of malaria are identified and it is clinically indicated. Blood may also be collected for malaria 18S qPCR and parasite lifecycle stage qRTPCR. Thick films may also be performed at the Investigator's discretion.

In-patient observation, apheresis and antimalarial treatment phase (confinement)

Subjects will be admitted to the clinical unit for 72 hours (or less - 48h minimum - at the discretion of the investigator if the subject is deemed clinically well and the subject is happy for early release) when parasitaemia is >20,000 parasites/mL or the Malaria Clinical Score is >6 (within 24 hours of notification) or at the Investigator's discretion.

Admission

The following procedures will occur at admission to the clinical unit (or just prior to the apheresis procedure for the blood sample collection):

1. Perform symptom-directed physical examination.
2. Record vital signs.
3. Perform alcohol breath test.
4. Collect urine for urinalysis and drug screen.

5. Collect blood samples for haematology, biochemistry, malaria 18S qPCR (pre-apheresis), and parasite lifecycle stage qRT-PCR. In the case where apheresis is not performed soon after admission but later in the day, blood samples collection will be postponed and performed just prior to the apheresis procedure.
   Blood samples must not be taken from veins located in the ante-cubital fossa.
6. Record Malaria Clinical Score.
7. Record AEs and use of concomitant medications.

Apheresis, rescue treatment and observation

The following procedures will occur during the apheresis, rescue treatment and observation period:

1. The subject will be escorted to the apheresis unit in the Cancer Care Services department at RBWH by a staff member. The subject will then undergo the apheresis procedure as per the (SOP) (see Appendix 3) whilst being supervised by the apheresis specialist nurse. Time on the apheresis machine will be approximately 2-4 hours. The subject may have to remain in the apheresis unit slightly longer to allow connection to the apheresis apparatus. Once the procedure is over the subject will be escorted back to the clinical site (Q-Pharm). A QPharm study doctor will be available by phone for the duration of the apheresis and be able to attend the subject within 10 minutes if needed. If the subject develops signs or symptoms of low calcium levels the apheresis specialist may advise the subject verbally to commence a high calcium diet.
2. Cannulate subjects with an indwelling intravenous cannula.
3. Collect blood samples for haematology, biochemistry (including calcium and magnesium) and malaria 18S qPCR (post-apheresis). A blood sample can also be collected for parasite lifecycle stage qRT-PCR (at the discretion of the investigator and if the maximum whole blood volume is not exceeded).
4. Obtain a single 12-lead ECG.
5. Administer artemether/lumefantrine treatment under direct observation.
6. Follow-up subjects as in-patients for 72 hours (or shorter - 48h minimum - at the discretion of the investigator if the subject is deemed clinically well and the subject is happy for early release) to ensure tolerance of the therapy and adequate clinical response.
7. Perform symptom-directed physical examination when signs or symptoms of malaria are identified and it is clinically indicated at the Investigator’s discretion.
8. Record vital signs 3 times a day whilst confined.
9. Record Malaria Clinical Score 3 times a day whilst confined.
10. Collect blood samples for malaria 18S qPCR at 12, , 24, 36, and 48 (if the subject remains in confinement for 72 h), hours following artemether/lumefantrine treatment initiation. Additional blood samples can be collected at the discretion of the investigator if required to ensure the safety of the subject.

11. Record AEs and use of concomitant medications.

Prior to exit from clinical unit

Subjects will be allowed to leave the unit 48 to 72 hours after initiation of artemether/lumefantrine treatment at the Investigator's discretion if they are asymptomatic and have a normal examination and no clinically significant laboratory abnormalities. Subjects may be requested to stay in confinement longer than 72 hours at the Investigator's discretion if deemed in their clinical interest.

The following procedures will occur prior to discharge from the clinical unit:

1. Perform symptom-directed physical examination.
2. Record vital signs.
3. Obtain a single 12-lead ECG.
4. Collect blood samples for haematology, biochemistry, malaria 18S qPCR if the subject is released around 48 or 72h. A blood sample can also be collected for parasite lifecycle stage qRT-PCR (at the discretion of the investigator and if the maximum whole blood volume is not exceeded).
5. Record AEs and use of concomitant medications.

Chloroquine phosphate rescue treatment (if required)

It is hypothesised that artemether/lumefantrine treatment will be curative considering that it is a registered drug used for treatment of *P. vivax* malaria infection. However, there is a possibility that resistance to artemether/lumefantrine by the *P. vivax* isolate HMPBS02-*Pv* may occur. In this case subjects will receive a standard course of therapy with chloroquine phosphate within 72 hours of administration of the first dose of artemether/lumefantrine treatment, at the discretion of the Investigator. Full details on the decision process to commence chloroquine treatment are presented in Section 6.1.5. If Chloroquine dosing is required, safety bloods (haematology and biochemistry) will be collected and assessed if not already scheduled prior to first dose and after last dose (or within 1 day of last dose). Monitoring of subjects post-chloroquine treatment will occur as presented above for artemether/lumefantrine treatment.

Artesunate rescue treatment (if required)

Treatment of subjects with i.v. artesunate will only occur in the event that subjects are unable to complete oral treatment with either artemether/lumefantrine or chloroquine (e.g. the subject is vomiting). Details on artesunate dosing are presented in Section 6.1.5. Subjects will be administered i.v artesunate on site and monitored as described above.

Out-patient monitoring post-artemether/lumefantrine treatment (post confinement until safety visit)

If the subject is released from confinement before 72h post Riamet® treatment, a follow-up visit will be undertaken at 72h for clinical evaluation and blood sampling. If the 18S PCR result is positive, the subject will be followed up until a minimum of one negative qPCR is detected.

The following procedures will take place during these visits:

1. Collect blood samples for malaria 18S qPCR monitoring. Blood sampling for malaria monitoring will occur at the investigator's discretion if there is concern regarding the possible recrudescence of parasitaemia.
2. Collect blood samples for parasite lifecycle stage qRT-PCR at the Investigator's discretion.
3. Collect blood samples for haematology and biochemistry, and urine for urinalysis at the Investigator's discretion.
4. Perform symptom-directed physical examination when signs and symptoms of malaria are identified and it is clinically indicated at the Investigator's discretion.
5. Record vital signs.
6. Record Malaria Clinical Score if vital signs are abnormal or at the Investigator's discretion.
7. Record AEs and use of concomitant medications.

Day 28±3

The following procedures will occur on Day 28±3:

1. Perform full physical examination.
2. Record vital signs.
3. Obtain a single 12-lead ECG.
4. Collect urine for urinalysis.
5. At the discretion of the investigator and if required for safety reasons, collect blood samples for haematology, biochemistry, malaria 18S qPCR and parasite lifecycle stage qRT-PCR (if required), RBC alloantibodies, and serology. If not required at Day 28±3, these samples will be collected at Day 90±7 to limit the volume of whole blood collected within 30 days of the apheresis procedure.
6. Collect blood samples for HMP bank assessment (**at Investigator's discretion** and if the maximum whole blood volume is not exceeded).
7. Record AEs and use of concomitant medications.

Follow-up phone call (Day 56±7)

A phone call will be made on Day 56±7 to the participants by clinic staff to monitor participant well-being and to solicit any adverse events.

7.3.4 FINAL STUDY VISIT

The following procedures will occur at the EOS visit (Day 90±7):

1. Perform symptom-directed physical examination.
2. Record vital signs.
3. If not already done on day 28±3, collect blood samples for haematology, biochemistry, malaria 18S qPCR and parasite lifecycle stage qRT-PCR (if required), RBC alloantibodies, and serology.
4. Collect blood samples for safety serum storage.
5. Collect blood samples for HMP bank assessment (**at Investigator's discretion** and if the maximum whole blood volume is not exceeded).
6. Record adverse events and use of concomitant medications.

7.3.5 EARLY TERMINATION VISIT

If withdrawal occurs at any stage of the study, the subject will be asked to complete an EOS evaluation. **In addition, subjects are informed on the essential requirement to complete the antimalarial drug treatment for their safety, via the Participant Information Sheet.**

Participation in an EOS evaluation by each subject is voluntary. Procedures during the early termination visit will include the following if withdrawal occurs prior to Day 28 visit:

1. Perform full physical examination.
2. Record vital signs.
3. Obtain a 12-lead ECG.
4. Collect urine sample for urinalysis.
5. Obtain blood for haematology, biochemistry, malaria qPCR and parasite lifecycle stage qRT-PCR, RBC alloantibodies and safety serum storage.
6. Record AEs and use of concomitant medications.

If withdrawal occurs after the Day 28 visit, the procedures outlined for the final study visit will be performed.

7.3.6 UNSCHEDULED VISIT

Unscheduled visits for malaria 18S qPCR or safety monitoring may be required at the

Investigator's discretion based on parasitaemia, clinical symptoms or laboratory results. Subjects will be contacted by phone to arrange these visits. Where possible, visits will be

arranged at a time that is both convenient for the subject and meets any clinical urgency as determined by the Investigator. Unscheduled visits will be documented in the source documents and CRF.

7.3.7 SCHEDULE OF EVENTS TABLE

The Schedule of Events Table summarises the procedures to be conducted as per this protocol during screening, confinement and post-confinement. Section 7.1 and 7.2 provide detailed information on the procedures.

Table 1 Schedule of Events

Procedure	Screening	Eligibility confirmation[a]	Malaria inoculation	Phone contact[p]	Malaria monitoring[b]	Apheresis and rescue treatment	Out-patient monitoring[t]	Safety visit	EOS visit
	D-28 to D-1	D-3 to D-1	D0	D1 to D3	D4 to apheresis	Apheresis to end of confinement[c]	Post confinement to Safety visit[t]	D28±3	D90±7
Eligibility and safety assessments									
Informed consent	X								
Beck Depression Inventory	X								
Medical history, eligibility & prior medications	X		X						
Drug & alcohol screen	X		X			X[d]			
Full physical examination	X							X	
Symptom-directed physical examination[e]			X		X	X	X[t]		X
Vital signs assessment	X		X		X	X	X[t]	X	X
ECG	X		X			X[f]		X	
Urinalysis	X	X				X[d]	X[t]	X	
Haematology & biochemistry	X	X			X[o]	X[f]	X[t]	X[u]	X[v]
G6PD test & Duffy antigen	X								
Assessment for HMP bank[i]	X[q]	X[q]						X[r]	X[r]
Serology & RBC alloantibody	X							X[u]	X[v]
Safety serum storage			X						X
AEs & concomitant medications			X	X	X	X	X[t]	X	X
Malaria clinical score			X		X	X	X[ht]		
Malaria monitoring and apheresis									
Malaria 18S qPCR blood sampling			X		X[g]	X	X[t]	X[u]	X[v]
Parasite lifecycle stage qRTPCR blood sampling[i]			X		X	X	X[t]	X[u]	

Malaria transmission assays (DFA and/or MFA)					X[j]	X[j]			
Apheresis						X[k]			
Immunology/pathophysiology			X[s]			X[s]			
Rescue drug treatment									
Riamet® treatment						X[k]			
Chloroquine treatment						X[m]			
Artesunate treatment						X[n]			

ECG: Electrocardiogram; G6PD: Glucose 6-phosphate dehydrogenase; RBC: Red blood cell; AEs: Adverse events; qPCR: quantitative polymerase chain reaction; qRT-PCR: quantitative reverse-transcriptase polymerase chain reaction; HMP: human malaria parasite; DFA: direct feed assay; MFA: membrane feed assay; EOS: End of study.

[a] This visit is not required in the event that the screening visit is conducted within this period. [b] Daily visits until Day 9. Up to 3 visits per day may be required, at the discretion of the investigator, between day 9 and treatment day. [c] Confinement is expected to start on day 9, 10, 11 or 12 and last between 48 to 72h. [d] At time of admission to clinical unit only. [e] Physical examinations should be performed prior to inoculation, upon admission to the clinical unit for apheresis and rescue treatment, and at Day 90±14/EOS. At all other times, symptom-directed physical examinations will only be performed when signs or symptoms of malaria are identified and it is clinically indicated at the Investigator's discretion. Before exit from confinement, nursing staff must confirm with the study doctor or investigator if any subject requires symptom-driven examination pre discharge. [f] On day of admission (just prior to the apheresis procedure) and exit from clinical unit only. Haematology and biochemistry (including calcium and magnesium) will be taken upon return to Q-pharm immediately post apheresis. [g] Blood sampling for malaria may be done up to 3 times a day from Day 9 to treatment day (at the discretion of the investigator) [h] Only if vital signs are abnormal, or at the Investigator's discretion. [i] At the Investigator's discretion, if in doubt contact Investigator. [j] Transmission assays may be performed at up to 2 time points prior to apheresis and rescue treatment, at the discretion of the Investigator. [k] Apheresis and initiation of rescue treatment with artemether/lumefantrine will be performed on the first day of admission to the clinical unit (expected to occur on Day 10, 11 or 12). [m] Chloroquine will only be administered to subjects in the case of failure of artemether/lumefantrine therapy. [n] Intravenous artesunate treatment will only occur in the event that a subject is unable to complete oral treatment with either artemether/lumefantrine or chloroquine (e.g. the subject is vomiting). [o] Haematology and biochemistry (including calcium and magnesium) will be performed on the day before apheresis. [p] A phone call will also be made on Day 56±7 to monitor participant well-being and to solicit any adverse events. [q] Assessment for HMP bank at screening or Day -3 to -1 eligibility confirmation visit will include a questionnaire to be completed by each subject.

[r] Blood for HMP bank may be taken at the Investigator's discretion on day 28 or day 90 but not both and if the maximum whole blood volume is not exceeded. [s] At the Investigator's discretion and if the maximum whole blood volume is not exceeded. If in doubt contact Investigator. [t] If subject is released from confinement before 72h post Riamet® treatment.

[u] At the discretion of the investigator and if required for safety reasons. Otherwise, blood sample will be collected on day 90±7 to limit the volume of whole blood collected within 30 days of the apheresis procedure. [v] If not performed on day 28 ±3.

7.4 JUSTIFICATION FOR SENSITIVE PROCEDURES

Not applicable.

7.5 CONCOMITANT MEDICATIONS, TREATMENTS, AND PROCEDURES

Concomitant medications, treatments and procedures are those occurring from administration of the malaria inoculum until the end of the study (last visit). Those occurring prior to administration of the inoculum are classified as prior medications, treatments and procedures. Medications taken within 28 days before the malaria inoculation will be recorded as prior medication. Prior and concomitant medications, treatments and procedures permitted in this study are outlined in the inclusion/exclusion criteria (section 5.1 and 5.2).

On inoculation day, subjects will be questioned in relation to relevant aspects of compliance with the study protocol, including drug intake since their screening clinic visit. Details of all other drugs taken (prescription and over-the-counter, systemic and topical administration) will be recorded at this time and appropriate action taken. The Investigator may permit the use of ibuprofen up to 1.2 g/day (except during the 48 hour period prior to apheresis) or acetaminophen up to 4 g/day, for treatment of headache or other pain if required. Any medication taken during the study for treatment of a medical condition or adverse event is to be recorded in the concomitant medication pages in the CRF (exact dose and timing of each dose to be specified).

7.5.1 PRECAUTIONARY MEDICATIONS, TREATMENTS, AND PROCEDURES

Ibuprofen should not be taken in the 48 hour period prior to apheresis.

7.6 PROHIBITED MEDICATIONS, TREATMENTS, AND PROCEDURES

All concomitant medications other than those routinely used for symptom relief in IBSM trials (i.e. acetaminophen, ibuprofen, ondansetron) or routine medications approved at screening (e.g. oral contraceptive) should be discussed with the Investigator before being approved unless deemed medically urgent. If the medication has already been taken it should be reviewed by the Investigator at the next opportunity and a decision should be made to continue, to stop, to switch to an alternative, or to withdraw the subject from the trial.

Subjects should not eat any poppy seeds in the 24 hours before the following time-points:

screening, inoculation day, and day of admission for antimalarial treatment.

Subjects should not eat or drink any food or beverages that contain alcohol (e.g. beer, wine, and mixed drinks) from 24 hours prior to each alcohol breath test and from inoculation until the end of antimalarial treatment.

Subjects should not consume more than 400 mg caffeine per day, equivalent to more than 4 cups of coffee, from inoculation until the end of the antimalarial treatment.

7.7 PROPHYLACTIC MEDICATIONS, TREATMENTS, AND PROCEDURES

Not applicable.

7.8 RESCUE MEDICATIONS, TREATMENTS, AND PROCEDURES

Rescue medications used in this study are defined in section 6 and details on their administration are given in section 7.3.

7.9 SUBJECT ACCESS TO STUDY AGENT AT STUDY CLOSURE

Not applicable.

8 ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

Safety assessments to be performed in this study include recording of AEs, clinical laboratory measurements (haematology, biochemistry, urinalysis), physical examinations, vital signs recording, ECGs, and Malaria Clinical Score recording.

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

An AE is any adverse change, i.e., any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease that occurs in a subject during the course of the study, whether or not considered by the Investigator as related to study treatment.

AEs include:

- A new symptom, sign or medical condition.
- A disease or medical condition detected or diagnosed during the course of the study even though it may have been present prior to the start of the study.
- An exacerbation of a pre-existing medical condition or disease.
- An increase in frequency or intensity of a pre-existing episodic disease or medical condition.
- Continuous persistent disease or symptoms present at study start that worsen following the start of the study.
- An abnormal assessment (e.g. change on physical examination, ECG findings) if it represents a clinically significant finding that was not present at study start or worsened during the course of the study.
- An abnormal laboratory test result if it represents a clinically significant finding, symptomatic or not, which was not present at study start or worsened during the course of the study or led to dose reduction, interruption or permanent discontinuation of study treatment.

Mosquito bite reactions (if DFA are performed) regardless of clinical significance.

Borderline abnormal laboratory findings and other objective assessments should NOT be routinely captured and reported as AEs, as they will be collected and analysed separately. However, abnormal

laboratory findings or other objective measurements that meet the following criteria should be captured and reported in the AE section of the CRF:

- The result meets the criteria for reporting as an SAE
- The test result is associated with accompanying symptoms, and/or
- It requires additional diagnostic testing or medical/surgical intervention, and/or
- It leads to a change in trial dosing outside of protocol-stipulated dose adjustments, or discontinuation from the trial, significant additional concomitant drug treatment, or other therapy, and/or
- It is considered by the Investigator or Sponsor to be clinically significant or represent a clinically significant change from baseline.

Merely repeating an abnormal test, in the absence of any of the above conditions, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE.

If a clinical diagnosis is associated with an abnormal laboratory finding, the relevant adverse event should be recorded as the diagnosis rather than the incidental laboratory finding (e.g. “hepatitis” should be recorded rather than “elevated transaminases”).

Surgical procedures themselves are not AEs; they are therapeutic measures for conditions which may, or may not, be AEs.

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

A serious adverse event (SAE) is defined as an AE which fulfils at least one of the following criteria:

- Results in death
- Is life-threatening ○ The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- Requires inpatient hospitalisation or prolongs existing hospitalisation, unless this is for:
  - ○ Elective or pre-planned treatment or standard monitoring for a pre-existing condition that is unrelated to the study and has not worsened since the start of the study. ○ Cosmetic surgery or for social reasons or respite care in the absence of any deterioration in the subject’s general condition.
- Results in persistent or significant disability/incapacity

Is a congenital abnormality/birth defect

- Is considered medically important ○ Medical and scientific judgement should be exercised in deciding whether other AEs are to be considered serious, such as important medical events that may not be immediately life-threatening but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are: intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias; convulsions that do not result in hospitalisation; development of drug dependency or drug abuse.
- Constitutes a possible Hy's Law case ○ Hy's Law case is defined as a subject with any value of alanine or aspartate aminotransferase greater than 3 × ULN together with an increase in total bilirubin to a value greater than 2 × ULN and not associated to an alkaline phosphatase value greater than 2 × ULN (FDA Guidance on Drug Induced Liver Injury: Premarketing Clinical Evaluation [2009]).

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is any SAE where a causal relationship with the malaria challenge agent (*P. vivax* HMPBS02-*Pv*) or the antimalarial rescue drugs (artemether/lumefantrine, Chloroquine, or artesunate) is at least a reasonable possibility, but the event is not listed in the Investigator Brochure(s) and/or Summary of Product Characteristics.

8.1.3 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

Not applicable.

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

In addition to determining whether an AE fulfils the criteria for a SAE or not, the severity of AEs experienced by study subjects will be graded according to the Common Terminology Criteria for Adverse Events v4.03 published 14 June 2010 (CTCAE v4.03). This guidance provides a common language to describe levels of severity, to analyse and interpret data, to scale the aggregate AE score, and to articulate the clinical significance of all AEs.

The severity of adverse events will be graded as follows:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or non-invasive intervention indicated; limiting age appropriate instrumental activities of daily living.
  Grade 3: Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care activities of daily living.

- Grade 4: Life-threatening consequences; urgent intervention indicated. Grade 5: Death related to AE.

A mild, moderate, or severe AE may or may not be serious (see Section 8.1.2). These terms are used to describe the intensity of a specific event. Medical judgment should be used on a case-by case basis.

Seriousness, rather than severity assessment, determines the regulatory reporting obligations.

8.2.2 RELATIONSHIP TO STUDY AGENT

The Investigator will decide if AEs are related to any of the study agents or procedures. Where possible, a distinction should be made between events considered related to the malaria challenge agents or the apheresis procedure and those related to protocol-mandated procedure (including mosquito feeding, and rescue medication).

The assessment of causality will be made using the following definitions:

Unrelated

This category is applicable to those AEs which are judged to be clearly and incontrovertibly due to extraneous causes (disease, environment, etc.) and do not meet the criteria for the relationship listed under unlikely, possible or probable.

Unlikely

In general, this category is applicable to an AE which meets the following criteria (must have the first two):

1. It does not follow a reasonable temporal sequence from administration of any of the study agents.
2. It may readily have been produced by the subject's clinical state, environment or toxic factors, or other modes of therapy administered to the subject.
3. It does not follow a known pattern of response to the study agents.
4. It does not reappear or worsen when any of the study agents are re-administered.

Possible

This category applies to those AEs in which the connection with any of the study agents appears unlikely but cannot be ruled out with certainty. An adverse event may be considered possible if or when (must have the first two):

1. It follows a reasonable temporal sequence from administration of any of the study agents.
2. It may have been produced by the subject's clinical state, environment or toxic factors, or other modes of therapy administered to the subject.
3. It follows a known pattern of response to any of the study agents.

Probable

This category applies to those adverse events which are considered, with a high degree of certainty, to be related to the study agents. An adverse event may be considered probable if (must have the first three):

1. It follows a reasonable temporal sequence from administration of any of the study agents.
2. It cannot be reasonably explained by the known characteristics of the subject's clinical state, environment or toxic factors, or other modes of therapy administered to the subject.
3. It disappears or decreases on cessation or reduction in dose.
4. It follows a known pattern of response to any of the study agents.
5. It reappears on re-administration.

8.2.3 EXPECTEDNESS

An AE is regarded as an unexpected event if its nature or severity is not consistent with the applicable reference safety information (Investigator's Brochures or approved manufacturer's prescribing information for marketed drugs). Events that add significant information on the specificity, severity or frequency of previously described reactions, are also regarded as unexpected.

Expected AEs from the malaria infection are listed in Appendix 2 and the Investigator's Brochure for the *P. vivax* HMPBS02-*Pv* challenge agent. Expected AEs from the antimalarial drugs used are listed in the artemether/lumefantrine and Chloroquine Consumer Medicine Information (see Appendix 4) and artesunate product insert.

8.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

All AEs must be documented and followed up by the Investigator until:

- the event is resolved, or
- no further medically relevant information in relation to the event can be expected, and
- the Investigator considers it justifiable to terminate the follow-up.

Events that are unresolved at the time of the subject's last follow-up visit should continue to be followed up by the Investigator for as long as medically indicated. The Sponsor retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

All AEs should be treated appropriately. The Investigator will decide upon the appropriate action to be taken in response to an AE, which may include one or more of the following:

- no action taken (i.e. further observation only)
- apheresis is withheld and the subject withdrawn from the study
- administration of a concomitant medication
- hospitalisation or prolongation of current hospitalisation (event to be reported as an SAE)
- other.

In a case of occurrence of SAEs, regardless of whether or not it is judged to be challenge agent- or antimalarial drug-related, the subject will receive appropriate care under clinical supervision until all the symptoms of the SAEs have diminished or resolved and the subject's condition improved.

For ongoing AEs, care will be provided for a period of time as specified in the clinical site work instruction protocols. However, if the nature of the ongoing AE is determined by the Investigator as not being inoculum-, apheresis-, or antimalarial drug-associated, the subject will be advised to visit his/her own general practitioner for further clinical care that he might require.

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

It is the Investigator's responsibility to document and report all AEs occurring in the clinical trial whether spontaneously reported by the subject, observed by the Investigator (either directly or by laboratory or other assessments), or elicited by general questioning. The period of observation for collection of AEs extends from the time of inoculation up to the end of the study. Events reported prior to this will be recorded as medical history, unless the symptoms worsen during the study.

The following information should be recorded for all AEs:

- a description of the AE
- the dates and times of onset and resolution of the event
- the duration of the event in hours
- the time of onset relative to the administration of the inoculum and/or apheresis
- the seriousness and severity of the event
- the action take in response to the event (including treatment required)
- the outcome of the event

- the relationship of the event to the study agents (causality assessment), including inoculum, apheresis, mosquito feeding, rescue medication, or any other treatment or procedure conducted during the study.

Changes in the severity of an AE will be documented to allow assessment of the duration of the event at each level of severity. AEs changing severity will be documented as separate AEs; those worsening in severity will be considered unresolved and those reducing in severity will be considered resolving. AEs characterised as intermittent require documentation of onset and duration at each episode.

All malaria-specific AEs will be tabulated and results graded according to a purpose-designed Malaria Clinical Score (Section 7.1.1).

8.4.2 SERIOUS ADVERSE EVENT REPORTING

The Investigator will take immediate appropriate action in response to SAEs to ensure subject safety and in an attempt to identify the causes of the event. Review and reporting of SAEs will be in accordance with the Sponsor's and QPharm's SAE reporting procedures. The Investigator will notify the Sponsor representative (QIMR Berghofer Regulatory Affairs), the QIMR Berghofer HREC, and the IMM of the occurrence of any SAE within 24 hours of becoming aware of the event. The notification should be in writing by email or fax, and documented on a standard SAE reporting form.

Sponsor Representative

QIMR Berghofer Regulatory Affairs

Email: clinical.trials@qimrberghofer.edu.au

QIMR Berghofer-HREC Contact

QIMR Berghofer HREC Secretary

E-mail: HREC.Secretariat@qimrberghofer.edu.au
Phone: +61 7 3362 0117

Independent Medical Monitor:
Professor Dennis Shanks

E-mail: Dennis.SHANKS@defence.gov.au

Phone: +61 7 3332 493

The Investigator will complete a follow-up SAE report within 14 days of the SAE, unless no further information is available in which case the follow-up report will be provided as soon as new information becomes available. The follow-up SAE report will be sent to QIMR Berghofer

Regulatory Affairs, the QIMR Berghofer HREC, and the IMM. Other supporting documents may be requested by these parties and will be provided by the Investigator or a delegate as soon as possible.

Any SAE that meets the criteria of a SUSAR (Section 8.1.2) will be reported to the TGA by QIMR Berghofer Regulatory Affairs in accordance with the Sponsor's reporting procedures.

8.4.3 UNANTICIPATED PROBLEM REPORTING

Not applicable.

8.4.4 EVENTS OF SPECIAL INTEREST

Not applicable.

8.4.5 REPORTING OF PREGNANCY

Not applicable.

8.5 STUDY HALTING RULES

See Section 5.5.

8.6 SAFETY OVERSIGHT

Safety oversight will be undertaken by the Principal Investigator , the collaborating PI, and the IMM who will serve as an independent expert to advise on clinical safety specifically in the situation where expert external advice is required regarding the need for administration of alternative/rescue antimalarial treatment in the circumstance of suboptimal response.

The Safety Review Team (SRT) will be responsible for decisions related to the safety of subjects and the continuation of the study. The role and composition of the SRT is outlined in the study specific SRT Charter. The SRT will meet approximately 4 weeks after the inoculation of each subject to review safety data before proceeding with the inoculation of a subsequent subject. A final SRT will also take place at completion of the study which will review safety parameters for all the subjects involved. The SRT will be composed of the Principal Investigator, the collaborating PI, the IMM, and a physician with expertise in clinical trials or infectious diseases. The SRT will review the clinical and laboratory safety data as well as the recorded AEs and SAEs. The SRT makes recommendations to the Sponsor. These recommendations are approved by the SRT Chair who signs a letter of recommendation that is sent to the Principal Investigator and the Sponsor.

Additionally, the SRT may meet to assess any events that trigger the stopping rules (Section 5.5) or as needed to provide a recommendation and findings to QIMR Berghofer HREC and the Principal Investigator, in accordance with the approved SRT Charter.

Whether at a scheduled or unscheduled meeting, the SRT will consider safety signals to determine whether or not they can recommend that the study continue.

9 CLINICAL MONITORING

It will be the Sponsor's responsibility to ensure that the study is monitored in accordance with the requirements of GCP. The conduct of the study will be reviewed internally by the clinical unit (QPharm) in accordance with their standard procedures and work instructions, and GCP guidelines. The study will be monitored according to the Sponsor's SOPs and all protocol deviations will be reported to the Sponsor (see Section 14.3 for more detail). Protocol deviations that impact subject safety or data integrity will also be reported to the QIMR Berghofer HREC.

During the study, appointed study monitor(s) (on behalf of the Sponsor) will visit the site to check completeness of subject records, accuracy of CRF entries, adherence to the protocol and to GCP, progress of enrolment, and to ensure that study agents were stored, dispensed, and accounted for according to specifications. Key study personnel are required to be available to assist the study monitor during these visits.

The Investigator will be required to give the monitor access to all relevant source documents to confirm their consistency with the CRF entries. The Sponsor will require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs, and the recording of data that is used for all primary and safety variables. Additional checks of the consistency of the source data with the CRFs will be performed according to the study specific monitoring plan. No information in source documents about the identity of the subjects will be disclosed.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL AND ANALYTICAL PLANS

This study is a Phase I exploratory study to investigate the safety and plausibility of apheresis as a method for harvesting parasites from healthy subjects experimentally infected with blood stage malaria. As such, no formal statistical analysis plan will be generated.

10.2 STATISTICAL HYPOTHESES

Not applicable.

10.3 ANALYSIS DATASETS

The safety analysis dataset will include all subjects who receive the malaria inoculum. This population will be used to analyse all safety data as well as demographic and baseline data.

10.4 DESCRIPTION OF STATISTICAL METHODS

10.4.1 GENERAL APPROACH

All measured variables and derived values will be listed. Continuous data will be summarised using descriptive statistics (mean and standard deviation, or median and interquartile range).

Categorical data will be presented using N and % (using the number of subjects without missing data in the calculation).

10.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

Not applicable.

10.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

No formal statistical analysis of the secondary endpoints will be performed, see Section 10.4.1.

10.4.4 SAFETY ANALYSES

The overall number and percentage of subjects with at least one AE (and SAE) will be tabulated over the entire study period. All AE data will be summarised by MedDRA system organ class and preferred term, and maximum severity. Vital signs, routine safety laboratory data and ECG parameters will be summarised descriptively by time-point. Both absolute values and change from baseline (inoculation) will be presented.

10.4.5 ADHERENCE AND RETENTION ANALYSES

Not applicable.

10.4.6 BASELINE DESCRIPTIVE STATISTICS

Demographic data will be summarised by descriptive statistics and will include total number of observations (n), mean, standard deviation (SD) and range for continuous variables and number and percentages with characteristics for dichotomous variables.

The subject disposition will be summarised. Study completion, study withdrawals, exclusions and violations will be summarised and the reasons for withdrawal, exclusions and violations will be listed.

Medical history, current medical conditions, previous and concomitant medications, results of laboratory screening tests, drug tests and any other relevant baseline information will be listed by subject.

10.4.7 PLANNED INTERIM ANALYSES

10.4.7.1 SAFETY REVIEW

There will be an SRT meeting approximately 4 weeks after the inoculation of each subject to review safety data before proceeding with the inoculation of a subsequent subject. There will also be a final SRT after the last subject which will review any SAEs, AEs, blood tests, vital signs or other investigations of concern. The SRT members must agree on the safety of the trial before going forward with the inoculation of the subsequent subject.

10.4.7.2 EFFICACY REVIEW

The SRT meeting undertaken approximately 4 weeks after the inoculation of the each subject will also assess the progress of the each subject in relation to the success or failure in extracting and concentrating all stages of malaria parasites by apheresis. The SRT may decide not to proceed with inoculating further subjects if they deem it futile and highly unlikely to be successful.

10.4.8 ADDITIONAL SUB-GROUP ANALYSES

Not applicable.

10.4.9 MULTIPLE COMPARISON/MULTIPLICITY

Not applicable.

10.4.10 TABULATION OF INDIVIDUAL RESPONSE DATA

All individual subject data will be listed by measure and time point.

10.4.11 EXPLORATORY ANALYSES

Not applicable.

10.5 SAMPLE SIZE

This study is a exploratory study to investigate the safety and plausibility of apheresis as a method for extracting and concentrating parasites from healthy subjects experimentally infected with blood stage *P. vivax*. Therefore, statistical considerations regarding sample size do not apply. We estimate that a total of up to 8 subjects inoculated in a sequential manner will be sufficient to meet the objectives of this study. Approximately 4 weeks after the inoculation of each subject an assessment of all data collected will be performed. If it is deemed by the PI that there is no need to proceed further as all objectives of the study have been met, the study may be considered complete with fewer than 8 subjects enrolled.

10.6 MEASURES TO MINIMIZE BIAS

10.6.1 ENROLLMENT/ RANDOMIZATION/ MASKING PROCEDURES

Treatment numbers will be allocated prior to inoculation and will serve as subject identifiers and for the purpose of distinction between cohorts, and will not correspond to a randomisation schedule.

Treatment numbers will be defined according to the CRU standard operating procedures (SOPs) and noted in a work instruction prior to study start. These numbers will be assigned to subjects on the morning of the dose administration after confirmation of their continued eligibility, in ascending, sequential order corresponding to the sequence of screening numbers for the subject in the admitted cohort. A log will be maintained at the site cross-referencing each subject's screening number to the treatment number assigned.

After allocation, treatment numbers will be recorded in the CRFs and displayed behind each subject's bed and on a wrist band at all times during the admission period.

10.6.2 EVALUATION OF SUCCESS OF BLINDING

Not applicable.

10.6.3 BREAKING THE STUDY BLIND/SUBJECT CODE

Not applicable.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The Investigator will maintain source documents for each subject in the study. Information entered into CRFs will be traceable to these source documents in the subject's file. The Investigator must certify that the data entered into the CRFs are complete and accurate. Documentation of the apheresis procedure will occur as normal in the specialist centre (see Appendix 3) and will be retained as a source document in the subject's file. After database lock, the Investigator will retain copies of the subject data for archiving at the investigational site.

Upon request, the Investigator(s)/institution(s) will permit direct access to source data/documents for trial-related monitoring, audits, Ethics Committee review, and regulatory inspection(s) by the Sponsor (or their appropriately qualified delegate) and Regulatory Authorities. Direct access includes examination, analysis, verification and reproduction of records and reports that are important to the evaluation of the trial.

12 QUALITY ASSURANCE AND QUALITY CONTROL

Data management will be performed in accordance with regulatory requirements. The data entered into the CRFs by investigational staff will be reviewed for completeness and accuracy.

The site personnel will clarify any apparent erroneous entries or inconsistencies and additional information will be requested from the site as required.

Medical history/current medical conditions and adverse events will be coded using the Medical dictionary for regulatory activities (MedDRA) terminology (version 19.0 or later).

After all data have been captured and reviewed, all queries have been resolved with the site and any protocol non-compliances that were identified during the data management processes have been confirmed by the site, the database will be declared to be complete and accurate, it will be locked and made available for data analysis. Any changes to the database after that time may only be made by the data manager, in consultation with the Sponsor and in accordance with documented database unlock and relock procedures.

Clinical monitoring will be conducted as described in Section 9.

Audits may be carried out by Sponsor quality assurance representatives, local authorities or authorities to whom information on this study has been submitted. All documents pertinent to this study must be made available for such inspections after adequate notice of intention to audit.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 ETHICAL STANDARD

The study will be conducted in accordance with the protocol approved by QIMR Berghofer HREC and the Royal Brisbane and Women's Hospital HREC, the principles of the Declaration of Helsinki (Recommendations guiding Medical Doctors in Biomedical Research Involving Human Subjects, Fortaleza, Brazil 2013), the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Notes for Guidance on Good Clinical Practice (GCP) (CPMP/ICH/135/95), as adopted by the Australian Therapeutic Goods Administration (2000).

The Investigator will minimise any discomfort experienced by subjects during the study. The only invasive procedures will be the intravenous inoculation of the malaria inoculum and the blood collection by cannulation/venepuncture and apheresis.

The maximum amount of blood to be collected from an individual in the study would be up to approximately 286 mL if HMP bank production is not performed (i.e. a volume which will not be more than the equivalent to a standard blood bank donation and it will be taken over at least a 4-8 week interval). See Appendix 1.

The total volume of blood drawn from each subject will not exceed 450 mL in any given 30 day period. This volume includes allowance for unscheduled safety and qPCR assessments that may be required at the discretion of the Principal Investigator or the Sponsor to ensure subject safety.

13.2 ETHICAL REVIEW

The protocol, consent forms and participant information sheets will be reviewed by the QIMR

Berghofer-HREC and the Royal Brisbane and Women's Hospital HREC. No study activities will be initiated prior to the approval of these documents by those committees. All amendments and addenda to the protocol will similarly be submitted to the QIMR Berghofer-HREC and the Royal Brisbane and Women's Hospital HREC for approval prior to their implementation.

Changes to the final study protocol can only be made with the prior consent of the Principal Investigator, the Sponsor and the HREC. All such changes must be attached to, or incorporated into, the final protocol, and communicated to all relevant members of Q-Pharm staff and, if appropriate, to study subjects. All deviations from this study protocol will be included in the trial master file and included in the CSR. An assessment of the significance of each protocol deviation will be given in the CSR. All deviations/amendments will be reported to the Sponsor. The different types of amendments are discussed below.

Non-substantial amendment

Administrative or logistical minor changes require a non-substantial amendment. Such changes include but are not limited to changes in study staff or contact details (e.g., Sponsor instead of CRO monitors) or minor changes in the packaging or labelling of study drug. An amendment deemed to be non-substantial must have no ethical implications.

The implementation of a non-substantial amendment may be done without notification to the HREC. It does not require their approval or to be signed by the Investigator. The HREC will be notified for these non-substantial changes in the next submission round, with the annual study report or study close out report which ever comes sooner that will be submitted to HREC.

Substantial amendment

Significant changes require a substantial amendment. Significant changes include but are not limited to: new data affecting the safety of subjects, change of the objectives/endpoints of the study, eligibility criteria, dose regimen, study assessments/procedures, treatment or study duration, with or without the need to modify the Participant Information Sheet and Informed Consent.

Substantial amendments are to be approved by the HREC. The implementation of a substantial amendment can only occur after formal approval by the HREC and must be signed by the Investigator.

Urgent amendment

An urgent amendment might become necessary to preserve the safety of the subjects included in the study. The requirements for approval should in no way prevent any immediate action being taken by the Investigator or the Sponsor in the best interests of the subjects. Therefore, if deemed necessary, an Investigator can implement an immediate change to the protocol for safety reasons. This means that, exceptionally, the implementation of urgent amendments will occur before submission to and approval by the HREC.

In such cases, the Investigator must notify the Sponsor within 24 hours. A related substantial amendment will be written within 10 working days and submitted to the HREC, together with a description of the steps that have already been taken in regard to implementation of this amendment.

HREC approval of future research

In the event that the Principal Investigator or the Sponsor want to perform testing on the samples that is not described in the protocol, additional HREC approval will be sought. This may be done if a subject consents to blood storage for use in future research (Section 13.3.1).

13.3 INFORMED CONSENT PROCESS

13.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO SUBJECTS

Subjects will be fully informed of the nature of the study, the properties and adverse effects of the inoculum, apheresis procedure, Chloroquine treatment and potential rescue treatment with artemether/lumefantrine and all relevant aspects of study procedures in the 'Participant Information Sheet'.

The Participant Information Sheet and informed Consent Form describes in detail the study agents, study procedures, and risks. Subjects will also receive an Informed Consent for Blood Storage and an option to grant permission to be contacted about future studies involvement.

Subjects will also receive the Consumer Medicine Information for artemether/lumefantrine. Subjects may also receive the Consumer Medicine Information for chloroquine (Appendix 4), and the product insert for artesunate if required.

13.3.2 CONSENT PROCEDURES AND DOCUMENTATION

During the initial screening visit/recruitment, potential subjects will read the Participant Information Sheet. The Investigator or clinical unit staff will explain the study via the Participant Information Sheet and the potential subjects will be encouraged to ask questions. Individuals willing to be considered for inclusion in the study will sign and date the informed Consent Form in the presence of an Investigator. Subjects will be given a copy of their signed informed Consent Form. Once the subject has consented to the study, the trial-specific screening activities may commence. See Section 7.3.1 for further details.

13.4 SUBJECT AND DATA CONFIDENTIALITY

Subjects will be informed that their data will be held on file by Q-Pharm and that these data may be viewed by staff of Q-Pharm (including, where necessary, staff of Q-Pharm other than the named Investigators).

Upon request, the Investigator(s)/institution(s) will permit direct access to source data/documents for trial-related monitoring, audits, Ethics Committee review, and regulatory inspection(s) by the Sponsor (or their appropriately qualified delegates) and Regulatory Authorities (see Section 11).

Subjects will be informed that a report of the study will be submitted to the Sponsor and may also be submitted to government agencies and perhaps for publication, but that they will only be identified in such reports by their study identification number, and their gender and age. The Investigators undertake to hold all personal information in confidence.

Subjects will be informed that samples collected for the purposes described in the protocol will be sent to Sponsor's nominated national or international laboratory for assessment.

13.4.1 RESEARCH USE OF STORED HUMAN SAMPLES,SPECIMENS OR DATA

Samples and data collected during this study will be used to achieve the study objectives. Samples and data will be stored according to Q-Pharm and QIMR Berghofer SOPs, and access will be limited to authorised personnel. Biological samples will be retained for the time required for assessment for analysis, and may then be discarded.

13.5 FUTURE USE OF STORED SPECIMENS

As part of the study, safety serum samples will be stored indefinitely at Q-Pharm/QIMR Berghofer for retrospective safety assessments that may later be indicated. Subjects consent to this storage and the use of the sample for safety assessments, when they sign the informed Consent Form for the study.

For all other samples, consent must be obtained from the subjects to store and use their samples for future research. This is done via the Informed Consent for Blood Storage that subjects receive during recruitment/screening. Subjects can decide if they want their samples to be used for future research or have their samples destroyed at the EOS. A subject's decision can be changed at any time prior to the EOS by notifying the study doctors or nurses in writing. However, if a subject consents to future use and some of their blood has already been used for research purposes, the information from that research may still be used.

Any future research using the stored samples that is beyond the current study will be reviewed by the QIMR Berghofer HREC (Section 13.2). All samples will be stored at QIMR Berghofer in accordance with the laboratory SOPs. The Investigator will ensure that confidentiality will be maintained continuously in all future research that involves use of these samples. The vials containing the samples of the consented subjects will be coded and the identifying information will not be released to any unauthorised third party. The subjects can also choose (via the Informed Consent for Blood Storage Form) for the samples to be re-labelled with only the study number, malaria strain and visit. No genetic testing will be performed on the stored samples without obtaining consent from the subjects. The stored samples will not be sold or used directly for production of any commercial product. There are no benefits to subjects in the collection, storage and subsequent research use of their samples. Reports about future

research done with subject samples will NOT be kept in their health records, but a subject's samples may be kept with the study records or in other secure areas.

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Each subject will have a clinical file (source data) and case report form (CRF, for protocol specific data) into which relevant data will be recorded. All recording will be done only in black ink. Corrections will only be made by drawing a single line through the incorrect entry, writing the correction in the nearest practicable space, and initialling and dating the correction. Correction fluids are not allowed.

A log of names, signatures and initials of all staff authorised to enter data into a subject's Clinic File and CRF will be kept. Upon completion of each study visit, all CRFs will be reviewed internally by the clinic for omissions or apparent errors so that these can be corrected without delay. Any corrections made after the review and signature of the Principal Investigator will be noted in the audit trail and will required reauthorisation (electronic sign off) by the Principal Investigator.

14.2 STUDY RECORDS RETENTION

All source data, clinical records and laboratory data relating to the study will be retained in the archive of the clinical unit (Q-Pharm) for a minimum of 15 years after the completion of the study. Data will be available for retrospective review or audit by arrangement with the Chief Executive Officer of the clinical unit (Q-Pharm). Written agreement from the Sponsor must precede destruction of the same.

14.3 PROTOCOL DEVIATIONS

A protocol deviation is any change, divergence or departure from the study design or procedures defined in the protocol.

All protocol deviations will be documented in the trial master file and included in the CSR. An assessment of the significance of each protocol deviation will be discussed in the CSR.

All protocol deviations will be reported to the Sponsor. Protocol deviations that are not approved by the Sponsor and QIMR Berghofer HREC prior to implementation should be reported to the Sponsor by the clinical unit. Protocol deviations should be recorded on a protocol deviation log by the clinical unit staff. Protocol deviation logs should be submitted to the Sponsor and QIMR Berghofer HREC via inclusion with the annual report.

Protocol deviations that impact subject safety or data integrity will be reported to both the Sponsor and the QIMR Berghofer HREC in a timely manner. A protocol deviation report form will be used for this purpose.

14.4 PUBLICATION AND DATA SHARING POLICY

The data management, statistical and medical writing team appointed by the Sponsor will collaborate to provide a detailed CSR upon conclusion of the study. This will include appendices

of all tables and listings generated during the analyses of data. The Sponsor undertakes to ensure that all safety observations made during the conduct of the trial are documented in this report.

Publication and reporting of results and outcomes of this trial will be accurate and honest, undertaken with integrity and transparency and in accordance with the relevant clauses outlined in the QIMR Berghofer Policy on Criteria for Authorship. QIMR Berghofer and the Principal Investigator have a responsibility to ensure that results of scientific interest arising from the clinical trials are appropriately published and disseminated. Publication of results will be subjected to fair peer-review. Authorship will be given to all persons providing significant input into the conception, design, and execution or reporting of the research according to the QIMR Berghofer Policy on Criteria of Authorship. No person who is an author, consistent with this definition, will be excluded as an author without his/her permission in writing. Authorship will be discussed between researchers prior to study commencement (or as soon as possible thereafter) and reviewed whenever there are changes in participation. Joint publications between QIMR Berghofer and the Royal Brisbane & Women's Hospital are envisaged. Acknowledgment will be given to collaborating institutions and hospitals and other individuals and organisations providing finance or facilities. All conflicts arising through disputes about authorship will be reviewed by the QIMR Berghofer Director.

Data will not be released publicly until the manuscript is accepted for publication. In the case of no publication, information will only be released to the public and media in accordance with the QIMR Berghofer Corporate Media Strategy Policy. However, the Investigator undertakes not to make any publication or release pertaining to the study and/or results of the study without the

Sponsor's prior written consent, being understood that the Sponsor will not unreasonably withhold its approval. The Sponsor has the right to publish the results of the study at any time.

The Investigator shall not use the name(s) of the Sponsor and/or of its employees in advertising or promotional material or publication without the prior written consent of the Sponsor. The Sponsor shall not use the name(s) of the Investigator and/or the collaborators in advertising or promotional material or publication without having received his/her and/or their prior written consent(s).

QIMR Berghofer will ensure that the key design elements of this protocol are posted in a publicly accessible database such as Australian New Zealand Clinical Trials Registry (ANZCTR) or Clinicaltrials.gov. In addition, upon study completion and finalisation of the study report the results of this trial will be either submitted for publication in an open access journal and/or posted in a publicly accessible database of clinical trial results.

15 STUDY ADMINISTRATION

15.1 STUDY LEADERSHIP

See Section 1 for key roles.

15.2 LIABILITY/INDEMNITY/INSURANCE

The study Sponsor will ensure sufficient insurance is available to enable it to indemnify and hold the investigator(s) and relevant staff as well as any hospital, institution, Ethics Committee or the like, harmless from any claims for damages for unexpected injuries, including death, that may be caused by the participant's participation in the study but only to the extent that the claim is not caused by the fault or negligence of the subjects or investigator(s). The Sponsor adheres to the guidelines of Medicines Australia for injury resulting from participation in a company sponsored trial, including the provision of 'No-fault clinical trial insurance'.

16 CONFLICT OF INTEREST POLICY

No conflicts of interest are applicable in this study.

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Appendix 1: Total whole blood volume in addition to Apheresis procedure

Procedure	**Sample**	**Volume per sample (mL)**	**No. samples per subject for the first 30 days**	**Total volume per subject (mL) for the first 30 days**
Laboratory Safety Assessment	Haematology (including G6PD)	2	4	8
		4	1	4
	Biochemistry (including LFT)	5	5	25
	Serology	3.5	0	0
	Safety Serum storage	5	1	5
Cannulation	Discard	2	8 (+2 if required)	16 (+4 if required)
Malaria Monitoring	Malaria qPCR (18S)	2	15 (+5 if required)	30 (+10 if required)
	Microscopy	2	0	0
	Parasite life-cycle stage qRT-PCR	2	5 (+3 if required)	10 (+6 if required)
Malaria Research	Immunology/ pathophysiology sample	20	0	0
MFA (in selected subjects but not those selected for HMP bank production)		Up to 80	Up to 1	80 (total over either 1 or 2 timepoints)
HMP bank blood borne infection testing (Appendix 7)		55	Up to 1	55
(in selected subjects but not those selected for MFA)				
Study Total (mL) no MFA no HMP Bank				98 (+20 if required)
Study Total (mL) MFA no HMP Bank				178 (+20 if required)
Study Total (mL) HMP Bank no MFA				up to - 153 (+20 if required)

Most subjects (not involved into membrane feeding or HMP bank procedures) will have 118 mL or less of whole blood taken during the first 30 days of the study (post screening) in addition to the 386 mL of red blood cells collected during the Apheresis procedure. An additional 20 mL may be collected on day 90±7.

Subjects involved in membrane feeding or HMP bank procedure will have 200 mL or less of whole blood taken during the first 30 days of the study (post screening) in addition to the 386 mL of red blood cells collected during the Apheresis procedure. An additional 75 mL may be collected on day 90±7.

Appendix 2: Symptoms and Signs of Malaria

Following challenge via the intravenous malaria challenge inoculation and during the post challenge period, the following signs and symptoms of malaria will be monitored:

Signs of Malaria

Fever (≥38°C)

Chills/Shivering/Rigors

Tachycardia

Hypotension

Symptoms of Malaria

Headache

Myalgia (muscle ache)

Arthralgia (joint ache)

Fatigue/lethargy

Malaise (general discomfort/uneasiness)

Sweating/hot spells

Anorexia

Nausea

Vomiting

Abdominal discomfort

Appendix 4: Antimalarial Rescue drugs approved manufacturer's prescribing information

Riamet® TGA July 2012

- Product Information
- Consumer Medicine Information

Appendix 5: Tubes for HMP bank blood borne infection testing

Tube Type and Number of Tubes	Tube Lot Number	Tube Expiry Date	Collection Time and Date	Operator initials
5 x 4 mL EDTA				
3 x 5 mL SST				
3 x 5 mL SST 4 x 5 mL PPT				

Appendix 6: Version History

Version	Date	Author(s)/Reviewer(s)	Significant Revisions
1.0	29 Aug 2017	A. Odedra	Initial version
1.1	17 Oct 2017	R. Watts/A. Odedra	Changed Principal Investigator to Prof. James McCarthy. Made changes in response to the RBWH HREC comments. See summary of changes document for more details.
2.0	1 Nov 2017	R. Watts/A. Odedra	Added an additional exploratory objective for antibody research. Added a baseline blood sample at Day 0 for immunology and pathophysiology malaria research. Changed blood volume for membrane feeding assays to 80 mL total over 1-2 time-points. See summary of changes document for more details.
2.1	22 March 2018	E. Rossignol/ A. Odedra	Addition of an additional coprincipal investigator

3.0	15 May 2018	E. Rossignol/ A. Odedra	- Added the possibility to reduce confinement to 48h if the subjects are deemed clinically well by the investigator and are happy to be released early. - Clarified that apheresis may occur on a day other than day 10. Earlier cohorts results have shown that, 24h before day 10, parasite levels may be much below the 20,000 parasites/mL threshold and the malaria score much below 6. - Added the possibility to collect samples more frequently for 18s PCR analysis from Day 9 to treatment day. - Added the possibility to confine subjects from day 9 until treatment day. - Clarified that the duration of stay at the apheresis unit may be longer than 4h (to allow preparation for the procedure).

Version	Date	Author(s)/Reviewer(s)	Significant Revisions
			- Clarified that immunology exploratory endpoints are optional. - Modified the inclusion criteria to exclude females. A very good venous access is required for the apheresis procedure and females often fail this criteria. It is deemed inappropriate to invite females for screening, considering the low likelihood that they may be included in the study. -Corrected the intravenous artesunate treatment duration. - Clarified that, before exit from confinement, nursing staff must confirm with the study doctor or investigator if any subject requires symptom-driven examination pre discharge.
3.1	9 July 2018	E. Rossignol/ A. Odedra	Addition of an additional coprincipal investigator
4.0	22 October 2018	E. Rossignol/ A. Odedra	Modification of the apheresis procedure and increase of the volume of red blood cells collected. Reduction of the volume of whole blood collected in compensation.
4.1	15 November 2018	E. Rossignol/ A. Odedra	Correct typos as per HREC request. Added the risk of low iron levels and fatigue in relation with the double red cell collection apheresis.

APPENDIX 3. MALARIA CLINICAL SCORE.

The malaria clinical score was calculated to quantify the clinical symptoms of malaria experienced by the subject. The following symptoms were scored as either absent (0), mild (1), moderate (2), or severe (3) at each specified timepoint to generate a total score (maximum score possible = 42).

- Headache
- Myalgia (muscle ache)
- Arthralgia (joint ache)
- Fatigue/lethargy
- Malaise (general discomfort/uneasiness)
- Chills/Shivering/Rigors
- Sweating/hot spells
- Anorexia
- Nausea
- Vomiting
- Abdominal discomfort
- Fever
- Tachycardia
- Hypotension

APPENDIX 4. APHERESIS COHORT 1 CLINICAL STANDARD OPERATING PROCEDURE.

1. Purpose

1.1. This SOP describes how to perform a continuous mononuclear cell (CMNC) procedure. It is intended to be used in conjunction with:

1.1.1. Spectra Optia Essentials

1.1.2. Spectra Optia Continuous Mononuclear Cell Collection (CMNC) Procedure Guide

2. Scope

2.1. The target audience for this resource are the registered nurses and medical officers who care for patients in the Apheresis Unit, Royal Brisbane & Women's Hospital (RBWH), who are involved and trained in apheresis.

2.2. Patient safety takes priority during a CMNC collection, therefore only competent nurses can perform this procedure.

2.3. All apheresis procedures will comply with 05009/WUG: Apheresis

2.4. All deviations and associated corrective actions shall be documented in the patient medical record.

3. Principle / Background

3.1. Apheresis refers to a number of procedures involving the removal of whole blood, separating it into the various blood components through centrifugation, removing a desired component (white/red cells, platelets and plasma with or without replacement fluids) and returning the rest of the components. Access to the intravascular compartment is required and efficacy of the treatment will depend on the volume of blood processed.

3.2. All patients requiring a CMNC collection are under the direct care of a Haematologist.

3.3. The RBWH Apheresis Unit is accredited by:

3.3.1. Australian Bone Marrow Donor Registry (ABMDR)

3.3.2. National Association of Testing Authorities (NATA)

3.3.3. Foundation for the Accreditation of Cellular Therapies (FACT)

3.4. The Royal Brisbane & Women's Hospital (RBWH) is accredited by the Australian Council on Healthcare Standards (ACHS)

3.5. The Disaster Plan (Section 11) addresses those elements most considered to be at risk and provides guidelines to cover some of the potential serious emergencies that may arise. All staff involved in apheresis procedures are responsible for following this plan as directed by senior personnel.

4. Objectives / Endpoints

4.1. All apheresis procedures shall be performed safely and appropriately.

5. Documentation

5.1. Policy and Standard/s

5.1.1. ACHS EQUiP National

5.1.2. Blood and Blood Products, Management of PROC103 Doc 110/15 (MNHHS)

5.1.3. Clinical Incident and Disclosure Management PROC007 Document 13/14 (MNHHS)

5.2. Procedures, Guidelines, Protocols

5.2.1. Australian Guidelines for the Prevention and Control of Infection in Healthcare (2010)

5.2.2. COLGDE001: RBWH Guidelines in the Management of Potential Adverse Events in Apheresis

5.2.3. 02002/Proc: Medications Management

5.2.4. 21605/Proc: Blood collection (Adult)

5.2.5. 05009/Proc: Apheresis

5.2.6. 05450/Proc: Peripheral Intravenous Cannulation, Venepuncture and Infusions – Adult and Paediatrics

5.2.7. 74100/Proc: Documentation in the Patient Record

5.2.8. 81000/Proc: Aseptic Non Touch Technique

5.2.9. 000342/Proc: Standard Precautions

5.2.10. 003139/Proc: Cleaning and Decontamination – patient environment and clinical equipment

5.3. Forms and Templates

5.3.1. CLIFRM013: RBWH Donor Consent for Bone Marrow Harvest or Apheresis Procedure

5.3.2. COLFRM009: RBWH Apheresis Patient Checklist label

5.3.3. COLFRM028: RBWH Apheresis Service Request

5.3.4. COLFRM030: RBWH Apheresis Product Collection Form

5.3.5. COLFRM053: RBWH Apheresis Procedure Run Sheet: Spectra Optia

6. Definitions and Abbreviations

6.1. AIM Automated interface management

6.2. ABMDR Australian Bone Marrow Donor Registry

6.3. ACD-A Acid citrate dextrose - solution A

6.4. BTS Biomedical Technical Services

6.5. CMNC Continuous mononuclear cell

6.6. CMV Cytomegalovirus

6.7. CP Collection preference

6.8. ELFT Electrolyte & liver function tests

6.9. FBC Full blood count

6.10. Hb Haemoglobin

6.11. HBV Hepatitis B virus

6.12. HCV Hepatitis C virus

6.13. HIV Human immunodeficiency virus

6.14. HTLV Human T-lymphotropic virus

6.15. IDM Infectious disease marker

6.16. IV Intravenous

6.17. MNC Mononuclear cell

6.18. MNHHS Metro North Hospital and Health Service

6.19. NUM Nurse Unit Manager

6.20. ODTU Oncology Day Therapy Unit

6.21. RBWH Royal Brisbane & Women's Hospital

6.22. RN Registered nurse

6.23. SOP Standard operating procedure

6.24. TBV Total blood volume

7. Materials

7.1. Equipment

7.1.1. Spectra Optia Apheresis System (version 11.3) – CMNC Collection.

7.1.2. IDL filler

7.1.3. Electronic blood pressure monitor

7.1.4. Volumetric infusion pump

7.1.5. Heat sealer

7.2. Disposables and Reagents

7.2.1. Spectra Optia IDL Set (Ref: 10310).

7.2.2. ACD-A anticoagulant (750ml)

7.2.3. 0.9% normal saline (1000ml)

7.2.4. Alcohol swab

7.2.5. 3 way taps (2)

7.2.6. IV cannulation or central line equipment. Refer to procedures: 05450/Proc: Peripheral Intravenous Cannulation, Venepuncture and Infusions – Adult and Paediatrics; 05501/Proc: Cannulation of Haemodialysis Access; 05600/Proc: Central Venous Access Devices (CVADs), Management – Adult. Ensure use of sufficient gauge cannulas (backeye needles are preferable for inlet venous access) to allow for adequate flow required for the procedure (preferably at least 16G for inlet and 18G for return)

7.2.7. Blood collection tubes

7.2.8. Intravenous giving set

7.2.9. Burette

8. Special Considerations

8.1. This procedure describes how to perform a CMNC procedure.

8.2. Ensure haematocrit is accurate, as this is used to calculate:

8.2.1. The limit for collect volumes

8.2.2. The plasma pump flow rate before the AIM system manages the concentration of cells in the collect port

8.2.3. Changing the haematocrit after the interface has been established will not change the interface position

8.3. The packing factor whilst collecting MNC defaults to 4.5

8.4. No plasma is to be collected

8.5. There MUST be FBC and ELFT blood test results available (taken within the previous 24hrs) PRIOR to commencement of procedure.

8.6. The minimum FBC criteria required in order to proceed with the procedure is described below.

QIMR volunteer	Platelet $\geq 100 \times 10^9$/L

	Hb ≥ 100G/L

8.7. There is a risk of deranged electrolytes due to the effects of the procedure. This effect is usually transient and resolves quickly upon cessation of CMNC collection. Electrolyte replacement may be required. Calcium gluconate is the most frequently required electrolyte replacement and the amount and rate is dependent on symptoms of hypocalcaemia during the procedure. Magnesium and potassium replacement can also be required, below is a guide for when supplementation may be required:

Pre-Procedure Electrolyte	Replacement guide
Magnesium < 60 mmol/L	10 – 20 mmols IV magnesium sulphate may be required during procedure
Potassium between 3.0 – 3.5 mmol/L	Oral potassium may be required e.g. 1-2 chlorvescent tablets
Potassium < 3.0 mmol	IV potassium chloride 20 mmols may be required during the procedure and / or 3 oral chlorvescent

9. Procedure

9.1. Confirm the following forms are current and valid:

9.1.1. CLIFRM013: RBWH Donor Consent for Bone Marrow harvest or Apheresis Procedure

9.1.2. COLFRM028: RBWH Apheresis Service Request

9.2. Prior to commencement of procedure, ensure patient has had appropriate IDMs taken.

9.2.1. HIV serology

9.2.2. Hepatitis B serology

9.2.3. Hepatitis C serology

9.2.4. Syphilis

9.3. All stock used to collect the product must be visually examined prior to use for signs of damage and contamination, as per COLSOP004: Apheresis Inventory Control Procedure. Record expiry date and lot number of all stock used on COLFRM030: RBWH Apheresis Product Collection form. Check expiry dates and lot numbers of procedure kit and intravenous fluid bags with 2nd RN and record on

COLFRM053: RBWH Apheresis Procedure Run Sheet: Spectra Optia

9.3.1. Confirm that all consumables and reagents used during the procedure are satisfactory for use; inspect for damage or evidence of contamination and mark the appropriate check box on COLFRM030: RBWH Apheresis Product Collection form

9.4. Set up, load and prime Spectra Optia IDL Set (Ref: 10310) as per on screen instructions

9.5. Follow machine prompts and enter patient data.

9.6. Educate patient of signs and symptoms of potential adverse reactions, emphasising importance of informing nursing staff if he/she experiences any such reactions

9.7. Perform baseline observations (temperature, pulse, respirations and blood pressure) and record on run sheet

9.8. Ensure COLFRM009: RBWH Apheresis Patient Checklist has been completed

9.9. Perform Cannulation according to 05450/Proc: Peripheral Intravenous Cannulation, Venepuncture and Infusions – Adult and Paediatrics

9.10. Collect blood samples if required.

9.11. Follow machine prompts to commence procedure

9.12. Review run screen to ensure pump flow rates are appropriate for the procedure. Monitor the AC infusion rate, ensuring that it does not exceed 0.9 ml/min

9.13. The Spectra Optia system will attempt to establish the interface at commencement of the procedure, during this time, the collect valve will remain closed.

9.14. The AIM system monitors the collect port and changes the plasma pump flow rate to manage the concentration of cells that flow through the collect port. When the AIM system detects cells in the collect port, the collect valve opens and starts collecting. Ensure the collect flow rate is set at 3ml/min

9.15. The CP defaults to 50 at commencement of procedure. The apheresis nurse can set the CP to collect lighter or darker from the buffy coat. Use the collection preference tool as a guide for the appropriate CP.

9.15.1. See appendix 1 for the appropriate CP, using the collection preference tool.

9.15.2. A higher CP results in collecting lighter in the buffy coat

9.15.3. A lower CP results in collecting darker in the buffy coat

9.16. Monitor and record patient's vital signs and run parameters q30 minutes or as clinically indicated. Monitor for signs of adverse reactions and record on procedure run sheet, reporting adverse events on PRIME.

9.17. Ensure patient comfort and safety throughout the procedure.

9.18. Apply a patient ID label to the collection bag. THE LABELS MUST BE APPLIED TO THE COLLECT BAG BEFORE THE PATIENT OR THE COLLECT BAG ARE DISCONNECTED FROMTHE PROCEDURE KIT.

9.19. At the end of the procedure, follow the system prompts to rinse back

9.20. Seal the collection bags using a heat sealer prior to disconnection of the patient. Ensure that there is approximately 20cm of sterile dockable tubing remaining after heat sealing.

9.21. The RN performing the collections shall record the date, time of completion and sign the collection bag label. Verify the identity of the patient with the patient ID label.

9.22. Contact QIMR to collect the cells. The time of collection completion shall be recorded on COLFRM030: RBWH Apheresis Product Collection form. The collection bags must be placed in a sealed plastic bag for transport to QIMR.

9.23. Release of the product from the Apheresis nurse to the QIMR scientist is recorded on COLFRM030: RBWH Apheresis Product Collection form. The Apheresis nurse releasing the collected product, must sign (stating time and date) that the product has been released, the QIMR scientist accepting the collected product must sign (stating date and time) that they have accepted product quarantine. Glove must be worn by all staff handling the product.

9.24. On completion of rinseback, collect routine blood samples (usually FBC and ELFT).

9.25. Perform final set of observations and record on procedure run sheet

9.26. Remove venous access as appropriate

9.27. Record final values on COLFRM053: RBWH Apheresis Procedure Run Sheet and complete COLFRM030: RBWH Apheresis Product Collection form.

9.28. Once paperwork is complete, scan / photocopy all procedural paperwork and send to QIMR.

9.29. Unload set and dispose of waste appropriately

9.30. Decontaminate Spectra Optia as per protocol (see COLSOP005: RBWH Apheresis Equipment Cleaning Procedure). Decontaminate all other equipment involved in performing the procedure, in accordance with 003139/Proc: Cleaning and Decontamination – patient environment and clinical equipment. Once all equipment used during the procedure has been

cleaned, tick appropriate area at the end of the run sheet, confirming completion of cleaning tasks.

9.31. Document all care given in patient medical notes. Include all completed paperwork with patient's medical notes.

9.32. Record relevant statistical information in: G:\Oncology\Apheresis\Log and Statistics

10. End Points

10.1. A maximum of 500mls is to be collected, unless clinically indicated

11. Disaster Plan

11.1. This section describes the actions to be taken in the event of serious problems which may arise in the Apheresis Unit which may impact on either:

11.1.1. The ability to perform the CMNC collection to the required specification 11.1.2. The quality of the collected product

11.2. Air-Conditioning System Failure – in the event that the air-conditioning system malfunctions, contact hospital maintenance and arrange for urgent repairs (ext. 67963).

11.3. Loss of Power – All critical equipment used to perform apheresis procedures should be connected to red emergency power points. These power points are supplied with the electricity from the emergency backup power generators, and in the event of a power failure, are automatically activated.

11.3.1. Critical equipment used within the apheresis unit includes:

11.3.1.1. Cell separators

11.3.1.2. Blood warmers

11.3.1.3. Electronic blood pressure monitors

11.3.1.4. Intravenous volumetric pumps

11.3.1.5. Heat sealers

11.3.2. In the event of an interruption to normal power supply, there may be a temporary loss of power before the emergency generator is activated. If there is an interruption of the power supply:

11.3.2.1. Close clamps on collect line immediately

11.3.2.2. If the emergency power supply is activated, power will resume, and the Spectra Optia system will reset. After system resets, a screen will appear with instructions for how to proceed.

11.3.2.3. To restart the procedure, follow onscreen instructions

11.3.2.4. To abort procedure, press RINSEBACK (if appropriate) or DISCONNECT and follow on screen instructions

11.3.3. In the event of a complete loss of power with no emergency power supply:

11.3.3.1. The Spectra Optia cell separator will shut down completely. Close clamps on collect line immediately. Seal the collection bags using a heat sealer (these should still be functional if battery charged)

11.3.3.2. Contact the patient physician and inform him of events and that the procedure may be aborted

11.3.3.3. Consult with the Director of the Haematology & BMT

11.3.3.4. The extracorporeal volume in the kit will be 297ml

11.3.3.5. The patient may be disconnected without performing RINSEBACK or perform MANUAL RINSEBACK as per instructions in Spectra Optia Essentials Guide, pp.73-76 (in the event that a blood prime was performed, RINSEBACK is not necessary)

11.4. Immediate evacuation – in the event that an immediate evacuation is required:

11.4.1. Close clamps on collect line if possible. NURSE AND PATIENT SAFETY IS PARAMOUNT, therefore do not complete this step if completion may endanger the health and safety of the nurse or the patient

11.4.2. Disconnect patient immediately and evacuate as per RBWH Code Orange Evacuation procedure. N.B. since RINSEBACK has not been performed, the patient will have a 297ml fluid deficit and may become hypotensive, monitor patient accordingly

11.5. Structural damage (e.g. fire, water / flood damage) – in the event of structural damage, relocate product, material and patient files as required, to another area of ODTU if possible. Depending on the scale of the problem, a final decision on a temporary location may need to be determined on a case-by-case assessment by a structural engineer and the Director of the BMT program

11.5.1. All critical equipment and/or consumables which may have incurred damage should be discarded and replaced as soon as possible

11.5.2. Contact hospital maintenance (ext. 67963) and organise for them to inspect critical equipment as soon as possible

11.5.3. Suspend all operational activities until repairs are complete and the facility is signed off as safe to reoccupy

11.6. Critical equipment failure – Perform troubleshooting as per relevant equipment manuals. If unable to resolve problem, contact BTS or manufacturer representative.

11.7. Critical consumable shortage – if a critical consumable shortage occurs, contact the following Apheresis Units to source consumables:

11.7.1. Greenslopes Private Hospital (Speed dial *2521).

11.7.2. Lady Cilento Childrens' Hospital (Jo Ritchie – 3068 5686, or Jill Shergold – 3068 5767)

11.7.3. Once alternative supply has been sourced, organise transport of the

required consumables. Liaise with the NUM, ODTU to organise payment of delivery e.g. courier service

11.8. Critical staff shortage – The Apheresis Nurse Practitioner shall liaise with the NUM, ODTU regularly to ensure staffing requirements of the apheresis unit are met. In the event that key personnel are unavailable to perform critical procedures:

11.8.1. Contact Apheresis Nurse Practitioner (pager# 26299) and NUM, ODTU (ext. 68754 / 73991) informing them of the situation

11.8.2. The NUM / Apheresis Nurse Practitioner will attempt to adequately staff the Apheresis Unit

11.8.3. If appropriate, the situation can be escalated to the Director of the BMT program and the Nursing Director, CCS

11.8.4. If adequate staffing cannot be accomplished, the Apheresis Nurse Practitioner shall liaise with the Director of the BMT Program to discuss requesting the assistance of other apheresis units in the area. Alternative units may be affiliated with the RBWH e.g. Greenslopes Private Hospital, or other similarly accredited Apheresis Units e.g. Princess Alexandra Hospital or the Mater Public Hospital

12. Responsibility

	Final Approval	Implement	Quality Assurance	Review	Perform Procedure
Apheresis Nurse Practitioner		X	X	X	X
Medical Director	X	X			
Nursing Staff					X

13. Occupational Health and Safety

13.1. Standard precautions shall be maintained throughout the procedure. Refer to 000342/Proc: Standard Precautions.

14. Risk

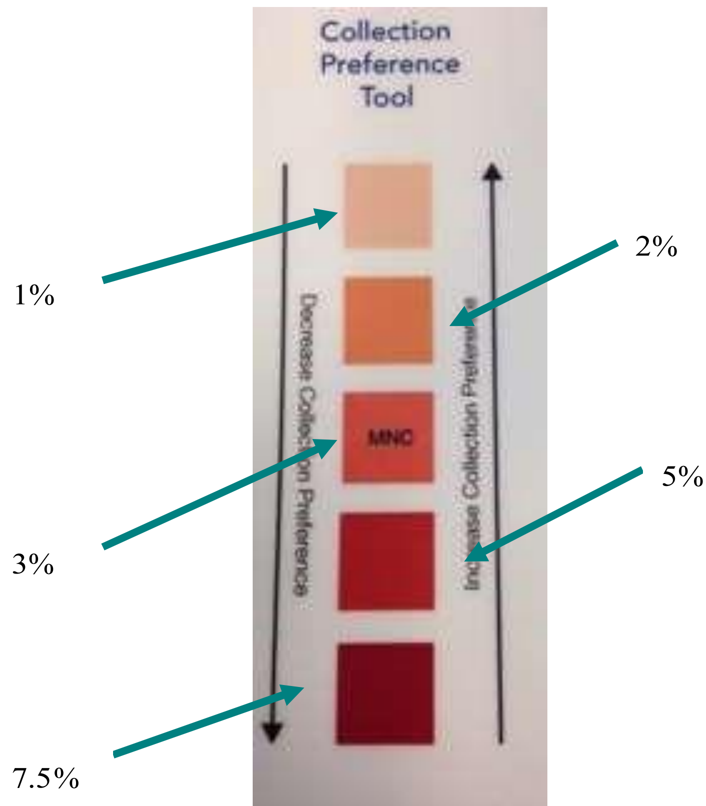
14.1. Low to Medium (case dependant).

15. Consultation

15.1. Director Bone Marrow Transplant Glen Kennedy

15.2. Apheresis Nurse Practitioner Kari Mudie

Appendix 1 – Collection preference guide



APPENDIX 5. APHERESIS COHORT 2 CLINICAL STANDARD OPERATING PROCEDURE.

1. Purpose

1.1. This SOP describes how to perform a continuous mononuclear cell (CMNC) procedure. It is intended to be used in conjunction with:

1.1.1. Spectra Optia Essentials

1.1.2. Spectra Optia Continuous Mononuclear Cell Collection (CMNC) Procedure Guide

2. Scope

2.1. The target audience for this resource are the registered nurses and medical officers who care for patients in the Apheresis Unit, Royal Brisbane & Women's Hospital (RBWH), who are involved and trained in apheresis.

2.2. Patient safety takes priority during a CMNC collection, therefore only competent nurses can perform this procedure.

2.3. All apheresis procedures will comply with 05009/WUG: Apheresis

2.4. All deviations and associated corrective actions shall be documented in the patient medical record.

3. Principle / Background

3.1. Apheresis refers to a number of procedures involving the removal of whole blood, separating it into the various blood components through centrifugation, removing a desired component (white/red cells, platelets and plasma with or without replacement fluids) and returning the rest of the components. Access to the intravascular compartment is required and efficacy of the treatment will depend on the volume of blood processed.

3.2. All patients requiring a CMNC collection are under the direct care of a Haematologist.

3.3. The RBWH Apheresis Unit is accredited by:

3.3.1. Australian Bone Marrow Donor Registry (ABMDR)

3.3.2. National Association of Testing Authorities (NATA)

3.3.3. Foundation for the Accreditation of Cellular Therapies (FACT)

3.4. The Royal Brisbane & Women's Hospital (RBWH) is accredited by the Australian Council on Healthcare Standards (ACHS)

3.5. The Disaster Plan (Section 11) addresses those elements most considered to be at risk and provides guidelines to cover some of the potential serious emergencies that may arise. All staff involved in apheresis procedures are responsible for following this plan as directed by senior personnel.

4. Objectives / Endpoints

4.1. All apheresis procedures shall be performed safely and appropriately.

5. Documentation

5.1. Policy and Standard/s

5.1.1. ACHS EQUiP National

5.1.2. Blood and Blood Products, Management of PROC103 Doc 110/15 (MNHHS)

5.1.3. Clinical Incident and Disclosure Management PROC007 Document 13/14 (MNHHS)

5.2. Procedures, Guidelines, Protocols

5.2.1. Australian Guidelines for the Prevention and Control of Infection in Healthcare (2010)

5.2.2. COLGDE001: RBWH Guidelines in the Management of Potential Adverse Events in Apheresis

5.2.3. 02002/Proc: Medications Management

5.2.4. 21605/Proc: Blood collection (Adult)

5.2.5. 05009/Proc: Apheresis

5.2.6. 05450/Proc: Peripheral Intravenous Cannulation, Venepuncture and Infusions – Adult and Paediatrics

5.2.7. 74100/Proc: Documentation in the Patient Record

5.2.8. 81000/Proc: Aseptic Non Touch Technique

5.2.9. 000342/Proc: Standard Precautions

5.2.10. 003139/Proc: Cleaning and Decontamination – patient environment and clinical equipment

5.3. Forms and Templates

5.3.1. CLIFRM013: RBWH Donor Consent for Bone Marrow Harvest or Apheresis Procedure

5.3.2. COLFRM009: RBWH Apheresis Patient Checklist label

5.3.3. COLFRM028: RBWH Apheresis Service Request

5.3.4. COLFRM030: RBWH Apheresis Product Collection Form

5.3.5. COLFRM053: RBWH Apheresis Procedure Run Sheet: Spectra Optia

6. Definitions and Abbreviations

6.1. AIM Automated interface management

6.2. ABMDR Australian Bone Marrow Donor Registry

6.3. ACD-A Acid citrate dextrose - solution A

6.4. BTS Biomedical Technical Services

6.5. CMNC Continuous mononuclear cell

6.6. CMV Cytomegalovirus

6.7. CP Collection preference

6.8. ELFT Electrolyte & liver function tests

6.9. FBC Full blood count

6.10. Hb Haemoglobin

6.11. HBV Hepatitis B virus

6.12. HCV Hepatitis C virus

6.13. HIV Human immunodeficiency virus

6.14. IDM Infectious disease marker

6.15. IV Intravenous

6.16. MNC Mononuclear cell

6.17. MNHHS Metro North Hospital and Health Service

6.18. NUM Nurse Unit Manager

6.19. ODTU Oncology Day Therapy Unit

6.20. QIMR Queensland Institute of Medical Research

6.21. RBWH Royal Brisbane & Women's Hospital

6.22. RN Registered nurse

6.23. SOP Standard operating procedure

6.24. TBV Total blood volume

7. Materials

7.1. Equipment

7.1.1. Spectra Optia Apheresis System (version 11.3) – CMNC Collection.

7.1.2. IDL filler

7.1.3. Electronic blood pressure monitor

7.1.4. Volumetric infusion pump

7.1.5. Heat sealer

7.2. Disposables and Reagents

7.2.1. Spectra Optia IDL Set (Ref: 10310).

7.2.2. ACD-A anticoagulant (750ml)

7.2.3. 0.9% normal saline (1000ml)

7.2.4. Alcohol swab

7.2.5. 3 way taps (2)

7.2.6. IV cannulation or central line equipment. Refer to procedures: 05450/Proc: Peripheral Intravenous Cannulation, Venepuncture and Infusions – Adult and Paediatrics; 05501/Proc: Cannulation of Haemodialysis Access; 05600/Proc: Central Venous Access Devices (CVADs), Management – Adult. Ensure use of sufficient gauge cannulas (backeye needles are preferable for inlet venous access) to allow for adequate flow required for the procedure (preferably at least 16G for inlet and 18G for return)

7.2.7. Blood collection tubes

7.2.8. Intravenous giving set

7.2.9. Burette

8. Special Considerations

8.1. This procedure describes how to perform a CMNC procedure.

8.2. Ensure haematocrit is accurate, as this is used to calculate:

8.2.1. The limit for collect volumes

8.2.2. The plasma pump flow rate before the AIM system manages the concentration of cells in the collect port

8.2.3. Changing the haematocrit after the interface has been established will not change the interface position

8.3. The packing factor whilst collecting MNC defaults to 4.5

8.4. No plasma is to be collected

8.5. There MUST be FBC and ELFT blood test results available (taken within the previous 24hrs) PRIOR to commencement of procedure.

8.6. The minimum FBC criteria required in order to proceed with the procedure is described below.

QIMR volunteer	Platelet $\geq 100 \times 10^9$/L
	Hb $\geq$ 100G/L

8.7. There is a risk of deranged electrolytes due to the effects of the procedure. This effect is usually transient and resolves quickly upon cessation of CMNC collection. Electrolyte replacement may be required. Calcium gluconate is the most frequently required electrolyte replacement and the amount and rate is dependent on symptoms of hypocalcaemia during the procedure. Magnesium and potassium replacement can also be required, below is a guide for when supplementation may be required:

Pre-Procedure Electrolyte	Replacement guide
Magnesium < 60 mmol/L	10 – 20 mmols IV magnesium sulphate may be required during procedure
Potassium between 3.0 – 3.5 mmol/L	Oral potassium may be required e.g. 1-2 chlorvescent tablets
Potassium < 3.0 mmol	IV potassium chloride 20 mmols may be required during the procedure and / or 3 oral chlorvescent

9. Procedure

9.1. Confirm the following forms are current and valid:

9.1.1. CLIFRM013: RBWH Donor Consent for Bone Marrow harvest or Apheresis Procedure

9.1.2. COLFRM028: RBWH Apheresis Service Request

9.2. Prior to commencement of procedure, ensure patient has had appropriate IDMs taken.

9.2.1. HIV serology

9.2.2. Hepatitis B serology

9.2.3. Hepatitis C serology

9.2.4. Syphilis

9.3. All stock used to collect the product must be visually examined prior to use for signs of damage and contamination, as per COLSOP004: Apheresis Inventory Control Procedure. Record expiry date and lot number of all stock used on COLFRM030: RBWH Apheresis Product Collection form. Check expiry dates and lot numbers of procedure kit and intravenous fluid bags with 2nd RN and record on

COLFRM053: RBWH Apheresis Procedure Run Sheet: Spectra Optia

9.3.1. Confirm that all consumables and reagents used during the procedure are satisfactory for use; inspect for damage or evidence of contamination and mark the appropriate check box on COLFRM030: RBWH Apheresis Product Collection form

9.4. Set up, load and prime Spectra Optia IDL Set (Ref: 10310) as per on screen instructions

9.5. Follow machine prompts and enter patient data.

9.6. Educate patient of signs and symptoms of potential adverse reactions, emphasising importance of informing nursing staff if he/she experiences any such reactions

9.7. Perform baseline observations (temperature, pulse, respirations and blood pressure) and record on run sheet

9.8. Ensure COLFRM009: RBWH Apheresis Patient Checklist has been completed

9.9. Perform Cannulation according to 05450/Proc: Peripheral Intravenous Cannulation, Venepuncture and Infusions – Adult and Paediatrics

9.10. Collect blood samples if required.

9.11. Follow machine prompts to commence procedure

9.12. Review run screen to ensure pump flow rates are appropriate for the procedure. Monitor the AC infusion rate, ensuring that it does not exceed 0.9 ml/min

9.13. The Spectra Optia system will attempt to establish the interface at commencement of the procedure, during this time, the collect valve will remain closed.

9.14. The AIM system monitors the collect port and changes the plasma pump flow rate to manage the concentration of cells that flow through the collect port. When the AIM system detects cells in the collect port, the collect valve opens and starts collecting. Ensure the collect flow rate is set at 3ml/min

9.15. The CP defaults to 50 at commencement of procedure. The apheresis nurse can set the CP to collect lighter or darker from the buffy coat. Use the collection preference tool as a guide for the appropriate CP.

9.15.1. See appendix 1 for the appropriate CP, using the collection preference tool.

9.15.2. A higher CP results in collecting lighter in the buffy coat

9.15.3. A lower CP results in collecting darker in the buffy coat

9.16. Monitor and record patient's vital signs and run parameters q30 minutes or as clinically indicated. Monitor for signs of adverse reactions and record on procedure run sheet, reporting adverse events on PRIME.

9.17. Ensure patient comfort and safety throughout the procedure.

9.18. Apply a patient ID label to the collection bag. THE LABELS MUST BE APPLIED TO THE COLLECT BAG BEFORE THE PATIENT OR THE COLLECT BAG ARE DISCONNECTED FROMTHE PROCEDURE KIT.

9.19. At the end of the procedure, follow the system prompts to rinseback

9.20. Seal the collection bags using a heat sealer prior to disconnection of the patient. Ensure that there is approximately 20cm of sterile dockable tubing remaining after heat sealing.

9.21. The RN performing the collections shall record the date, time of completion and sign the collection bag label. Verify the identity of the patient with the patient ID label.

9.22. Contact QIMR to collect the cells. The time of collection completion shall be recorded on COLFRM030: RBWH Apheresis Product Collection form. The collection bags must be placed in a sealed plastic bag for transport to QIMR.

9.23. Release of the product from the Apheresis nurse to the QIMR scientist is recorded on COLFRM030: RBWH Apheresis Product Collection form. The Apheresis nurse releasing the collected product, must sign (stating time and date) that the product has been released, the QIMR scientist accepting the collected product must sign (stating date and time) that they have accepted product quarantine. Glove must be worn by all staff handling the product.

9.24. On completion of rinseback, collect routine blood samples (usually FBC and ELFT).

9.25. Perform final set of observations and record on procedure run sheet

9.26. Remove venous access as appropriate

9.27. Record final values on COLFRM053: RBWH Apheresis Procedure Run Sheet and complete COLFRM030: RBWH Apheresis Product Collection form.

9.28. Once paperwork is complete, scan / photocopy all procedural paperwork and send to QIMR.

9.29. Unload set and dispose of waste appropriately

9.30. Decontaminate Spectra Optia as per protocol (see COLSOP005: RBWH Apheresis Equipment Cleaning Procedure). Decontaminate all other equipment involved in performing the procedure, in accordance with

003139/Proc: Cleaning and Decontamination – patient environment and clinical equipment. Once all equipment used during the procedure has been cleaned, tick appropriate area at the end of the run sheet, confirming completion of cleaning tasks.

9.31. Document all care given in patient medical notes. Include all completed paperwork with patient's medical notes.

9.32. Record relevant statistical information in: G:\Oncology\Apheresis\Log and Statistics

10. End Points

10.1. QIMR will inform the apheresis nursing staff of the target collect volume which will not exceed 450ml

10.1.1. QIMR may request a specified volume of red cells be added to collection at end of procedure. The volume of red cells collected must not be more than 162ml and must be included in the maximum target collect volume of 450ml

10.1.1.1. The total collect volume must not exceed 450ml

11. Disaster Plan

11.1. This section describes the actions to be taken in the event of serious problems which may arise in the Apheresis Unit which may impact on either:

11.1.1. The ability to perform the CMNC collection to the required specification 11.1.2. The quality of the collected product

11.2. Air-Conditioning System Failure – in the event that the air-conditioning system malfunctions, contact hospital maintenance and arrange for urgent repairs (ext. 67963).

11.3. Loss of Power – All critical equipment used to perform apheresis procedures should be connected to red emergency power points. These power points are supplied with the electricity from the emergency backup power generators, and in the event of a power failure, are automatically activated.

11.3.1. Critical equipment used within the apheresis unit includes:

11.3.1.1. Cell separators

11.3.1.2. Blood warmers

11.3.1.3. Electronic blood pressure monitors

11.3.1.4. Intravenous volumetric pumps

11.3.1.5. Heat sealers

11.3.2. In the event of an interruption to normal power supply, there may be a temporary loss of power before the emergency generator is activated. If there is an interruption of the power supply:

11.3.2.1. Close clamps on collect line immediately

11.3.2.2. If the emergency power supply is activated, power will resume, and the Spectra Optia system will reset. After system resets, a screen will appear with instructions for how to proceed.

11.3.2.3. To restart the procedure, follow onscreen instructions

11.3.2.4. To abort procedure, press RINSEBACK (if appropriate) or DISCONNECT and follow on screen instructions

11.3.3. In the event of a complete loss of power with no emergency power supply:

11.3.3.1. The Spectra Optia cell separator will shut down completely. Close clamps on collect line immediately. Seal the collection bags using a heat sealer (these should still be functional if battery charged)

11.3.3.2. Contact the patient physician and inform him of events and that the procedure may be aborted

11.3.3.3. Consult with the Director of the Haematology & BMT

11.3.3.4. The extracorporeal volume in the kit will be 297ml

11.3.3.5. The patient may be disconnected without performing RINSEBACK or perform MANUAL RINSEBACK as per instructions in Spectra Optia Essentials Guide, pp.73-76 (in the event that a blood prime was performed, RINSEBACK is not necessary)

11.4. Immediate evacuation – in the event that an immediate evacuation is required:

11.4.1. Close clamps on collect line if possible. NURSE AND PATIENT SAFETY IS PARAMOUNT, therefore do not complete this step if completion may endanger the health and safety of the nurse or the patient

11.4.2. Disconnect patient immediately and evacuate as per RBWH Code Orange Evacuation procedure. N.B. since RINSEBACK has not been performed, the patient will have a 297ml fluid deficit and may become hypotensive, monitor patient accordingly

11.5. Structural damage (e.g. fire, water / flood damage) – in the event of structural damage, relocate product, material and patient files as required, to another area of ODTU if possible. Depending on the scale of the problem, a final decision on a temporary location may need to be determined on a case-by-case assessment by a structural engineer and the Director of the BMT program

11.5.1. All critical equipment and/or consumables which may have incurred damage should be discarded and replaced as soon as possible

11.5.2. Contact hospital maintenance (ext. 67963) and organise for them to inspect critical equipment as soon as possible

11.5.3. Suspend all operational activities until repairs are complete and the facility is signed off as safe to reoccupy

11.6. Critical equipment failure – Perform troubleshooting as per relevant equipment manuals. If unable to resolve problem, contact BTS or manufacturer representative.

11.7. Critical consumable shortage – if a critical consumable shortage occurs, contact the following Apheresis Units to source consumables:

11.7.1. Greenslopes Private Hospital (Speed dial *2521).

11.7.2. Lady Cilento Childrens' Hospital (Jo Ritchie – 3068 5686, or Jill Shergold – 3068 5767)

11.7.3. Once alternative supply has been sourced, organise transport of the required consumables. Liaise with the NUM, ODTU to organise payment of delivery e.g. courier service

11.8. Critical staff shortage – The Apheresis Nurse Practitioner shall liaise with the NUM, ODTU regularly to ensure staffing requirements of the apheresis unit are met. In the event that key personnel are unavailable to perform critical procedures:

11.8.1. Contact Apheresis Nurse Practitioner (pager# 26299) and NUM, ODTU (ext. 68754 / 73991) informing them of the situation

11.8.2. The NUM / Apheresis Nurse Practitioner will attempt to adequately staff the Apheresis Unit

11.8.3. If appropriate, the situation can be escalated to the Director of the BMT program and the Nursing Director, CCS

11.8.4. If adequate staffing cannot be accomplished, the Apheresis Nurse Practitioner shall liaise with the Director of the BMT Program to discuss requesting the assistance of other apheresis units in the area. Alternative units may be affiliated with the RBWH e.g. Greenslopes Private Hospital, or other similarly accredited Apheresis Units e.g. Princess Alexandra Hospital or the Mater Public Hospital

12. Responsibility

	Final Approval	Implement	Quality Assurance	Review	Perform Procedure
Apheresis Nurse Practitioner		X	X	X	X
Medical Director	X	X			
Nursing Staff					X

13. Occupational Health and Safety

13.1. Standard precautions shall be maintained throughout the procedure. Refer to 000342/Proc: Standard Precautions.

14. Risk

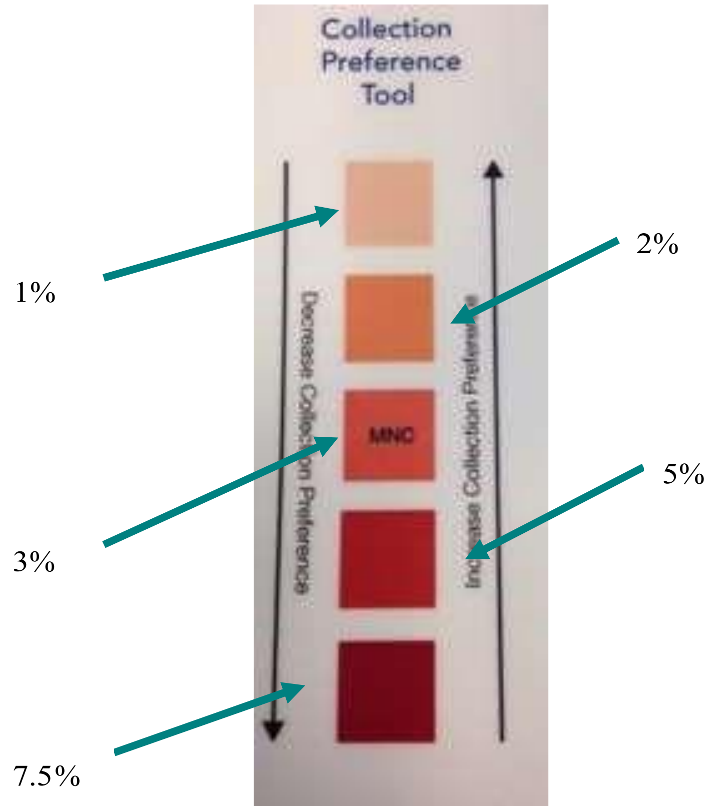
14.1. Low to Medium (case dependant).

15. Consultation

15.1. Director Bone Marrow Transplant Glen Kennedy

15.2. Apheresis Nurse Practitioner Kari Mudie

Appendix 1 – Collection preference guide


Collection
Preference
Tool
1%
2%
MNC
3%
5%
7.5%
Decrease Collection Preference

APPENDIX 6. APHERESIS COHORT 3 CLINICAL STANDARD OPERATING PROCEDURE.

1. Purpose

1.1. This SOP describes the apheresis procedures used for this study. It is intended to be used in conjunction with:

1.1.1. Spectra Optia Essentials

2. Scope

2.1. The target audience for this resource are the registered nurses and medical officers who care for patients in the Apheresis Unit, Royal Brisbane & Women's Hospital (RBWH), who are involved and trained in apheresis.

2.2. Patient safety takes priority during all apheresis procedures, therefore only competent nurses can perform this procedure.

2.3. All apheresis procedures will comply with 05009/WUG: Apheresis

2.4. All deviations and associated corrective actions shall be documented in the patient medical record.

3. Principle / Background

3.1. Apheresis refers to a number of procedures involving the removal of whole blood, separating it into the various blood components through centrifugation, removing a desired component (white/red cells, platelets and plasma with or without replacement fluids) and returning the rest of the components. Access to the intravascular compartment is required and efficacy of the treatment will depend on the volume of blood processed.

3.2. This apheresis procedure will consist of 2 stages:

3.2.1. Stage 1: RBC Depletion of QIMR volunteer

3.2.2. Stage 2: PMN procedure of concentrated red blood cell product

3.3. During a red cell depletion, red blood cells are removed from the patient until the target haematocrit has been attained, and replaced with desired replacement fluid

3.4. All patients requiring an apheresis procedure are under the direct care of a Haematologist.

3.5. The RBWH Apheresis Unit is accredited by:

3.5.1. Australian Bone Marrow Donor Registry (ABMDR)

3.5.2. National Association of Testing Authorities (NATA)

3.5.3. Foundation for the Accreditation of Cellular Therapies (FACT)

3.6. The Royal Brisbane & Women's Hospital (RBWH) is accredited by the Australian Council on Healthcare Standards (ACHS)

3.7. The Disaster Plan (Section 11) addresses those elements most considered to be at risk and provides guidelines to cover some of the potential serious emergencies that may arise. All staff involved in apheresis procedures are responsible for following this plan as directed by senior personnel.

4. Objectives / Endpoints

4.1. All apheresis procedures shall be performed safely and appropriately.

5. Documentation

5.1. Policy and Standard/s

5.1.1. ACHS EQUiP National

5.1.2. Blood and Blood Products, Management of PROC103 Doc 110/15 (MNHHS)

5.1.3. Clinical Incident and Disclosure Management PROC007 Document 13/14 (MNHHS)

5.2. Procedures, Guidelines, Protocols

5.2.1. Australian Guidelines for the Prevention and Control of Infection in Healthcare (2010)

5.2.2. COLGDE001: RBWH Guidelines in the Management of Potential Adverse Events in Apheresis

5.2.3. 02002/Proc: Medications Management

5.2.4. 21605/Proc: Blood collection (Adult)

5.2.5. 05009/Proc: Apheresis

5.2.6. 05450/Proc: Peripheral Intravenous Cannulation, Venepuncture and Infusions – Adult and Paediatrics

5.2.7. 74100/Proc: Documentation in the Patient Record

5.2.8. 81000/Proc: Aseptic Non Touch Technique

5.2.9. 000342/Proc: Standard Precautions

5.2.10. 003139/Proc: Cleaning and Decontamination – patient environment and clinical equipment

5.3. Forms and Templates

5.3.1. CLIFRM013: RBWH Donor Consent for Bone Marrow Harvest or Apheresis Procedure

5.3.2. COLFRM009: RBWH Apheresis Patient Checklist label

5.3.3. COLFRM028: RBWH Apheresis Service Request

5.3.4. COLFRM030: RBWH Apheresis Product Collection Form

5.3.5. COLFRM053: RBWH Apheresis Procedure Run Sheet: Spectra Optia

6. Definitions and Abbreviations

6.1. AIM — Automated interface management

6.2. ABMDR — Australian Bone Marrow Donor Registry

6.3. ACD-A — Acid citrate dextrose - solution A

6.4. BMP — Bone Marrow Processing

6.5. BTS — Biomedical Technical Services

6.6. ELFT Electrolyte & liver function tests

6.7. FBC Full blood count

6.8. Hb Haemoglobin

6.9. HBV Hepatitis B virus

6.10. HCV Hepatitis C virus

6.11. HIV Human immunodeficiency virus

6.12. IDM Infectious disease marker

6.13. IV Intravenous

6.14. MNHHS Metro North Hospital and Health Service

6.15. NUM Nurse Unit Manager

6.16. ODTU Oncology Day Therapy Unit

6.17. PMN Polymorphonuclear (abbreviation used for granulocytes)

6.18. QIMR Queensland Institute of Medical Research

6.19. RBC Red blood cell

6.20. RBCX Red blood cell exchange

6.21. RBWH Royal Brisbane & Women's Hospital

6.22. RN Registered nurse

6.23. SOP Standard operating procedure

6.24. TBV Total blood volume

7. Materials

7.1. Equipment

7.1.1. Spectra Optia Apheresis System (version 11.3)

7.1.1.1. RBCX (Depletion)

7.1.1.2. PMN procedure

7.1.2. IDL filler

7.1.3. Standard filler

7.1.4. Electronic blood pressure monitor

7.1.5. Volumetric infusion pump

7.1.6. Heat sealer

7.2. Disposables and Reagents

7.2.1. Spectra Optia IDL Set (Ref: 10310).

7.2.2. Spectra Optia Exchange Set (Ref: 12220)

7.2.3. BMP Accessory Set (Ref: 11300)

7.2.4. ACD-A anticoagulant (750ml)

7.2.5. 0.9% normal saline (1000ml)

7.2.6. Alcohol swab

7.2.7. 3 way taps

7.2.8. IV cannulation or central line equipment. Refer to procedures: 05450/Proc: Peripheral Intravenous Cannulation, Venepuncture and Infusions – Adult and Paediatrics; 05501/Proc: Cannulation of Haemodialysis Access; 05600/Proc: Central Venous Access Devices (CVADs), Management – Adult. Ensure use of sufficient gauge cannulas (backeye needles are preferable for inlet venous access) to allow for adequate flow required for the procedure (preferably at least 16G for inlet and 18G for return)

7.2.9. Blood collection tubes

7.2.10. Intravenous giving set

7.2.11. Burette

8. Special Considerations

8.1. This procedure describes how to perform:

8.1.1. Stage 1 - Red cell depletion procedure.

8.1.2. Stage 2 - PMN procedure

8.2. Ensure haematocrit is accurate, as this is used to calculate:

8.2.1. The limit for collect volumes

8.2.2. The plasma pump flow rate before the AIM system manages the concentration of cells in the collect port

8.2.3. Changing the haematocrit after the interface has been established will not change the interface position

8.3. The packing factor during a red cell depletion defaults to 20

8.4. The packing factor during a PMN procedure defaults to 4.5

8.5. The Inlet:AC ratio defaults to 13:1 during a RBC depletion procedure

8.6. There MUST be FBC and ELFT blood test results available (taken within the previous 24hrs) PRIOR to commencement of procedure.

8.7. The minimum FBC criteria required in order to proceed with the procedure is described below.

QIMR volunteer	Platelet $\geq 100 \times 10^9$/L
	Hb $\geq$ 100G/L

8.8. There is a risk of deranged electrolytes due to the effects of the procedure. This effect is usually transient and resolves quickly upon cessation of CMNC collection. Electrolyte replacement may be required. Calcium gluconate is the most frequently required electrolyte replacement and the amount and rate is dependent on symptoms of hypocalcaemia during the procedure. Magnesium and potassium replacement can also be required, below is a guide for when supplementation may be required:

Pre-Procedure Electrolyte	Replacement guide
Magnesium < 60 mmol/L	10 – 20 mmols IV magnesium sulphate

	may be required during procedure
Potassium between 3.0 – 3.5 mmol/L	Oral potassium may be required e.g 1-2 chlorvescent tablets
Potassium < 3.0 mmol	IV potassium chloride 20 mmols may be required during the procedure and / or 3 oral chlorvescent

9. Stage 1 - Red Cell Depletion Procedure

9.1. Confirm the following forms are current and valid:

9.1.1. CLIFRM013: RBWH Donor Consent for Bone Marrow harvest or Apheresis Procedure

9.1.2. COLFRM028: RBWH Apheresis Service Request

9.2. Prior to commencement of procedure, ensure patient has had appropriate IDMs taken.

9.2.1. HIV serology

9.2.2. Hepatitis B serology

9.2.3. Hepatitis C serology

9.2.4. Syphilis

9.3. All stock used to collect the product must be visually examined prior to use for signs of damage and contamination, as per COLSOP004: Apheresis Inventory Control Procedure. Record expiry date and lot number of all stock used on COLFRM030: RBWH Apheresis Product Collection form. Check expiry dates and lot numbers of procedure kit and intravenous fluid bags with 2nd RN and record on COLFRM053: RBWH Apheresis Procedure Run Sheet: Spectra Optia

9.3.1. Confirm that all consumables and reagents used during the procedure are satisfactory for use; inspect for damage or evidence of

contamination and mark the appropriate check box on COLFRM030: RBWH Apheresis Product Collection form

9.4. On initial set up screen, choose RBCX procedure

9.5. Set up, load and prime Spectra Optia Exchange Set (Ref: 12220) as per on screen instructions

9.6. Follow machine prompts and enter patient data.

9.7. Exchange type - Depletion

9.8. Depletion fluid type – Saline / Albumin

9.9. Fluid balance – 100%

9.10. The default end point is target hct

9.11. Enter target hct required to collect 450ml of concentrated red cell product

9.11.1. Collect volume should not exceed 500ml

9.11.2. Target hct shall not exceed 10% drop from pre procedure hct

9.12. Educate patient of signs and symptoms of potential adverse reactions, emphasising importance of informing nursing staff if he/she experiences any such reactions

9.13. Perform baseline observations (temperature, pulse, respirations and blood pressure) and record on run sheet

9.14. Ensure COLFRM009: RBWH Apheresis Patient Checklist has been completed

9.15. Perform Cannulation according to 05450/Proc: Peripheral Intravenous Cannulation, Venepuncture and Infusions – Adult and Paediatrics

9.16. Collect blood samples if required.

9.17. Follow machine prompts to commence procedure

9.18. Review run screen to ensure pump flow rates are appropriate for the procedure. Monitor the AC infusion rate, ensuring that it does not exceed 0.9 ml/min

9.19. The Spectra Optia system will attempt to establish the interface at commencement of the procedure, during this time, the collect valve will remain closed.

9.20. The AIM system monitors for RBC spill over.

9.21. The replace pump pumps replacement fluid into the reservoir where it combines with the plasma for return to the patient

9.22. Monitor and record patient's vital signs and run parameters q15 minutes or as clinically indicated. Monitor for signs of adverse reactions and record on procedure run sheet, reporting adverse events on Riskman.

9.23. Ensure patient comfort and safety throughout the procedure.

9.24. During a RBC depletion procedure, RBC are removed until the target Hct has been attained.

9.25. At the end of the procedure, follow the system prompts to rinseback

9.26. Seal the collection bag prior to disconnection of the patient, in preparation for transfer to BMP Accessory Set

9.27. The time of collection completion shall be recorded on COLFRM030: RBWH Apheresis Product Collection form.

9.28. On completion of rinseback, collect routine blood samples (usually FBC and ELFT).

9.29. Perform final set of observations and record on procedure run sheet

9.30. Remove venous access as appropriate

9.31. Record final values on COLFRM053: RBWH Apheresis Procedure Run Sheet and complete COLFRM030: RBWH Apheresis Product Collection form.

9.32. Unload set and dispose of waste appropriately

9.33. Document all care given in patient medical notes. Include all completed paperwork with patient's medical notes.

9.34. Record relevant statistical information in: G:\Oncology\Apheresis\Log and Statistics

10. Stage 2 - PMN Procedure

10.1. Transfer collected red cell concentrate into BMP Accessory Set (Ref: 12220)

10.2. All stock used to collect the product must be visually examined prior to use for signs of damage and contamination, as per COLSOP004: Apheresis Inventory Control Procedure. Check expiry dates and lot numbers of consumables with 2nd

RN and record on COLFRM053: RBWH Apheresis Procedure Run Sheet: Spectra Optia

10.3. Set up, load and prime Spectra Optia IDL Set (Ref: 10310) as per on screen instructions for PMN procedure

10.3.1. If custom prime is required, use Albumin 4% to complete

10.4. This procedure is intended for patients, not a bag of concentrated red cells, therefore requires patient data to be entered into program in order to proceed with procedure.

10.4.1. Follow machine prompts and enter patient data.

10.4.2. When prompted, enter 60% for patient hct

10.5. Follow machine prompts to commence procedure

10.6. The Spectra Optia system will attempt to establish the interface at commencement of procedure, during this time, the collect valve will remain closed

10.7. The AIM system monitors the collect port and changes the plasma pump flow rate to manage the concentration of cells that flow through he collect port. When the AIM system detects cells in the collect port, the collect valve opens. Ensure the collect flow rate is set at 2ml/min.

10.8. Ensure the main run screen accurately displays that no HES is being used

10.9. Review AC infusion rate regularly, ensuring that it does not exceed 0.9mls/min

10.10. The CP defaults to 60 at commencement of procedure. The apheresis nurse can set the CP to collect lighter or darker from the buffy coat. Use the collection preference tool for the appropriate CP

10.10.1. See Appendix 1 for the appropriate CP, using the collection preference tool, for PMN collections

10.10.2. A higher CP results in collecting lighter in the buffy coat

10.10.3. A lower CP results in collecting darker in the buffy coat

10.11. If the interface is lost during the procedure, the collect flow rate can be reduced to 1ml/min until interface has been regained. Ensure the collect flow rate is then returned to 2ml/min

10.12. QIMR staff are responsible for labelling of collection bags.

10.13. Seal the collection bag using heat sealer - QIMR staff will indicate the most appropriate point to heat seal line.

10.14. On completion of procedure select DISCONNECT when prompted

10.15. Record final procedural values on run sheet

10.16. QIMR staff are responsible for transporting collected product to QIMR for further testing.

10.17. Record relevant statistical information located in: G:\Oncology\Apheresis\Log and Statistics

10.18. Unload set and dispose of waste appropriately

10.19. Decontaminate Spectra Optia as per protocol (see COLSOP005: RBWH Apheresis Equipment Cleaning Procedure). Decontaminate all other equipment involved in performing the procedure, in accordance with 003139/Proc: Cleaning and Decontamination – patient environment and clinical equipment. Once all equipment used during the procedure has

been cleaned, tick appropriate area at the end of the run sheet, confirming completion of cleaning tasks.

10.20. Once paperwork is complete, scan / photocopy all procedural paperwork and send to QIMR.

11. End Points

11.1. The total collect volume of red cell depletion will be approximately 450ml

11.1.1. Total collect volume shall not exceed 500ml

11.1.2. Discuss with QIMR staff if total collect volume is expected to exceed 500ml. QIMR staff will advise apheresis nursing staff of most appropriate action

11.2. Target hct shall not exceed 10% drop from pre procedure hct

11.3. RBC depletion procedure time should not exceed 180 minutes

11.3.1. Discuss with QIMR staff if total procedure time is expected to exceed 180 minutes. QIMR staff will advise apheresis nursing staff of most appropriate action

12. Disaster Plan

12.1. This section describes the actions to be taken in the event of serious problems which may arise in the Apheresis Unit which may impact on either:

12.1.1. The ability to perform the CMNC collection to the required specification

12.1.2. The quality of the collected product

12.2. Air-Conditioning System Failure – in the event that the air-conditioning system malfunctions, contact hospital maintenance and arrange for urgent repairs (ext. 67963).

12.3. Loss of Power – All critical equipment used to perform apheresis procedures should be connected to red emergency power points. These power points are supplied with the electricity from the emergency backup

power generators, and in the event of a power failure, are automatically activated.

12.3.1. Critical equipment used within the apheresis unit includes:

12.3.1.1. Cell separators

12.3.1.2. Blood warmers

12.3.1.3. Electronic blood pressure monitors

12.3.1.4. Intravenous volumetric pumps

12.3.1.5. Heat sealers

12.3.2. In the event of an interruption to normal power supply, there may be a temporary loss of power before the emergency generator is activated. If there is an interruption of the power supply:

12.3.2.1. Close clamps on collect line immediately

12.3.2.2. If the emergency power supply is activated, power will resume, and the Spectra Optia system will reset. After system resets, a screen will appear with instructions for how to proceed.

12.3.2.3. To restart the procedure, follow onscreen instructions

12.3.2.4. To abort procedure, press RINSEBACK (if appropriate) or DISCONNECT and follow on screen instructions

12.3.3. In the event of a complete loss of power with no emergency power supply:

12.3.3.1. The Spectra Optia cell separator will shut down completely. Close clamps on collect line immediately. Seal the collection bags using a heat sealer (these should still be functional if battery charged)

12.3.3.2. Contact the patient physician and inform him of events and that the procedure may be aborted

12.3.3.3. Consult with the Director of the Haematology & BMT

12.3.3.4. The extracorporeal volume in the kit will be 297ml

12.3.3.5. The patient may be disconnected without performing RINSEBACK or perform MANUAL RINSEBACK as per instructions in Spectra Optia Essentials Guide, pp.73-76 (in the event that a blood prime was performed, RINSEBACK is not necessary)

12.4. Immediate evacuation – in the event that an immediate evacuation is required:

12.4.1. Close clamps on collect line if possible. NURSE AND PATIENT SAFETY IS PARAMOUNT, therefore do not complete this step if completion may endanger the health and safety of the nurse or the patient

12.4.2. Disconnect patient immediately and evacuate as per RBWH Code Orange Evacuation procedure. N.B. since RINSEBACK has not been performed, the patient will have a 297ml fluid deficit and may become hypotensive, monitor patient accordingly

12.5. Structural damage (e.g. fire, water / flood damage) – in the event of structural damage, relocate product, material and patient files as required, to another area of ODTU if possible. Depending on the scale of the problem, a final decision on a temporary location may need to be determined on a case-by-case assessment by a structural engineer and the Director of the BMT program

12.5.1. All critical equipment and/or consumables which may have incurred damage should be discarded and replaced as soon as possible

12.5.2. Contact hospital maintenance (ext. 67963) and organise for them to inspect critical equipment as soon as possible

12.5.3. Suspend all operational activities until repairs are complete and the facility is signed off as safe to reoccupy

12.6. Critical equipment failure – Perform troubleshooting as per relevant equipment manuals. If unable to resolve problem, contact BTS or manufacturer representative.

12.7. Critical consumable shortage – if a critical consumable shortage occurs, contact the following Apheresis Units to source consumables:

12.7.1. Greenslopes Private Hospital (Speed dial *2521).

12.7.2. Lady Cilento Childrens' Hospital (Jo Ritchie – 3068 5686, or Jill Shergold – 3068 5767)

12.7.3. Once alternative supply has been sourced, organise transport of the required consumables. Liaise with the NUM, ODTU to organise payment of delivery e.g. courier service

12.8. Critical staff shortage – The Apheresis Nurse Practitioner shall liaise with the NUM, ODTU regularly to ensure staffing requirements of the apheresis unit are met. In the event that key personnel are unavailable to perform critical procedures:

12.8.1. Contact Apheresis Nurse Practitioner (pager# 26299) and NUM, ODTU (ext. 68754 / 73991) informing them of the situation

12.8.2. The NUM / Apheresis Nurse Practitioner will attempt to adequately staff the Apheresis Unit

12.8.3. If appropriate, the situation can be escalated to the Director of the BMT program and the Nursing Director, CCS

12.8.4. If adequate staffing cannot be accomplished, the Apheresis Nurse Practitioner shall liaise with the Director of the BMT Program to discuss requesting the assistance of other apheresis units in the area. Alternative units may be affiliated with the RBWH e.g. Greenslopes Private Hospital, or other similarly accredited Apheresis Units e.g. Princess Alexandra Hospital or the Mater Public Hospital

13. Responsibility

	Final Approval	Implement	Quality Assurance	Review	Perform Procedure
Apheresis Nurse Practitioner		X	X	X	X
Medical Director	X	X			
QIMR staff				X	
Nursing Staff					X

14. Occupational Health and Safety

14.1. Standard precautions shall be maintained throughout the procedure. Refer to 000342/Proc: Standard Precautions.

15. Risk

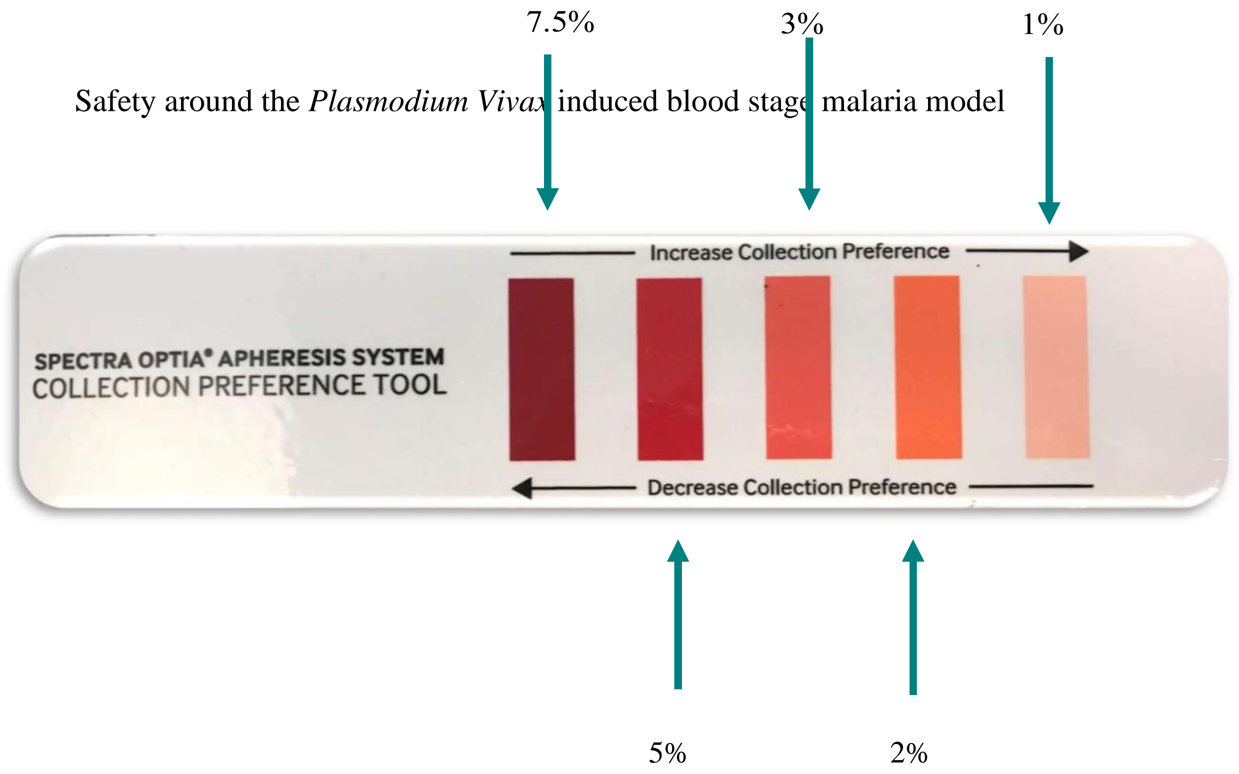
15.1. Low to Medium (case dependant).

16. Consultation

16.1. Director Haematology & Bone Marrow Transplant Glen Kennedy

16.2. Apheresis Nurse Practitioner Kari Mudie

16.3. QIMR Registrar Anand Odedra


7.5%
3%
1%
Increase Collection Preference
SPECTRA OPTIA® APHERESIS SYSTEM
COLLECTION PREFERENCE TOOL
Decrease Collection Preference
5%
2%

APPENDIX 7. APHERESIS COHORT 4 CLINICAL STANDARD OPERATING PROCEDURE.

1. Purpose

1.1. This SOP describes the apheresis procedures used for this study. It is intended to be used in conjunction with:

1.1.1. Spectra Optia Essentials

2. Scope

2.1. The target audience for this resource are the registered nurses and medical officers who care for patients in the Apheresis Unit, Royal Brisbane & Women's Hospital (RBWH), who are involved and trained in apheresis.

2.2. Patient safety takes priority during all apheresis procedures, therefore only competent nurses can perform this procedure.

2.3. All apheresis procedures will comply with 05009/WUG: Apheresis

2.4. All deviations and associated corrective actions shall be documented in the patient medical record.

3. Principle / Background

3.1. Apheresis refers to a number of procedures involving the removal of whole blood, separating it into the various blood components through centrifugation, removing a desired component (white/red cells, platelets and plasma with or without replacement fluids) and returning the rest of the components. Access to the intravascular compartment is required and efficacy of the treatment will depend on the volume of blood processed.

3.2. This apheresis procedure will consist of 2 stages:

3.2.1. Stage 1: RBC Depletion of QIMR volunteer

3.2.2. Stage 2: PMN procedure of concentrated red blood cell product

3.3. During a red cell depletion, red blood cells are removed from the patient until the target haematocrit has been attained, and replaced with desired replacement fluid

3.4. All patients requiring an apheresis procedure are under the direct care of a Haematologist.

3.5. The RBWH Apheresis Unit is accredited by:

3.5.1. Australian Bone Marrow Donor Registry (ABMDR)

3.5.2. National Association of Testing Authorities (NATA)

3.5.3. Foundation for the Accreditation of Cellular Therapies (FACT)

3.6. The Royal Brisbane & Women's Hospital (RBWH) is accredited by the Australian Council on Healthcare Standards (ACHS)

3.7. The Disaster Plan (Section 11) addresses those elements most considered to be at risk and provides guidelines to cover some of the potential serious emergencies that may arise. All staff involved in apheresis procedures are responsible for following this plan as directed by senior personnel.

4. Objectives / Endpoints

4.1. All apheresis procedures shall be performed safely and appropriately.

5. Documentation

5.1. Policy and Standard/s

5.1.1. ACHS EQUiP National

5.1.2. Blood and Blood Products, Management of PROC103 Doc 110/15 (MNHHS)

5.1.3. Clinical Incident and Disclosure Management PROC007 Document 13/14 (MNHHS)

5.2. Procedures, Guidelines, Protocols

5.2.1. Australian Guidelines for the Prevention and Control of Infection in Healthcare (2010)

5.2.2. COLGDE001: RBWH Guidelines in the Management of Potential Adverse Events in Apheresis

5.2.3. 02002/Proc: Medications Management

5.2.4. 21605/Proc: Blood collection (Adult)

5.2.5. 05009/Proc: Apheresis

5.2.6. 05450/Proc: Peripheral Intravenous Cannulation, Venepuncture and Infusions – Adult and Paediatrics

5.2.7. 74100/Proc: Documentation in the Patient Record

5.2.8. 81000/Proc: Aseptic Non Touch Technique

5.2.9. 000342/Proc: Standard Precautions

5.2.10. 003139/Proc: Cleaning and Decontamination – patient environment and clinical equipment

5.3. Forms and Templates

5.3.1. CLIFRM013: RBWH Donor Consent for Bone Marrow Harvest or Apheresis Procedure

5.3.2. COLFRM009: RBWH Apheresis Patient Checklist label

5.3.3. COLFRM028: RBWH Apheresis Service Request

5.3.4. COLFRM030: RBWH Apheresis Product Collection Form

5.3.5. COLFRM053: RBWH Apheresis Procedure Run Sheet: Spectra Optia

6. Definitions and Abbreviations

6.1. AIM Automated interface management

6.2. ABMDR Australian Bone Marrow Donor Registry

6.3. ACD-A Acid citrate dextrose - solution A

6.4. BMP Bone Marrow Processing

6.5. BTS Biomedical Technical Services

6.6. ELFT Electrolyte & liver function tests

6.7. FBC Full blood count

6.8. Hb Haemoglobin

6.9. HBV Hepatitis B virus

6.10. HCV Hepatitis C virus

6.11. HIV Human immunodeficiency virus

6.12. IDM Infectious disease marker

6.13. IV Intravenous

6.14. MNHHS Metro North Hospital and Health Service

6.15. NUM Nurse Unit Manager

6.16. ODTU Oncology Day Therapy Unit

6.17. PMN Polymorphonuclear (abbreviation used for granulocytes)

6.18. QIMR Queensland Institute of Medical Research

6.19. RBC Red blood cell

6.20. RBCX Red blood cell exchange

6.21. RBWH Royal Brisbane & Women's Hospital

6.22. RN Registered nurse

6.23. SOP Standard operating procedure

6.24. TBV Total blood volume

7. Materials

7.1. Equipment

7.1.1. Spectra Optia Apheresis System (version 11.3)

7.1.1.1. RBCX (Depletion)

7.1.1.2. PMN procedure

7.1.2. IDL filler

7.1.3. Standard filler

7.1.4. Electronic blood pressure monitor

7.1.5. Volumetric infusion pump

7.1.6. Heat sealer

7.2. Disposables and Reagents

7.2.1. Spectra Optia IDL Set (Ref: 10310).

7.2.2. Spectra Optia Exchange Set (Ref: 12220)

7.2.3. BMP Accessory Set (Ref: 11300)

7.2.4. ACD-A anticoagulant (750ml)

7.2.5. 0.9% normal saline (1000ml)

7.2.6. Alcohol swab

7.2.7. 3 way taps

7.2.8. IV cannulation or central line equipment. Refer to procedures: 05450/Proc: Peripheral Intravenous Cannulation, Venepuncture and Infusions – Adult and Paediatrics; 05501/Proc: Cannulation of Haemodialysis Access; 05600/Proc: Central Venous Access Devices (CVADs), Management – Adult. Ensure use of sufficient gauge cannulas (backeye needles are preferable for inlet venous access) to allow for adequate flow required for the procedure (preferably at least 16G for inlet and 18G for return)

7.2.9. Blood collection tubes

7.2.10. Intravenous giving set

7.2.11. Burette

8. Special Considerations

8.1. This procedure describes how to perform:

8.1.1. Stage 1 - Red cell depletion procedure.

8.1.2. Stage 2 - PMN procedure

8.2. Ensure haematocrit is accurate, as this is used to calculate:

8.2.1. The limit for collect volumes

8.2.2. The plasma pump flow rate before the AIM system manages the concentration of cells in the collect port

8.2.3. Changing the haematocrit after the interface has been established will not change the interface position

8.3. The packing factor during a red cell depletion defaults to 20

8.4. The packing factor during a PMN procedure defaults to 4.5

8.5. The Inlet:AC ratio defaults to 13:1 during a RBC depletion procedure

8.6. There MUST be FBC and ELFT blood test results available (taken within the previous 24hrs) PRIOR to commencement of procedure.

8.7. The minimum FBC criteria required in order to proceed with the procedure is described below.

QIMR volunteer	Platelet $\geq 100 \times 10^9$/L
	Hb $\geq$ 100G/L

8.8. There is a risk of deranged electrolytes due to the effects of the procedure. This effect is usually transient and resolves quickly upon cessation of CMNC collection. Electrolyte replacement may be required. Calcium gluconate is the most frequently required electrolyte replacement and the amount and rate is dependent on symptoms of hypocalcaemia during the procedure. Magnesium and potassium replacement can also be required, below is a guide for when supplementation may be required:

Pre-Procedure Electrolyte	Replacement guide
Magnesium < 60 mmol/L	10 – 20 mmols IV magnesium sulphate may be required during procedure
Potassium between 3.0 – 3.5 mmol/L	Oral potassium may be required e.g 1-2 chlorvescent tablets
Potassium < 3.0 mmol	IV potassium chloride 20 mmols may be required during the procedure and / or 3 oral chlorvescent

9. Stage 1 - Red Cell Depletion Procedure

9.1. Confirm the following forms are current and valid:

9.1.1. CLIFRM013: RBWH Donor Consent for Bone Marrow harvest or Apheresis Procedure

9.1.2. COLFRM028: RBWH Apheresis Service Request

9.2. Prior to commencement of procedure, ensure patient has had appropriate IDMs taken.

9.2.1. HIV serology

9.2.2. Hepatitis B serology

9.2.3. Hepatitis C serology

9.2.4. Syphilis

9.3. All stock used to collect the product must be visually examined prior to use for signs of damage and contamination, as per COLSOP004: Apheresis Inventory Control Procedure. Record expiry date and lot number of all stock used on COLFRM030: RBWH Apheresis Product Collection form. Check expiry dates and lot numbers of procedure kit and intravenous fluid bags with 2nd RN and record on COLFRM053: RBWH Apheresis Procedure Run Sheet: Spectra Optia

9.3.1. Confirm that all consumables and reagents used during the procedure are satisfactory for use; inspect for damage or evidence of contamination and mark the appropriate check box on COLFRM030: RBWH Apheresis Product Collection form

9.4. On initial set up screen, choose RBCX procedure

9.5. Set up, load and prime Spectra Optia Exchange Set (Ref: 12220) as per on screen instructions

9.6. Follow machine prompts and enter patient data.

9.7. Exchange type - Depletion

9.8. Depletion fluid type – Saline / Albumin

9.9. Fluid balance – 100%

9.10. The default end point is target hct

9.11. Enter target hct required to collect 450ml of concentrated red cell product

9.11.1. Collect volume should not exceed 500ml

9.11.2. Target hct shall not exceed 10% drop from pre procedure hct

9.12. Educate patient of signs and symptoms of potential adverse reactions, emphasising importance of informing nursing staff if he/she experiences any such reactions

9.13. Perform baseline observations (temperature, pulse, respirations and blood pressure) and record on run sheet

9.14. Ensure COLFRM009: RBWH Apheresis Patient Checklist has been completed

9.15. Perform Cannulation according to 05450/Proc: Peripheral Intravenous Cannulation, Venepuncture and Infusions – Adult and Paediatrics

9.16. Collect blood samples if required.

9.17. Follow machine prompts to commence procedure

9.18. Review run screen to ensure pump flow rates are appropriate for the procedure. Monitor the AC infusion rate, ensuring that it does not exceed 0.9 ml/min

9.19. The Spectra Optia system will attempt to establish the interface at commencement of the procedure, during this time, the collect valve will remain closed.

9.20. The AIM system monitors for RBC spillover.

9.21. The replace pump pumps replacement fluid into the reservoir where it combines with the plasma for return to the patient

9.22. Monitor and record patient's vital signs and run parameters q15 minutes or as clinically indicated. Monitor for signs of adverse reactions and record on procedure run sheet, reporting adverse events on Riskman.

9.23. Ensure patient comfort and safety throughout the procedure.

9.24. During a RBC depletion procedure, RBC are removed until the target Hct has been attained.

9.25. At the end of the procedure, follow the system prompts to rinseback

9.26. Seal the collection bag prior to disconnection of the patient, in preparation for transfer to BMP Accessory Set

9.27. The time of collection completion shall be recorded on COLFRM030: RBWH Apheresis Product Collection form.

9.28. On completion of rinseback, collect routine blood samples (usually FBC and ELFT).

9.29. Perform final set of observations and record on procedure run sheet

9.30. Remove venous access as appropriate

9.31. Record final values on COLFRM053: RBWH Apheresis Procedure Run Sheet and complete COLFRM030: RBWH Apheresis Product Collection form.

9.32. Unload set and dispose of waste appropriately

9.33. Document all care given in patient medical notes. Include all completed paperwork with patient's medical notes.

9.34. Record relevant statistical information in: G:\Oncology\Apheresis\Log and Statistics

10. Stage 2 - PMN Procedure

10.1. Transfer collected red cell concentrate into BMP Accessory Set (Ref: 12220)

10.2. All stock used to collect the product must be visually examined prior to use for signs of damage and contamination, as per COLSOP004: Apheresis

Inventory Control Procedure. Check expiry dates and lot numbers of consumables with 2nd
RN and record on COLFRM053: RBWH Apheresis Procedure Run Sheet: Spectra Optia

10.3. Set up, load and prime Spectra Optia IDL Set (Ref: 10310) as per on screen instructions for PMN procedure

10.3.1. If custom prime is required, use Albumin 4% to complete

10.4. This procedure is intended for patients, not a bag of concentrated red cells, therefore requires patient data to be entered into program in order to proceed with procedure.

10.4.1. Follow machine prompts and enter patient data.

10.4.2. When prompted, enter 60% for patient hct

10.5. Follow machine prompts to commence procedure

10.6. The Spectra Optia system will attempt to establish the interface at commencement of procedure, during this time, the collect valve will remain closed

10.7. The AIM system monitors the collect port and changes the plasma pump flow rate to manage the concentration of cells that flow through he collect port. When the AIM system detects cells in the collect port, the collect valve opens. Ensure the collect flow rate is set at 2ml/min.

10.8. Ensure the main run screen accurately displays that <u>no</u> HES is being used

10.9. Review AC infusion rate regularly, ensuring that it does not exceed 0.9mls/min

10.10. The CP defaults to 60 at commencement of procedure. The apheresis nurse can set the CP to collect lighter or darker from the buffy coat. Use the collection preference tool for the appropriate CP

10.10.1. See Appendix 1 for the appropriate CP, using the collection preference tool, for PMN collections

10.10.2. A higher CP results in collecting lighter in the buffy coat

10.10.3. A lower CP results in collecting darker in the buffy coat

10.11. If the interface is lost during the procedure, the collect flow rate can be reduced to 1ml/min until interface has been regained. Ensure the collect flow rate is then returned to 2ml/min

10.12. QIMR staff are responsible for labelling of collection bags.

10.13. Seal the collection bag using heat sealer - QIMR staff will indicate the most appropriate point to heat seal line.

10.14. On completion of procedure select DISCONNECT when prompted

10.15. Record final procedural values on run sheet

10.16. QIMR staff are responsible for transporting collected product to QIMR for further testing.

10.17. Record relevant statistical information located in: G:\Oncology\Apheresis\Log and Statistics

10.18. Unload set and dispose of waste appropriately

10.19. Decontaminate Spectra Optia as per protocol (see COLSOP005: RBWH Apheresis Equipment Cleaning Procedure). Decontaminate all other equipment involved in performing the procedure, in accordance with 003139/Proc: Cleaning and Decontamination – patient environment and clinical equipment. Once all equipment used during the procedure has been cleaned, tick appropriate area at the end of the run sheet, confirming completion of cleaning tasks.

10.20. Once paperwork is complete, scan / photocopy all procedural paperwork and send to QIMR.

11. End Points

11.1. The total collect volume of red cell depletion will be approximately 450ml

11.1.1. Total collect volume shall not exceed 500ml

11.1.2. Discuss with QIMR staff if total collect volume is expected to exceed 500ml. QIMR staff will advise apheresis nursing staff of most appropriate action

11.2. Target hct shall not exceed 10% drop from pre procedure hct

11.3. RBC depletion procedure time should not exceed 180 minutes

11.3.1. Discuss with QIMR staff if total procedure time is expected to exceed 180 minutes. QIMR staff will advise apheresis nursing staff of most appropriate action

12. Disaster Plan

12.1. This section describes the actions to be taken in the event of serious problems which may arise in the Apheresis Unit which may impact on either:

12.1.1. The ability to perform the CMNC collection to the required specification

12.1.2. The quality of the collected product

12.2. Air-Conditioning System Failure – in the event that the air-conditioning system malfunctions, contact hospital maintenance and arrange for urgent repairs (ext. 67963).

12.3. Loss of Power – All critical equipment used to perform apheresis procedures should be connected to red emergency power points. These power points are supplied with the electricity from the emergency backup power generators, and in the event of a power failure, are automatically activated.

12.3.1. Critical equipment used within the apheresis unit includes:

12.3.1.1. Cell separators

12.3.1.2. Blood warmers

12.3.1.3. Electronic blood pressure monitors

12.3.1.4. Intravenous volumetric pumps

12.3.1.5. Heat sealers

12.3.2. In the event of an interruption to normal power supply, there may be a temporary loss of power before the emergency generator is activated. If there is an interruption of the power supply:

12.3.2.1. Close clamps on collect line immediately

12.3.2.2. If the emergency power supply is activated, power will resume, and the Spectra Optia system will reset. After system resets, a screen will appear with instructions for how to proceed.

12.3.2.3. To restart the procedure, follow onscreen instructions

12.3.2.4. To abort procedure, press RINSEBACK (if appropriate) or DISCONNECT and follow on screen instructions

12.3.3. In the event of a complete loss of power with no emergency power supply:

12.3.3.1. The Spectra Optia cell separator will shut down completely. Close clamps on collect line immediately. Seal the collection bags using a heat sealer (these should still be functional if battery charged)

12.3.3.2. Contact the patient physician and inform him of events and that the procedure may be aborted

12.3.3.3. Consult with the Director of the Haematology & BMT

12.3.3.4. The extracorporeal volume in the kit will be 297ml

12.3.3.5. The patient may be disconnected without performing RINSEBACK or perform MANUAL RINSEBACK as per instructions in Spectra Optia Essentials Guide, pp.73-76 (in the event that a blood prime was performed, RINSEBACK is not necessary)

12.4. Immediate evacuation – in the event that an immediate evacuation is required:

12.4.1. Close clamps on collect line if possible. NURSE AND PATIENT SAFETY IS PARAMOUNT, therefore do not complete this step if completion may endanger the health and safety of the nurse or the patient

12.4.2. Disconnect patient immediately and evacuate as per RBWH Code Orange Evacuation procedure. N.B. since RINSEBACK has not been performed, the patient will have a 297ml fluid deficit and may become hypotensive, monitor patient accordingly

12.5. Structural damage (e.g. fire, water / flood damage) – in the event of structural damage, relocate product, material and patient files as required, to another area of ODTU if possible. Depending on the scale of the problem, a final decision on a temporary location may need to be determined on a case-by-case assessment by a structural engineer and the Director of the BMT program

12.5.1. All critical equipment and/or consumables which may have incurred damage should be discarded and replaced as soon as possible

12.5.2. Contact hospital maintenance (ext. 67963) and organise for them to inspect critical equipment as soon as possible

12.5.3. Suspend all operational activities until repairs are complete and the facility is signed off as safe to reoccupy

12.6. Critical equipment failure – Perform troubleshooting as per relevant equipment manuals. If unable to resolve problem, contact BTS or manufacturer representative.

12.7. Critical consumable shortage – if a critical consumable shortage occurs, contact the following Apheresis Units to source consumables:

12.7.1. Greenslopes Private Hospital (Speed dial *2521).

12.7.2. Lady Cilento Childrens' Hospital (Jo Ritchie – 3068 5686, or Jill Shergold – 3068 5767)

12.7.3. Once alternative supply has been sourced, organise transport of the required consumables. Liaise with the NUM, ODTU to organise payment of delivery e.g. courier service

12.8. Critical staff shortage – The Apheresis Nurse Practitioner shall liaise with the NUM, ODTU regularly to ensure staffing requirements of the apheresis unit are met. In the event that key personnel are unavailable to perform critical procedures:

12.8.1. Contact Apheresis Nurse Practitioner (pager# 26299) and NUM, ODTU (ext. 68754 / 73991) informing them of the situation

12.8.2. The NUM / Apheresis Nurse Practitioner will attempt to adequately staff the Apheresis Unit

12.8.3. If appropriate, the situation can be escalated to the Director of the BMT program and the Nursing Director, CCS

12.8.4. If adequate staffing cannot be accomplished, the Apheresis Nurse Practitioner shall liaise with the Director of the BMT Program to discuss requesting the assistance of other apheresis units in the area. Alternative units may be affiliated with the RBWH e.g. Greenslopes Private Hospital, or other similarly accredited Apheresis Units e.g. Princess Alexandra Hospital or the Mater Public Hospital

13. Responsibility

	Final Approval	Implement	Quality Assurance	Review	Perform Procedure
Apheresis Nurse Practitioner		X	X	X	X
Medical Director	X	X			
QIMR staff				X	

Nursing Staff					X

14. Occupational Health and Safety

14.1. Standard precautions shall be maintained throughout the procedure. Refer to 000342/Proc: Standard Precautions.

15. Risk

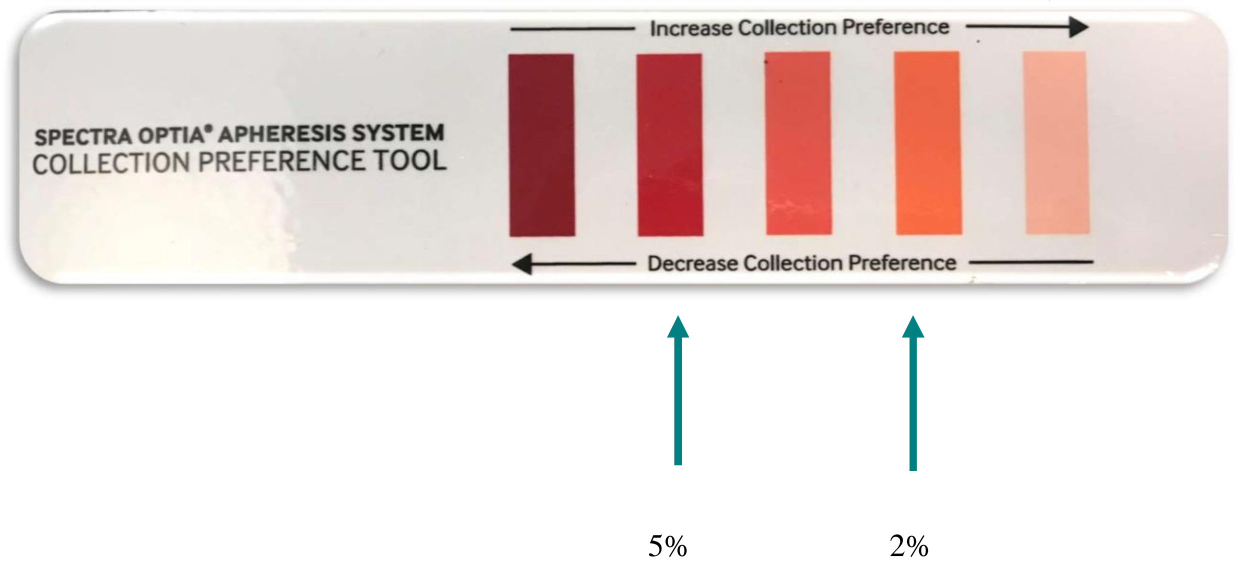
15.1. Low to Medium (case dependant).

16. Consultation

16.1. Director Haematology & Bone Marrow Transplant Glen Kennedy

16.2. Apheresis Nurse Practitioner Kari Mudie

16.3. QIMR Registrar Anand Odedra


7.5%
3%
1%
Increase Collection Preference
SPECTRA OPTIA® APHERESIS SYSTEM
COLLECTION PREFERENCE TOOL
Decrease Collection Preference
5%
2%

APPENDIX 8. APHERESIS COHORT 1 LABORATORY STANDARD OPERATING PROCEDURE.

Clinical Tropical Medicine, QIMR		
CTM QIMR Version:	**Malarial Enrichment via Apheresis - Form** Status:	Date Effective: Review Date: Two years after review date

Apheresis laboratory master SOP: Cohort 1

1. **NB Ensure the corresponding form is filled out in conjunction with the below method**
2. Ensure centrifuge is preheated using program 9 – 4500rpmr/HOLD/40°C
3. Once blood bag has been transported to the waterbath in CTM and process is ready to continue, fill two large silver thermos' with preheated water. Refer to "Appendix 1 – Table of Correct Water Volumes" for volume of water to add. Prepare one thermos for holding the blood bag and one thermos for holding 50ml falcons at temperature.
4. Check temperature of water and replace if below 38°C.
5. Place blood bag into thermos and move into BSC.
6. Move 50/250ml (for 1 and 2% HCT) collection tube into BSC and hold in thermos.
7. Use spike adaptor on sterile port of blood bag.
    7.1. **Note: Do not remove blood bag from thermos or lift above water level.**
8. Grab a 30/50ml preheated syringe from prewarming incubator.
9. Unscrew white cap of spike adaptor and place to the side.
10. Connect syringe to leur lock of spike adaptor.
11. Carefully syringe blood out of blood bag and dispense into 50ml/250ml collection tube.
12. Repeat process as necessary until all blood has been removed from blood bag.
13. Prepare a labelled 50ml falcon for sampling into a 50ml tube rack.

14. Mix collection tube well, either by gentle swirling or aspiration using a 25ml strippette.
15. Measure volume of HCT fraction received.
16. Aspirate 3.5 ml of whole blood using a 5ml strippette into sampling tube. Sampling tube can be moved into another BSC for aliquoting during process.
17. Spin 50ml/250ml collection tube in a preheated centrifuge at 530g/5-15min/40°C
18. Replace water in thermos just before centrifuge stops spinning.
19. Collect collection tube from centrifuge with thermos and set centrifuge to preheat. (Program 9 – 4500rpm, HOLD, 40°C)
20. Place thermos back into BSC.
21. Use a strippette to remove supernatant and place into a waste tube.
22. Resuspend pellet and measure.
    - 22.1. For 1%, 2% or 3% HCT: Resuspend pellet in equal volume of prewarmed AB serum.
        - 22.1.1. Take 600ul for Feed mix. Remainder is transferred for subsampling and cryopreservation
        - 22.1.2. Calculate volume of packed RBCs to add to prepare Feed Mix. Add equal volume of prewarmed AB serum.
        - 22.1.3. Aliquot 1.25ml of Feed Mix into prewarmed eppendorf.
        - 22.1.4. Prepare small thermos for transportation.
        - 22.1.5. Place FM into floatie and transport in small thermos to PC3.
        - 22.1.6. Conduct Membrane Feed as per CTM QIMR SOP 15 – Infection of Mosquitoes' with plasmodium by membrane feeding

1. Use remaining Feed Mix for slides & flow sampling.
2. For 5% and 7% HCT: Resuspend pellet in equal volume of RPMI and prepare thin and thick films.
3. PREEPARING CELL PELLETS FOR STORAGE PRIOR TO CRYOPRESERVATION (PER HCT FRACTION)
   3.1. Transfer remaining pellet to prepared 25mL aliquots of pre-warmed (37°C) AB complete media.
   3.2. Add additional media until total volume is 30mL.
   3.3. Store at 37°C until shortly prior to cryopreservation.
   3.4. Record details on Apheresis Fraction Processing CTM QIMR QF 75A
4. PRECHILLNG OF CELL SOLUTIONS FOR CRYOPRESERVATION (PER HCT FRACTION)
   4.1. Immediately before pre chilling solutions, prepare a dry ice/ethanol slurry in a plastic beaker
   4.2. Whilst rapidly mixing, prechill a dummy tube of WFI for a period of 50 seconds within slurry
   4.3. Measure temperature of dummy tube.
   4.4. If within 3-8°C, proceed with chilling of HCT Fraction cell suspension in the same manner as the dummy tube.
   4.5. If not alter slurry until desired specifications can be rechecked using fresh dummy tubes prior to proceeding with rapid chilling
   4.6. Wipe chilled cell suspension thoroughly with 80% v/v ethanol and transfer to ice slurry within clean biohazard hood.
   4.7. Evenly split cell suspension into two labelled tubes and transfer to fridge for storage at 2-8°C
   4.8. Record details on Apheresis Fraction Processing CTM QIMR QF 75A

5. ADDITION OF GLYCEROLYTE TO RBCS/WBCS FOR CRYOPRESERVATION (PER HCT FRACTION)

5.1. Calculate the total volume of glycerolyte needed (the pooled volume of the parasitised RBCs multiplied by 2.2).

5.2. Record glycerolyte lot number expiry date and volume required on Section

5.3. Centrifuge cell suspension at 530g/5-15min/ 2-8°C

5.4. Remove and discard supernatant

5.5. Agitate pellet (or gently pipette) to resuspend pellet.

5.6. Slowly add dropwise 1/5 of the total volume of glycerolyte to the pooled volume RBCs, whilst gently shaking the tubes to mix the content.

5.7. Note: Rapid introduction of glycerolyte can cause damage to RBCs. therefore glycerolyte should be added slowly and should be mixed well after addition.

5.8. Incubate at room temperature (18-24°C) for 5 minutes.

5.9. Add dropwise the remaining volume of glycerolyte.

5.10. Add 1ml volumes to prechilled labelled nunc tubes.

5.11. On completion of aliquoting, ensure tubes are tightly sealed and transfer to a preconditioned Mr Frosty.

5.12. Carefully transfer Mr Frosty to -80°C freezer for a minimum of twelve hours

5.13. Record details on Apheresis Fraction Processing CTM QIMR QF 75A

6. ADDITION OF CRYOSTOR CS2 TO WBC/RBC FOR CRYOPRESERVATION (PER HCT FRACTION)

6.1. Remove a 50µL sample from the cell suspension tube stored at 2-8°C

6.2. Perform total cell count (Refer to CTM QIMR SOP 38 – RBC Counting using a Haemocytometer) and record details of CTM QIMR QF 38A – RBC Calculation Form.

6.3. Calculate the volume of Cryostor CS2 that the pellet would have to be resuspended in to give a concentration of 10^6c/mL.

6.4. Centrifuge cell suspension at 530g/5-15min/ 2-8°C

6.5. Remove and discard supernatant

6.6. Agitate pellet (or gently pipette) to resuspend pellet.

6.7. Slowly add required volume of prechilled Cryostor CS2

6.8. Add 1ml volumes to prechilled labelled nunc tubes.

6.9. On completion of aliquoting, ensure tubes are tightly sealed and transfer to a preconditioned Mr Frosty.

6.10. Carefully transfer Mr Frosty to -80°C freezer for a minimum of twelve hours

6.11. Record details on Apheresis Fraction Processing CTM QIMR QF 75A

APPENDIX 9. APHERESIS COHORT 2 LABORATORY STANDARD OPERATING PROCEDURE.

Clinical Tropical Medicine, QIMR		
CTM QIMR Version:	**Malarial Enrichment via Apheresis - Form** Status:	Date Effective: Review Date: Two years after review date

Apheresis laboratory master SOP: Cohort 2

Pre-processing

23. Record all lot and catalogue numbers on QIMR- QF-75-B Malaria Parasite Isolation from Apheresis Product for volumes.

24. Spray or wipe labelled transfer bags, exchange couplers, CPDA-1 blood bags and necessary consumables into the hood using 80% v/v Ethanol.
25. Insert exchange coupler into CPDA-1 blood bag and aliquot citrate into labelled 50mL tubes.
26. Using an exchange coupler and pipette prefill labelled transferred bags with the correct citrate volumes.
    26.1. Refer to QIMR- QF-75-B Malaria Parasite Isolation from Apheresis Product for volumes.
27. Record times on QIMR-QF-75-B of when transfer bags containing citrate are prewarmed.

Collection and Waterbath setup for maintaining temperature of transfer bags at Apheresis unit

1. Refer to “Waterbath setup for blood bags”.
    1.1. Refer to and ensure to QIMR- QF-75-B Malaria Parasite Isolation from Apheresis Product for volumes is filled out for all fractions being collected.

Transfer of transfer bags from Apheresis unit to CTM

1. **“**Refer to Apheresis Thermos Blood Collection Method”.
2. Ensure “Apheresis_Thermos_Transport_Form” is filled out for each transfer of fraction of blood collected.

Processing of Apheresis Blood Fractions

1. **NB Ensure QIMR-QF-75-A Apheresis Fraction Processing is filled in .**
2. Ensure centrifuge is preheated using program 9 – 4500rpmr/HOLD/40°C.
3. Once blood bag has been transported to the waterbath in CTM and process is ready to continue, fill two large silver thermos’ with preheated water. Prepare one thermos for holding the blood bag and one thermos for holding 50ml falcons at temperature.
4. Check temperature of water and replace if below 38°C.
5. Place blood bag into thermos and move into BSC.
6. Move 50/250ml (for 1 and 2% HCT) collection tube into BSC and hold in thermos.
7. Use spike adaptor on sterile port of blood bag.
   7.1. **Note: Do not remove blood bag from thermos or lift above water level.**
8. Grab a 30/50ml preheated syringe from prewarming incubator.
9. Unscrew white cap of spike adaptor and place to the side.
10. Connect syringe to leur lock of spike adaptor.
11. Carefully syringe blood out of blood bag and dispense into 50ml/250ml collection tube.
12. Repeat process as necessary until all blood has been removed from blood bag.
13. Prepare a labelled 50ml falcon for sampling into a 50ml tube rack.
14. Mix collection tube well, either by gentle swirling or aspiration using a 25ml strippette.
15. Measure volume of HCT fraction received.
16. Aspirate an appropriate volume of sample into sampling tube. Sampling tube can be moved into another BSC for aliquoting during process.
    16.1. Refer to Master Sample list for details regarding subsampling
17. Spin 50ml/250ml collection tube in a preheated centrifuge at 1455g/5-15min/40°C

18. Replace water in thermos just before centrifuge stops spinning.
19. Collect collection tube from centrifuge with thermos and set centrifuge to preheat. (Program 9 – 4500rpm, HOLD, 40°C)
20. Place thermos back into BSC.
21. Use a strippette to remove plasma and place into a labelled collection tube.
22. Resuspend pellet and measure.
    22.1. For 1%, 2% or 3% HCT: Resuspend 650uL of pellet in 650uL of prewarmed AB serum for "Feed Mix". Remainder is to be used for subsampling.
        22.1.1.1. Refer Master Sample list for details regarding subsampling.
        22.1.2. Prepare small thermos for transportation.
        22.1.3. Place FM into floatie and transport in small thermos to PC3.
        22.1.4. Conduct Membrane Feed as per CTM QIMR SOP 15 –Infection of Mosquitoes' with plasmodium by membrane feeding
    22.2. For 5% and 7% HCT: Resuspend pellet in equal volume of RPMI and prepare thin and thick films.

Volume of Water to Add to Thermos:

PREEPARING CELL PELLETS FOR STORAGE PRIOR TO CRYOPRESERVATION (PER HCT FRACTION)

6.12. Transfer remaining pellet to prepared 25mL aliquots of pre-warmed (37°C) AB complete media.

6.13. Add additional media until total volume is 30mL.

6.14. Store at 37°C until shortly prior to cryopreservation.

6.15. Record details on Apheresis Fraction Processing CTM QIMR QF 75A

7. PRECHILLNG OF CELL SOLUTIONS FOR CRYOPRESERVATION (PER HCT FRACTION)

7.1. Immediately before pre chilling solutions, prepare a dry ice/ethanol slurry in a plastic beaker

7.2. Whilst rapidly mixing, prechill a dummy tube of WFI for a period of 50 seconds within slurry

7.3. Measure temperature of dummy tube.

7.4. If within 3-8°C, proceed with chilling of HCT Fraction cell suspension in the same manner as the dummy tube.

7.5. If not alter slurry until desired specifications can be rechecked using fresh dummy tubes prior to proceeding with rapid chilling

7.6. Wipe chilled cell suspension thoroughly with 80% v/v ethanol and transfer to ice slurry within clean biohazard hood.

7.7. Evenly split cell suspension into two labelled tubes and transfer to fridge for storage at 2-8°C

7.8. Record details on Apheresis Fraction Processing CTM QIMR QF 75A

8. ADDITION OF GLYCEROLYTE TO RBCS/WBCS FOR CRYOPRESERVATION (PER HCT FRACTION)

8.1. Calculate the total volume of glycerolyte needed (the pooled volume of the parasitised RBCs multiplied by 2.2).

8.2. Record glycerolyte lot number expiry date and volume required on Section

8.3. Centrifuge cell suspension at 530g/5-15min/ 2-8°C

8.4. Remove and discard supernatant

8.5. Agitate pellet (or gently pipette) to resuspend pellet.

8.6. Slowly add dropwise 1/5 of the total volume of glycerolyte to the pooled volume RBCs, whilst gently shaking the tubes to mix the content.

8.7. Note: Rapid introduction of glycerolyte can cause damage to RBCs. therefore glycerolyte should be added slowly and should be mixed well after addition.

8.8. Incubate at room temperature (18-24°C) for 5 minutes.

8.9. Add dropwise the remaining volume of glycerolyte.

8.10. Add 1ml volumes to prechilled labelled nunc tubes.

8.11. On completion of aliquoting, ensure tubes are tightly sealed and transfer to a preconditioned Mr Frosty.

8.12. Carefully transfer Mr Frosty to -80°C freezer for a minimum of twelve hours

8.13. Record details on Apheresis Fraction Processing CTM QIMR QF 75A

9. ADDITION OF CRYOSTOR CS2 TO WBC/RBC FOR CRYOPRESERVATION (PER HCT FRACTION)

9.1. Remove a 50µL sample from the cell suspension tube stored at 2-8°C

9.2. Perform total cell count (Refer to CTM QIMR SOP 38 – RBC Counting using a Haemocytometer) and record details of CTM QIMR QF 38A – RBC Calculation Form.

9.3. Calculate the volume of Cryostor CS2 that the pellet would have to be resuspended in to give a concentration of 10^6c/mL.

9.4. Centrifuge cell suspension at 530g/5-15min/ 2-8°C

9.5. Remove and discard supernatant

9.6. Agitate pellet (or gently pipette) to resuspend pellet.

9.7. Slowly add required volume of prechilled Cryostor CS2

9.8. Add 1ml volumes to prechilled labelled nunc tubes.

9.9. On completion of aliquoting, ensure tubes are tightly sealed and transfer to a preconditioned Mr Frosty.

9.10. Carefully transfer Mr Frosty to -80°C freezer for a minimum of twelve hours

9.11. Record details on Apheresis Fraction Processing CTM QIMR QF 75A

APPENDIX 10. APHERESIS COHORT 3 LABORATORY STANDARD OPERATING PROCEDURE.

Clinical Tropical Medicine, QIMR		
CTM QIMR Version:	**Malarial Enrichment via Apheresis - Form** Status:	Date Effective: Review Date: Two years after review date

Apheresis laboratory master SOP: Cohort 3

Pre-processing

28. Record all lot and catalogue numbers on QIMR- QF-75-B Malaria Parasite Isolation from Apheresis Product for volumes.

29. Spray or wipe labelled transfer bags, exchange couplers, CPDA-1 blood bags and necessary consumables into the hood using 80% v/v Ethanol.
30. Insert exchange coupler into CPDA-1 blood bag and aliquot citrate into labelled 50mL tubes.
31. Using an exchange coupler and pipette prefill labelled transferred bags with the correct citrate volumes.
    31.1. Refer to QIMR- QF-75-B Malaria Parasite Isolation from Apheresis Product for volumes.
32. Record times on QIMR-QF-75-B of when transfer bags containing citrate are prewarmed.

Processing of Apheresis Blood Fractions – Above 1%, 1%, 2%, 3%, 5% 7%, below 7% -20mL volumes

23. NB Ensure QIMR-QF-75-A Apheresis Fraction Processing is filled in .

24. Ensure centrifuge is preheated using program 9 – 4500rpmr/HOLD/40°C.
25. Following wiping down with 80% v/v Ethanol, place blood bag into BSC.
26. Move 50mL collection tube into BSC.
27. Use spike adaptor on sterile port of blood bag.

28. Unscrew white cap of spike adaptor and place to the side.
29. Dispense fractions into 50ml collection tube.
30. Repeat process as necessary until all blood has been removed from blood bag.
31. Prepare a labelled 50ml falcon for sampling into a 50ml tube rack.
32. Mix collection tube well, either by gentle swirling or aspiration using a 25ml strippette.
33. Measure volume of HCT fraction received.
34. Aspirate an appropriate volume of sample into sampling tube. Sampling tube can be moved into another BSC for aliquoting during process.
    34.1. Refer to Master Sample list for details regarding subsampling
35. Spin 50ml collection tube in a preheated centrifuge at 1455g/5-15min/RT
36. Use a strippette to remove plasma and place into a labelled collection tube
    36.1. Refer to Master Sample list for details regarding subsampling and slide manufacture

Pooling fractions for sampling– Above 1%, 1%, 2%, 3%, 5% 7%, below 7%: 2 mL volumes

2%,3% Pool – 5mL each, 5%,7% Pool – 5mL each

1. From each of the fractions, sample the appropriate volumes and pool into 50mL tubes
    1.1.1. Refer to Master Sample list for details regarding subsampling and slide manufacture

APPENDIX 11. APHERESIS COHORT 4 LABORATORY STANDARD OPERATING PROCEDURE.

Clinical Tropical Medicine, QIMR		
CTM QIMR Version:	**Malarial Enrichment via Apheresis - Form** Status:	Date Effective: Review Date: Two years after review date

Apheresis laboratory master SOP: Cohort 4

EXPERIMENTAL PLAN

APHERESIS DAY 1

TIME POINTS AND TASKS

PRE-APHERESIS T=0hr

- Blood collection (Q-Pharm; 1 x 6 mL EDTA, 2 x 10mL LiHep tubes)
- Pre-aliquot 19,600 µL and 39,200 µL of McCoy media into 12 x PRE-APHERESIS T75 (T=8hr, T=16hr, T=32hr, T=40hr and T=48hr (=/- NAG) and 3 x PRE-APHERESIS T225 flasks (T=4hr, T=24hr, T=48hr) respectively.
- 1 x 6mL EDTA TUBE
  - Aliquot samples for testing (18S SAF, pvs25, Sysmex)
  - Aliquot samples for and prepare thick/thin films
  - Aliquot sample for FLOW cytometry, laser capture microdissection slide (LCS)
- 1 x 10mL LIHEP TUBE:
  - Prepare feed mix and perform MFA
  - Set up 3 x T225 MFA culture flasks (T=4hr, T=24hr, T=48hr)
- 1x10mL LiHEP TUBE:
  - Set up 12 x T75 asexual/sexual culture flasks (+/- NAG) (T=8hr, T=16hr, T=24hr, T=32hr, T=40hr, T=48hr)
  - Aliquot sample for pLDH

INTERMEDIATE T=0hr, FINAL T=0hr, SPARE T=0hr (*optional*), WASTE T=0hr

- Intermediate, Final, Spare (*optional*)and Waste sample collection (Apheresis Unit)
- Aliquot samples for testing (18S SAF, pvs25, Sysmex)
- Aliquot samples for and prepare thick/thin films
- Aliquot sample for FLOW cytometry
- Prepare feed mix and perform MFA
- Set up 3 x T225 MFA cultures (T=4hr, T=24hr, T=48hr)
- Set up 4 x T75 Asexual/Sexual cultures (+/- NAG) (T=24hr, T=48hr)
- Aliquot sample for pLDH

PRE-APHERESIS T=4hr

- Prepare feed mix from 1 x T=4hr T225 flask and perform MFA
- Aliquot sample for and prepare thin films from T=4hr T225 flask
- Aliquot sample for DNA, RNA, FLOW cytometry from T=4hr T225 flask

INTERMEDIATE T=4hr

- Prepare feed mix from 1 x T=4hr T225 flask and perform MFA
- Aliquot sample for and prepare thin film from T=4hr T225 flask
- Aliquot sample for DNA, RNA, FLOW cytometry from T=4hr T225 flask

PRE-APHERESIS T=8hr

- Aliquot samples for pLDH from 2 x T=8hr T75 flasks (+/- NAG)
- Aliquot samples for and prepare thin film from 2 x T=8hr T75 flasks (+/- NAG)
- Aliquot samples for DNA, RNA, FLOW cytometry from 2 x T=8hr T75 flasks (+/- NAG)

PRE-APHERESIS T=16hr

- Aliquot samples for pLDH from 2 xT=16hr T75 flasks (+/- NAG)
- Aliquot sample for and prepare thin films from 2x T=16hr T75 flasks (+/- NAG)

- Aliquot sample for DNA, RNA, FLOW cytometry from 2 x T=16hr T75 flasks (+/- NAG)

APHERESIS DAY 2

TIME POINTS AND TASKS

PRE-APHERESIS T=24hr; INTERMEDIATE T=24hr; FINAL T=24hr; WASTE T=24hr

For each time point sample type:

- Prepare feed mix from T=24hr T225 flask and perform MFA
- Aliquot sample for and prepare thin film from T=24hr T225 flask
- Aliquot sample for DNA, RNA from T=24hr T225 flask
- Aliquot sample for pLDH from 2 x T=24hr T75 flasks (+/- NAG)
- Aliquot sample for and prepare thin films from 2 x T=24hr T75 flasks (+/- NAG)
- Aliquot sample for DNA, RNA, FLOW cytometry from 2 x T=24hr T75 flasks (+/- NAG)

PRE-APHERESIS T=32hr

- Aliquot sample for pLDH from 2 x T=32hr T75 flasks (+/- NAG)
- Aliquot sample for and prepare thin film from 2 x T=32hr T75 flasks (+/- NAG)
- Aliquot sample for DNA, RNA, FLOW cytometry from 2 x T=32hr T75 flasks (+/- NAG)

PRE-APHERESIS T=40hr

- Aliquot sample for pLDH from 2 x T=40hr T75 flasks (+/- NAG)
- Aliquot sample for and prepare thin film from 2 x T=40hr T75 flasks (+/- NAG)
- Aliquot sample for DNA, RNA, FLOW cytometry from 2 x T=40hr T75 flasks (+/- NAG)

MEDIA CHANGES

- Perform 50% media change on remaining T=48hr T225 flasks

- Perform 50% media change on remaining T=32hr; T=40hr; T=48hr T75 flasks

APHERESIS DAY 3

TIME POINTS AND TASKS

PRE-APHERESIS T=48hr; INTERMEDIATE T=48hr; FINAL T=24hr; SPARE (*optional*) T=24hr; WASTE T=24hr

For each time point sample type:

- Prepare feed mix from T=48hr T225 flask and perform MFA
- Aliquot sample for and prepare thin/thick films from T=48hr T225 flask
- Aliquot sample for DNA, RNA, FLOW cytometry from T=48hr T225 flask
- Aliquot sample for pLDH from 2 x T=48hr T75 flasks (+/- NAG)
- Aliquot sample for and prepare thin film from 2 x T=48hr T75 flasks (+/- NAG)
- Aliquot sample for DNA, RNA, FLOW cytometry from 2 x T=48hr T75 flask (+/- NAG)

PROCEDURE

APHERESIS DAY 1

PRE-APHERESIS T=0hr BLOOD COLLECTION (Q-Pharm; **1 x 6mL EDTA tube, 2 x10mL LiHep tubes**)

PRE-BLOOD COLLECTION PROCESS

1. Turn on water baths in the CTM general lab (set 38.5°C) and PC3 (set 40°C).
2. Turn on heat blocks in TC (set 40°C).
3. Configure Mediheat unit for sample transport.
4. Collect apheresis sampling labels, Mediheat unit, esky, APH kit and take over to Apheresis Ward.
5. Once at the ward, set up the Mediheat unit as per SOP and label collection bags.
6. Return to CTM lab and prepare for pre-apheresis blood sample transport from Q-Pharm.
7. Clean BSC with 80% ethanol.
8. Pre-aliquot 19,600 µL and 39,200 µL of McCoy media into pre-labelled 12 x PRE-APHERESIS T75 (T=8hr, T=16hr, T=32hr, T=40hr and T=48hr (+/- NAG) and 3 x PRE-APHERESIS T225 flasks (T=4hr, T=24hr, T=48hr) respectively.
9. Following phone call from Q-Pharm, prepare for blood collection.
10. Fill required number of thermos flasks with a pre-measured volume (650mL) of pre-heated water. Ensure a spare thermos flask is also filled.
11. Place no more than 2 thermos flasks per esky. Bring a spare esky for collection of blood tubes and beakers.
12. Head to Q-Pharm, Level 5 CBCRC and gain entry from reception. Collect pre-heated blood tubes and beakers. Transport to Level 5 CBCRC.
13. Place a maximum of 4 x 10mL tubes per thermos flask. Place a maximum of 5 x 6mL tubes per thermos flask.
14. ***Note: Tubes will need to be inverted upon collection from Q-Pharm staff. Invert tubes 3-4 times.***
15. **Record blood draw and collection time on transport form** QIMRB CTM QF-15-C.

16. Transport tubes to Tissue Culture lab on level 13 Bancroft and place blood tubes in waterbath set to 38.5°C.

ALIQUOT PRE-APHERESIS T=0hr SAMPLES FROM EDTA TUBE

1. Wipe blood tube into BSC with 80% ethanol.
2. Invert blood collection tubes to mix, then transfer the contents of the EDTA blood collection tube into a 50mL tube and the contents of the two LiHep blood collection tubes into two separate 50mL tubes.
3. Aliquot samples for **18S, pvs25, Sysmex, thin/thick films, laser capture (LCS) and FLOW** from EDTA 50 mL tube according to **Table 1**, mixing well between aliquots. **Record, Initial and date.**

Sample Source	Sample Type	No. Aliquots	Sample Volume (µL)	Test	Buffer Vol. (µL)	Initial & Date
Pre-Apheresis	QPID	**3**	**250**	18S_SAF	400	
Pre-Apheresis	QPID	**3**	**250**	pvs25	1250	
Pre-Apheresis	Sysmex	**1**	**1000**	Blood count	-	
Pre-Apheresis	Thin Film	**1**	**50**	Parasitemia	-	
Pre-Apheresis	Thick Film	**1**	**50**	Parasitemia	-	
Pre-Apheresis	Flow	**1**	**500**	Parasitemia	-	
Pre-Apheresis	LCS	**1**	**1500**	Molecular	-	

4. Store samples as per **Table 2**. **Record time stored, initial/date.**
5. Prepare 3 x thick and 3 x thin blood films from EDTA sub-sample tube. Refer to SOP-MBE-1.

6. Store films as per **Table 2**. **Record time stored, initial/date.**
7. Prepare blood films for LCS according to Experimental Plan (GR).

Table 2. PRE-APHERESIS T=0hr Sample Storage

Sample Source	Sample Type	Storage condition	Storage location	Storage location details (e.g. KT freezer, Shelf 1, Box 1)	Time stored	Initial & Date
Pre-Apheresis	QPID	-80°C	-80°C freezer			
Pre-Apheresis	QPID	-80°C	-80°C freezer			
Pre-Apheresis	Sysmex	4-8°C	TC blood fridge			
Pre-Apheresis	Flow	4-8°C	Flow room fridge			
Pre-Apheresis	Thin Films	Ambient	Labelled Slide Box			
Pre-Apheresis	Thick Films	Ambient	Labelled Slide Box			
Pre-Apheresis	LCS	Ambient	Labelled Slide Box			

PRE-APHERESIS T=0hr MEMBRAME FEED ASSAY (MFA) ~ 9:30AM

Note: Keep sample heated throughout process.

1. Centrifuge 1 x 50mL LiHep tube at 530xg for 5 mins at 40°C in a pre-heated centrifuge.
2. While sample is spinning, aliquot 1.3mLof AB serum into labelled 2mL eppendorf tube.
3. Remove supernatant from sample and discard.

4. Aliquot 650µl of packed infected red blood cells (RBCs) into eppendorf tube and mix.
5. Fill small thermos flask with pre-heated water.
6. Place feed mix tube in blue floaty and transport to PC3 in thermos flask.
7. Perform MFA as per CTM QIMR SOP 15.

Day 1 NF54 CONTROL MFA

Note: Prepare NF54 feed-mix at the same time as PRE-APHERESIS MFA process above.

1. Remove 15mL of culture from gametocyte plate into pre-warmed 50mL tube.
2. Centrifuge at 530xg for 5 mins at 40°C in a pre-warmed centrifuge.
3. Aliquot 1.3mL of AB serum into a 2mL Eppendorf tube.
4. Fill small thermos flask to 1/2 - 2/3 total volume with pre-heated water.
5. Once sample has been centrifuged, remove supernatant.
6. Add 650µL of packed infected red blood cells into 2mL tube and mix well.
7. Place 2mL eppendorf tube into blue floaty and then into small thermos flask.

PRE-APHERESIS T=0hr T225 MFA CULTURE SET-UP

1. After the feed-mix is prepared, set up 3 x Pre-Apheresis T225 MFA Culture flasks (T=4hr, T=24hr and T=48hr).
2. Mix the RBC pellet in the same 50mL tube used to prepare the feed mix and transfer 800µL packed RBCs into each of the T225 flasks (Pre-Apheresis T225 MFA Culture flask T=4hr, T=24hr and T=48hr).
3. Gas each flask for 1 min at ≥ 10L/min.
4. Wipe flasks with 80% ethanol before placing in the incubator.
5. **Place the T=48hr flask in the vertical position.**
6. **Record the volume of packed red cells and the actual culture set-up time/date.**

PRE-APHERESIS T=0hr T75 ASEXUAL/SEXUAL PARASITE CULTURE SET-UP

1. Set up 12 x PRE-APHERESIS T75 (T=8hr, T=16hr, T=32hr, T=40hr and T=48hr (+/- NAG).

2. Centrifuge the second 50mL LiHep tube at 530xg for 5 mins at 40°C in a pre-heated centrifuge.
3. Remove supernatant from sample and discard.
4. Mix pellet and transfer 400µL of packed red blood cells into each of the twelve T75 flasks pre-filled with media (T=8hr, T=16hr, T=24hr, T=32hr, T=40hr, T=48hr +/- NAG).
5. Gas each flask for 30sec at 7-10L/min.
6. Wipe flasks with 80% ethanol before placing in the incubator.
7. **Place T=32hr, T=40hr and T=48hr flask +/- NAG in vertical position** (6 T75 flasks in total).
7. **Record the volume of packed red cells and the actual culture set-up time/date.**
8. Aliquot sample for pLDH.

INTERMEDIATE T=0hr SUB-SAMPLE COLLECTION (Apheresis Ward)

Note: A Red Cell Depletion process will be run by the Apheresis Nurse on an apheresis unit (RBWH; Apheresis Ward). A sub-sample (INTERMEDIATE sample; 20-25mL) will be harvested from the collection bag. Lab staff from CTM Bancroft 13 lab will be called to retrieve the sample.

1. Using pre-heated water, fill a thermos flask of appropriate size with appropriate insert and place in an esky. Transport the esky to the Apheresis ward.
2. Transport the INTERMEDIATE sub-sample to CTM Bancroft Level 13 lab in the thermos flask within the esky.
   Note: Citrate may be sterilely injected into bag after process if concerns of clotting arise. **The Apheresis Nurse will transfer Red Blood Cell Depletion to a Bone Marrow Processing accessory set for the PMN apheresis process (i.e. for collection of FINAL AND WASTE samples).**

DETERMINE SAMPLE VOLUME AND THE HAEMATOCRIT OF THE INTERMEDIATE T=0hr SUB-SAMPLE

1. Following wiping down with 80% Ethanol, place sample into BSC.

2. Prepare a labelled 50mL falcon tube for sampling into a 50mL tube rack.
3. Move labelled 50mL collection tube into BSC.
4. Mix sample tube well, either by gentle swirling or aspiration using a strippette.
5. Measure volume of sample received using a strippette and **record in Table 5.**
6. Dispense the sample aseptically into 50mL collection tube.

Table 5. Sample volume received following apheresis process

Sample Source	Time Received	Total volume of sample (mL)	Date	Initials
Intermediate				
Final				
Spare				
Waste				

7. Mix collection tube well, either by gentle swirling or aspiration using a strippette.
8. Aliquot 100µL of sample into 1.5mL eppendorf tube for Coulter Counter sampling.
9. Perform Coulter Counter analysis on Level 9 CBCRC.
10. Once haematocrit is determined, contact either Anand (0411 041 391) or Kari (0438 736 371) to inform of the result.

ALIQUOT INTERMEDIATE T=0hr SAMPLES FOR TESTING

1. Aliquot samples for **18S, pvs25, Sysmex, thin/thick films, FLOW cytometry, MFA/T225 MFA culture set up, T75 Asexual/Sexual culture set up** according to **Table 6**, mixing well between aliquots. **Record Initial and date.**

Sample Source	Sample Type	No. Aliquots	Sample Volume (µL)	Test	Buffer Volume (µL)	Initial & Date

Intermediate	QPID	**3**	**250**	18S_SAF	400	
Intermediate	QPID	**3**	**250**	pvs25	1250	
Intermediate	Sysmex	**1**	**1000**	Blood count	-	
Intermediate	Thin Film	**1**	**50**	Parasitemia	-	
Intermediate	Thick Film	**1**	**50**	Parasitemia	-	
Intermediate	Flow	**1**	**500**	Parasitemia	-	
Intermediate	Asexual/Sexual Culture	**1**	**10,000**	Growth	-	
Intermediate	MFA	**1**	**10,000**	Transmission	-	

2. Store samples as per **Table 7**.
3. Prepare 3 x thick and 3 x thin blood films. Refer to SOP-MBE-1.
4. Store blood films as per **Table 7**.

Table 7. INTERMEDIATE T=0hr Sample Storage

Sample Source	Sample Type	Storage condition	Storage location	Time stored	Initial & Date
Intermediate	QPID	-80°C	-80°C freezer		
Intermediate	QPID	-80°C	-80°C freezer		
Intermediate	Sysmex	4-8°C	TC blood fridge		
Intermediate	Flow	4-8°C	Flow room fridge		
Intermediate	Thin Films	Ambient	Labelled Slide Box		
Intermediate	Thick Films	Ambient	Labelled Slide Box		

INTERMEDIATE T=0hr MFA ~ 12:00PM

Note: *Keep sample heated throughout process.*

1. Centrifuge 10mL of INTERMEDIATE sub-sample (in 50mL tube) at 530xg for 5 mins at 40°C in a pre-heated centrifuge.
2. While sample is spinning, aliquot 1.3mLof AB serum into labelled 2mL eppendorf tube.
3. Remove supernatant from sample and discard.
4. Aliquot 650µl of packed infected red blood cells into eppendorf tube and mix.
5. Fill small thermos flask with pre-heated water.
6. Place feed mix tube in blue floaty and transport to PC3 in thermos flask.
7. Perform MFA as per CTM QIMR SOP 15.

INTERMEDIATE T=0hr T225 MFA CULTURE SET-UP

1. After the feed-mix is prepared, aliquot 39,200 µL McCoy media into 3 x INTERMEDIATE T225 MFA Culture flasks (T=4hr, T=24hr and T=48hr).
2. Mix the RBC pellet in the same 50mL tube used to prepare the feed mix and transfer 800µL packed RBCs into each of the 3 x INTERMEDIATE T225 flasks (T=4hr, T=24hr and T=48hr).
3. Gas each flask for 1 min at ≥ 10L/min.
4. Wipe flasks with 80% ethanol before placing in the incubator.
5. **Place the T=48hr flask in the vertical position.**
6. **Record the volume of packed red cells and the actual culture set-up time/date.**

INTERMEDIATE T=0hr T75 ASEXUAL/SEXUAL PARASITE CULTURE SET-UP

1. Aliquot 19,600 µL McCoy media (+/- NAG) into 4 x INTERMEDIATE T75 flasks (T=24hr, T=48hr +/- NAG).
2. Centrifuge 10mL of INTERMEDIATE sub-sample at 530xg for 5 mins at 40°C in a pre-heated centrifuge.
3. Remove supernatant from sample and discard.

4. Mix pellet and transfer 400µL of packed red blood cells into 4 xT75 flasks pre-filled with media (T=24hr, T=48hr +/- NAG).
5. Gas each flask for 30 sec at 7-10L/min.
6. Wipe flasks with 80% ethanol before placing in the incubator.
6. **Record the volume of packed RBCs and the actual culture set-up time/date.**
7. **Place the T=48hr flasks (+/- NAG) in the vertical position** (2 T75 flasks in total).
8. Aliquot sample for pLDH.

PRE-APHERESIS T=4hr MFA AND SAMPLING (T225 FLASK) ~ 1:30PM

Note: *One hour prior to preparing feed mix, pre-warm the centrifuge, thaw an aliquot of AB serum, rack up pre-labelled tubes for sampling (DNA, RNA, pLDH, FLOW cytometry) and have slides available for preparing blood films in the work station.*

1. Remove the PRE-APHERESIS T225 T=4hr flask from incubator.
2. Wipe all the surfaces with 80% ethanol.
3. Transfer the entire contents of the flask into a 50 mL tube.
4. Centrifuge at 530xg for 5 mins at 40°C in a pre-warmed centrifuge.
5. While sample is spinning, aliquot 1.3mL of AB serum into labelled 2mL eppendorf tube.
6. Remove 90% of the supernatant from sample and discard.
7. Aliquot 650µl of packed infected red blood cells into eppendorf tube and mix.
8. Fill a small thermos flask with pre-heated water.
9. Place feed mix tube in blue floaty and transport to PC3 in thermos flask.
10. Perform membrane feed assay as per CTM QIMR SOP 15.
11. Aliquot the remaining packed RBC transferring 100µL in DNA tubes (containing 400 µL lysis buffer) and 100µL in RNA tubes (containing 1000 µL RNACell protect) and 50µL for FLOW cytometry.
12. Store samples

13. Aliquot 10µL for preparing thin blood film.
14. Prepare thin blood film. **Store blood film.**
15. **Record pRBC volume, actual date and time.**

FINAL, SPARE *(optional)* & WASTE T=0hr SAMPLE COLLECTION (Apheresis Ward)

1. Label BMP accessories set bag with "WASTE" label, ensure label is over existing label.
2. Place collection bags in Mediheat transport unit.
   Note: *While collecting sample, make sure to frequently mix the bag. The Apheresis Nurse will run PMN collection process on apheresis machine and collect 100mL of first fraction based on collection colour into a bag labelled "FINAL". A decision will be made to collect a further 100mL if possible, into a bag labelled "SPARE". Bags will be heat sealed prior to removal from Apheresis Ward.*
3. Place all bags including BMP accessories set/WASTE bag in Mediheat for transport to CTM Bancroft Level 13.

FINAL, SPARE *(optional)* & WASTE T=0hr SUB-SAMPLING FOR TESTING

1. Following wiping down with 80% v/v Ethanol, place bags into BSC.
2. Prepare a labelled 50mL falcon tubes for sampling into a 50mL tube rack.
3. Move labelled 50mL tubes into BSC.
4. Mix sample bag well.
5. Dispense sample aseptically into 250mL tube using a sterile spike adaptor.
6. Measure volume of sample received using a strippette/pipette and record in **Table 8.**

Table 8. Sample volume received following apheresis process

Sample Source	Time Received	Total volume of sample (mL)	Date	Initials
Intermediate				
Final				
Spare				

Waste				

7. For each sample source, aliquot samples for **18S, pvs25, Sysmex, thin/thick films, FLOW cytometry, MFA/T225 MFA culture set up, T75 Asexual/Sexual culture set up according to Table 9**, mixing well between aliquots. **Initial and date.**

Table 9. FINAL BAG, SPARE BAG (optional) and WASTE BAG T=0hr Sampling						
Sample Source	**Sample Type**	**No. Aliquots**	**Sample Volume (µL)**	**Test**	**Buffer Volume (µL)**	**Initial & Date**
Final	QPID	**3**	**250**	18S_SAF	400	
Final	QPID	**3**	**250**	pvs25	1250	
Final	Sysmex	**1**	**1000**	Blood count	-	
Final	Thin Film	**1**	**50**	Parasitemia	-	
Final	Thick Film	**1**	**50**	Parasitemia	-	
Final	Flow	**1**	**500**	Parasitaemia	-	
Final	T75 Asexual/Sexual Culture	**1**	**10,000**	Growth	-	
Final	MFA/T225 MFA Culture	**1**	**10,000**	Transmission	-	
Spare	QPID	**3**	**250**	18S_SAF	400	
Spare	QPID	**3**	**250**	pvs25	1250	
Spare	Sysmex	**1**	**1000**	Blood count	-	
Spare	Thin Film	**1**	**50**	Parasitemia	-	
Spare	Thick Film	**1**	**50**	Parasitemia	-	

Spare	Flow	**1**	**500**	Parasitaemia	-	
Waste	QPID	**3**	**250**	18S_SAF	400	
Waste	QPID	**3**	**250**	pvs25	1250	
Waste	Sysmex	**1**	**1000**	Blood count	-	
Waste	Thin Film	**1**	**50**	Parasitemia	-	
Waste	Thick Film	**1**	**50**	Parasitemia	-	
Waste	Flow	**1**	**500**	Parasitaemia	-	
Waste	T75 Asexual/Sexual Culture	**1**	**10,000**	Growth	-	
Waste	MFA/T225 MFA Culture	**1**	**10,000**	Transmission	-	

8. Store samples as per **Table 10**.
9. For each sample source, prepare 3 x thick and 3 x thin blood films. Refer to SOP-MBE-1.
10. **Store films as per Table 10. Record time stored, initial/date.**

Table 10. FINAL, SPARE (optional) AND WASTE T=0hr SAMPLE STORAGE					
Sample Source	**Sample Type**	**Storage condition**	**Storage location**	**Time stored**	**Initial & Date**
FINAL					
Final	QPID 18S_SAF	-80°C	-80°C freezer		

Final	QPID pvs25	-80°C	-80°C freezer		
Final	Sysmex	4-8°C	TC blood fridge		
Final	Flow	4-8°C	Flow room fridge		
Final	Thin Film	Ambient	Labelled Slide Box		
Final	Thick Film	Ambient	Labelled Slide Box		
WASTE					
Waste	QPID 18S_SAF	-80°C	-80°C freezer		
Waste	QPID pvs25				
Waste	Sysmex	4-8°C	TC blood fridge		
Waste	Flow	4-8°C	Flow room fridge		
Waste	Thin Film	Ambient	Labelled Slide Box		
Waste	Thick Film	Ambient	Labelled Slide Box		
OPTIONAL SPARE					
Spare	QPID 18S_SAF	-80°C	-80°C freezer		
Spare	QPID pvs25	-80°C	-80°C freezer		
Spare	Sysmex	4-8°C	TC blood fridge		

Spare	Flow	4-8°C	Flow room fridge		
Spare	Thin Film	Ambient	Labelled Slide Box		
Spare	Thick Film	Ambient	Labelled Slide Box		

FINAL & WASTE T=0hr MFA ~ 2:00PM

***Note**: Keep sample heated throughout process.*

1. Centrifuge 10mL of FINAL, SPARE (optional) and WASTE sub-sample at 530xg for 5 mins at 40°C in a pre-heated centrifuge.
2. While sample is spinning, aliquot 1.3mL of AB serum into labelled 2mL eppendorf tube.
3. Remove supernatant from sample and discard.
4. Aliquot 650µl of packed infected RBCs into eppendorf tubes and mix.
5. Fill small thermos flask with pre-heated water.
6. Place feed mix tubes in blue floaty and transport to PC3 in thermos flask.
7. Perform MFA as per CTM QIMR SOP 15.

FINAL & WASTE T=0hr T225 MFA CULTURE SET-UP

1. After the feed-mix is prepared, aliquot 39,200 µL McCoy media into 4 x FINAL and WASTE T225 MFA Culture flasks (T=24hr and T=48hr).
2. Mix the RBC pellet in the same 50mL tube used to prepare the feed mix and transfer 800µL packed RBCs into each of the T225 flasks (FINAL and WASTE T225 MFA Culture flask T=24hr and T=48hr).
3. Gas each flask for 1 min at 10L/min.
4. Wipe flasks with 80% ethanol before placing in the incubator.
5. **Place the T=48hr flask in the vertical position.**
6. **Record the volume of packed red cells and the actual culture set-up time/date.**

FINAL, SPARE (optional) & WASTE T=0hr T75 ASEXUAL/SEXUAL PARASITE CULTURE SET-UP

1. Aliquot 19,600 µL McCoy media (+/- NAG) into 8 x FINAL and WASTE T75 Asexual/Sexual Culture flasks (T=24hr, T=48hr +/- NAG).
2. Centrifuge 10mL of FINAL and WASTE sub-sample and centrifuge at 530xg for 5 mins at 40°C in a pre-heated centrifuge.
3. Remove supernatant from sample and discard.
4. Mix pellet and transfer 400µL of packed red blood cells into each of the 8 x T75 flasks pre-filled with media (T=24hr, T=48hr +/- NAG).
5. Gas each flask for 30 sec at 7-10L/min.
6. Wipe flasks with 80% ethanol before placing in the incubator.
7. **Record the volume of packed RBCs and the actual culture set-up time/date.**
8. **Place the T=48hr flasks (+/- NAG) in the vertical position (2 T75 flasks in total).**
9. Aliquot sample for pLDH**.**

DELIVER SYSMEX SAMPLES TO PATHOLOGY QUEENSLAND

1. Once all samples are collected for SYSMEX analysis, place relevant samples (with completed sample request form) in a bag in an esky and deliver to Pathology QLD no later than 4:00pm.

INTERMEDIATE T=4hr MFA (T225 FLASK) ~ 4:00PM

Note: *One hour prior to preparing feed mix, pre-warm the centrifuge, thaw an aliquot of AB serum, rack up pre-labelled tubes for sampling (DNA, RNA, pLDH, FLOW cytometry) and have slides available for preparing blood films in the work station.*

1. Remove the INTERMEDIATE T225 T=4hr MFA flask from incubator.
2. Wipe all the surfaces with 80% ethanol.
3. Transfer the entire contents of the flask into a 50 mL tube.
4. Centrifuge at 530xg for 5 mins at 40°C in a pre-warmed centrifuge.

5. While sample is spinning, aliquot 1.3mL of AB serum into labelled 2mL eppendorf tube.
6. Remove 90% of the supernatant from sample and discard.
7. Aliquot 650µl of packed infected red blood cells into eppendorf tube and mix.
8. Fill a small thermos flask with pre-heated water.
9. Place feed mix tube in blue floaty and transport to PC3 in thermos flask.
10. Perform membrane feed assay as per CTM QIMR SOP 15.
11. Aliquot the remaining packed RBC transferring 100µL in DNA tubes (containing 400 µL lysis buffer) and 100µL in RNA tubes (containing 1000 µL RNAcell protect).
12. Store samples.
13. Aliquot 10µL for preparing blood films.
14. Prepare thin blood slide.

PRE-APHERESIS T=8hr T75 SAMPLING ~ 5:30PM

Note: Sampling will be performed every 8 hours after culture set up (T=8hr, T=16hr, T=24hr, T=32hr, T=40hr and T=48hr). **Table 13** will be generated with actual times to harvest at the end of APHERESIS DAY 1 after all samples have been received.

1. Ten minutes before the set-up time, prepare/rack up sampling tubes (DNA, RNA, Flow and pLDH) and have slides available for preparing blood films in the workstation.
2. Verify time-point and the type of media in the label of the 2 X T75 flasks.
3. Remove the 2 x PRE-APHERESIS T=8hr T75 flasks (+/- NAG) from incubator.
4. Wipe all the surfaces with 80% ethanol.
1. Transfer the entire contents of each flask into a pre-labelled 50mL tube.
2. Centrifuge tubes at 530xg for 5 mins at 40°C and remove and discard 90% of supernatant
15. For each flask, aliquot packed RBCs transferring 150µL in pLDH tubes (O-Ring tubes empty) 100µL in DNA tubes (containing 400 µL lysis buffer) and

100µL in RNA tubes (containing 1000 µL RNAcell protect) and 50µL for FLOW cytometry**.**

16. Store samples.
17. Aliquot 10µL for preparing blood films.
18. Prepare 2 x thin blood films.
19. **Record pRBC volume, actual date and time.**

PRE-APHERESIS T=16hr T75 SAMPLING ~ 1:30AM

1. Ten minutes before the set-up time, prepare/rack up sampling tubes (DNA, RNA, flow and pLDH) and have slides available for preparing blood films in the workstation.
2. Verify time-point and the type of media in the label of the 2 X T75 flasks.
3. Remove the 2 x PRE-APHERESIS T=16hr T75 flasks (+/- NAG) from incubator.
4. Wipe all the surfaces with 80% ethanol.
3. Transfer the entire contents of each flask into a pre-labelled 50mL tube.
4. Centrifuge tubes at 530xg for 5 mins at 40°C and remove and discard 90% of supernatant
20. For each flask, aliquot packed RBCs transferring 150µL in pLDH tubes (O-Ring tubes empty) 100µL in DNA tubes (containing 400 µL lysis buffer) and 100µL in RNA tubes (containing 1000 µL RNAcell protect) and 50µL for FLOW cytometry.
21. Store samples as per
22. Aliquot 10µL for preparing blood films.
23. Prepare 2 x thin blood films.

APPENDIX 12. SUPPLEMENTARY MATERIAL.

Table 1. Asexual parasites/mL and asexual parasites/RBC apheresis clinical study

Subject	Sample Type	Asexual parasites/mL	fold enrichment asexual parasites compared to pre-apheresis	Red Cell Count	asexual parasites/RBC	fold enrichment asexual parasites/RBC compared to pre-apheresis
1	Pre-apheresis	11,497	1	4.35E+09	2.64E-06	1.00
	1% HCT	27,394	2.38	1.40E+08	1.96E-04	74.03
	2% HCT	37,064	3.22	1.90E+08	1.95E-04	73.81
	3% HCT	41,945	3.65	3.00E+08	1.40E-04	52.90
	5% HCT	46,105	4.01	4.30E+08	1.07E-04	40.57
	7% HCT	56,180	4.89	5.90E+08	9.52E-05	36.03
2	Pre-apheresis	24,100	1	4.81E+09	5.01E-06	1.00
	1% HCT	21,409	0.89	2.00E+08	1.07E-04	21.36
	2% HCT	27,022	1.12	3.50E+08	7.72E-05	15.41
	3%HCT	34,406	1.43	2.60E+08	1.32E-04	26.41
	5% HCT	38,646	1.6	4.30E+08	8.99E-05	17.94
	7% HCT	43,480	1.8	5.00E+08	8.70E-05	17.36
3	Pre-apheresis	25,475	1	5.34E+09	4.77E-06	1.00
	0.5% HCT	46,009	1.81	7.00E+07	6.57E-04	137.77
	1% HCT	46,212	1.81	1.40E+08	3.30E-04	69.19
	2% HCT	50,077	1.97	1.50E+08	3.34E-04	69.98
	3% HCT	68,441	2.69	3.50E+08	1.96E-04	40.99
	5% HCT	51,600	2.03	4.40E+08	1.17E-04	24.58
	7% HCT	80,024	3.14	7.70E+08	1.04E-04	21.78
	11% HCT	100,951	3.96	1.23E+09	8.21E-05	17.20
	2/3% HCT	90,662	3.56	2.60E+08	3.49E-04	73.09
	5/7% HCT	95,699	3.76	6.10E+08	1.57E-04	32.89
	0.5-11% HCT	94,785	3.72	4.50E+08	2.11E-04	44.15
4	Pre-apheresis	94,965	1	5.22E+09	1.82E-05	1.00
	Intermediate	108,041	1.15	7.18E+09	1.50E-05	0.83
	Final	107,251	1.14	2.90E+08	3.70E-04	20.33
	Spare	74,149	0.79	4.80E+08	1.54E-04	8.49
	Waste	36,059	0.38	3.94E+09	9.15E-06	0.50

Asexual parasites/mL and asexual parasites/RBC apheresis clinical study data from subjects 1 to 4 including fold enrichments as measured by 18S qPCR.

Table 2. Gametocytes/mL and gametocytes/RBC apheresis clinical study

Subject	Sample	gametocytes/ mL	fold enrichment gametocytes/ ml compared to pre-apheresis	Red Cell Count	gametocytes/ RBC	fold enrichment gametocytes/RBC compare to pre-apheresis
1	Pre-apheresis	26,458	1.00	4.35E+09	6.08E-06	1.00
	1% HCT	454	0.02	1.40E+08	3.24E-06	0.53
	2% HCT	952	0.04	1.90E+08	5.01E-06	0.82
	3% HCT	1,186	0.04	3.00E+08	3.95E-06	0.65
	5% HCT	1,388	0.05	4.30E+08	3.22E-06	0.53
	7% HCT	1,146	0.04	5.90E+08	1.94E-06	0.32
	Percoll	9,396	0.36	N/A	N/A	N/A
2	Pre-apheresis	88,670	1.00	4.81E+09	1.84E-05	1.00
	1% HCT	3,216	0.04	2.00E+08	1.61E-05	0.87
	2% HCT	2,871	0.03	3.50E+08	8.20E-06	0.44
	3%HCT	2,246	0.03	2.60E+08	8.64E-06	0.47
	5% HCT	2,429	0.03	4.30E+08	5.65E-06	0.31
	7% HCT	3,907	0.04	5.00E+08	7.81E-06	0.42
	Percoll	2,064,260	23.28	N/A	N/A	N/A
3	Pre-apheresis	114,495	1.00	5.34E+09	2.14E-05	1.00
	0.5% HCT	9,283	0.08	7.00E+07	1.33E-04	6.19
	1% HCT	12,573	0.11	1.40E+08	8.98E-05	4.19
	2% HCT	10,983	0.10	1.50E+08	7.32E-05	3.42
	3% HCT	9,390	0.08	3.50E+08	2.68E-05	1.25
	5% HCT	8,100	0.07	4.40E+08	1.84E-05	0.86
	7% HCT	10,999	0.10	7.70E+08	1.43E-05	0.67
	11% HCT	13,472	0.12	1.23E+09	1.10E-05	0.51
	2/3% HCT	11,035	0.10	2.60E+08	4.24E-05	1.98
	5/7% HCT	9,097	0.08	6.10E+08	1.49E-05	0.70
	0.5-11% HCT	15,054	0.13	4.50E+08	3.35E-05	1.56
	Percoll	3,618,500	44.8	N/A	N/A	N/A
4	Pre-apheresis	547,975	1.00	5.22E+09	1.05E-04	1.00
	Intermediate	794,329	1.45	7.18E+09	1.11E-04	1.05
	Final	102,192	0.19	2.90E+08	3.52E-04	3.36
	Spare	304,977	0.56	4.80E+08	6.35E-04	6.05
	Waste	90,743	0.17	3.94E+09	2.30E-05	0.22

Gametocytes/mL and gametocytes/RBC apheresis clinical study data from subjects 1 to 4 including fold enrichments as measured by *pvS25* qPCR.

Human malaria parasite bank formation calculations

The maximum level of asexual parasite enrichment per mL of sample compared to pre-apheresis was 4.9-fold. Percoll concentration increases the theoretical level of enrichment to 15.5-fold compared to pre-apheresis.

The HMP013 *P. vivax* parasite bank produces vials each of which contains 2.08×10^6 parasites. The following formulas were used to calculate the viability of apheresis as a method to concentrate parasites. Maximum enrichment values were used as the purpose was to assess the possibility of producing a HMP bank using apheresis rather than the practicality.

Parasites/mL of enriched blood = Pre-apheresis whole blood 18S qPCR/mL × Fold enrichment derived from percoll × Maximum fold enrichment from apheresis

Parasites/mL of packed blood (based on bank HCT of 0.39) = Parasites/mL of enriched blood/0.39

Number of parasites/vial compared based on 248 µL = (Parasites/mL of packed blood/1000) × 248

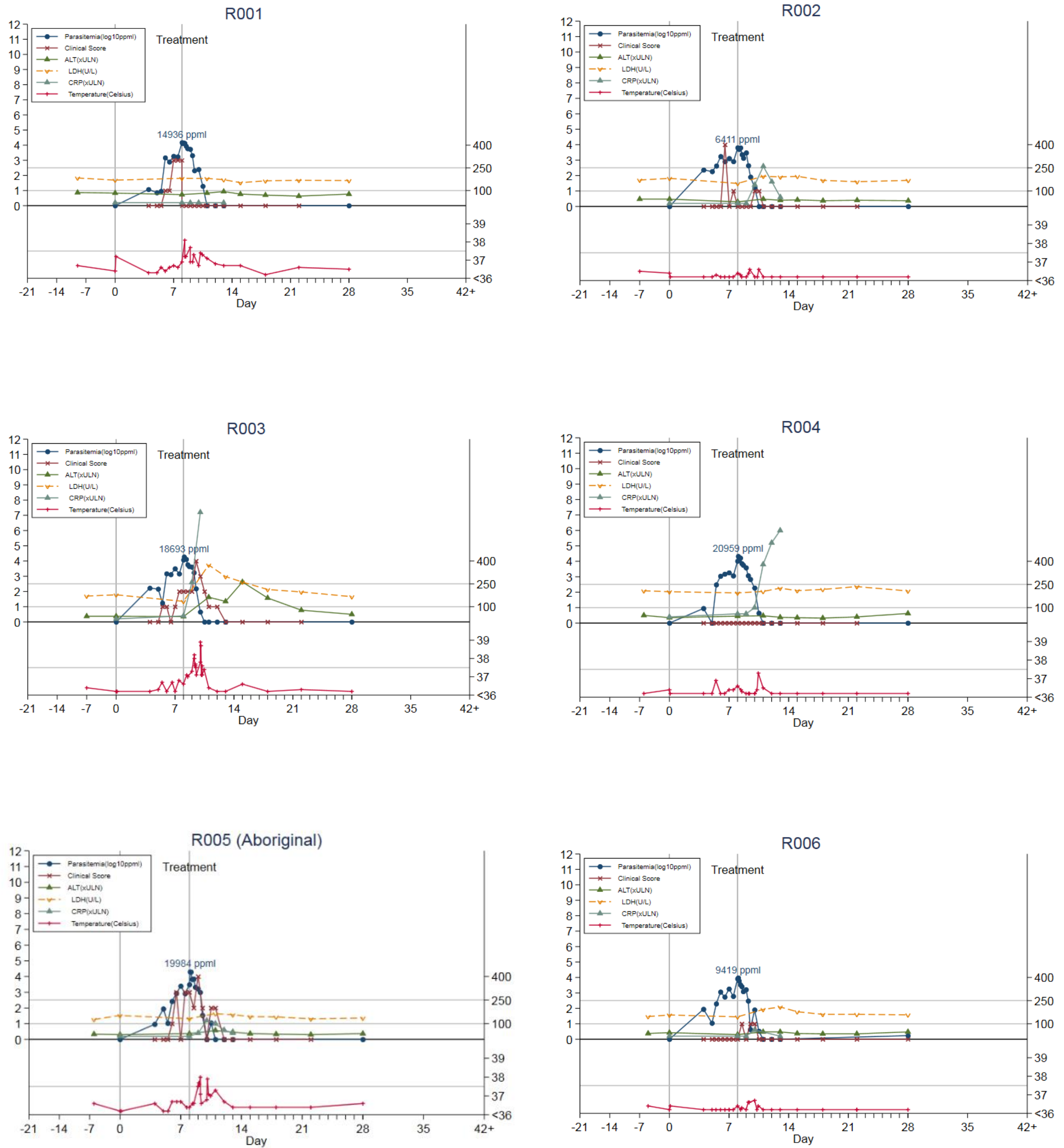
Parasites/mL of enriched blood = $25{,}475 \times 3.17 \times 4.89 = 3.95 \times 10^5$

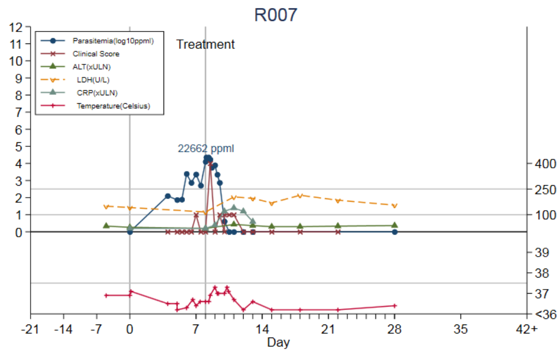
Parasites/mL of packed blood (based on bank HCT of 0.39) = $3.95 \times 10^5/0.39 = 1.01 \times 10^6$

Number of parasites/vial based on 248 µL = $(1.01 \times 10^6/1000) \times 248 = 2.51 \times 10^5$

Hence apheresis and percoll cannot concentrate parasites enough to create HMP bank vials equivalent to those used to infect subjects in this study (2.51×10^5 compared to 2.08×10^6). If only apheresis is used to concentrate parasites the vials would contain a maximum of 7.92×10^4 parasites.

Figure 1. Descriptive subject Graphs




R007
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
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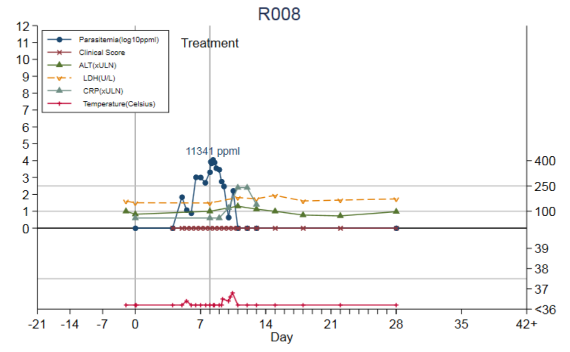
Day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ALT(xULN)
LDH(U/L)
CRP(xULN)
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Treatment
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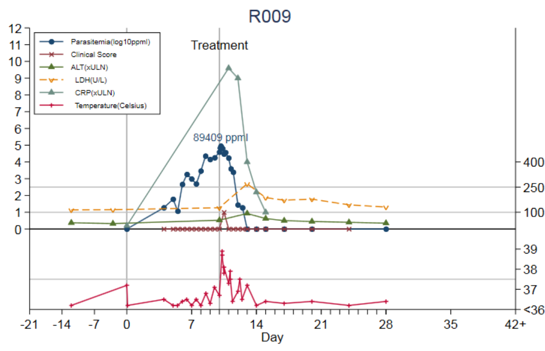
Day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ALT(xULN)
LDH(U/L)
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Temperature(Celsius)
Treatment
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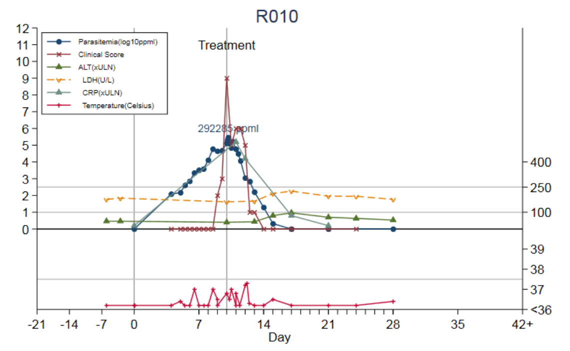
Day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ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
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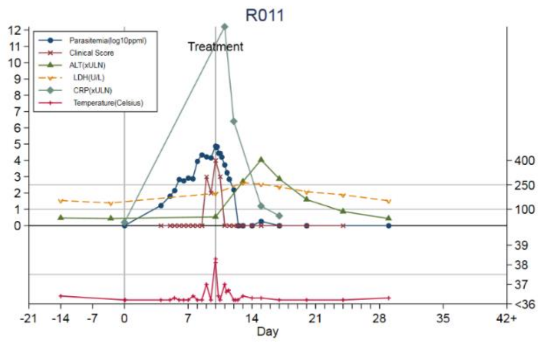
Day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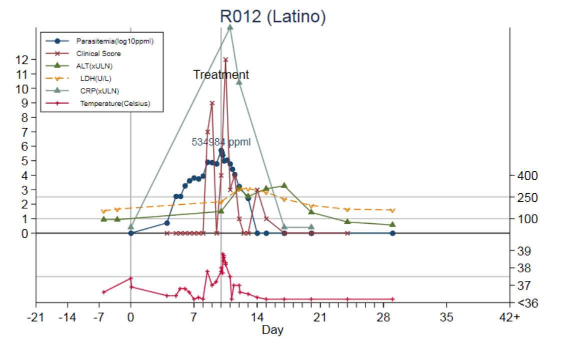
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Day


R012 (Latino)
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
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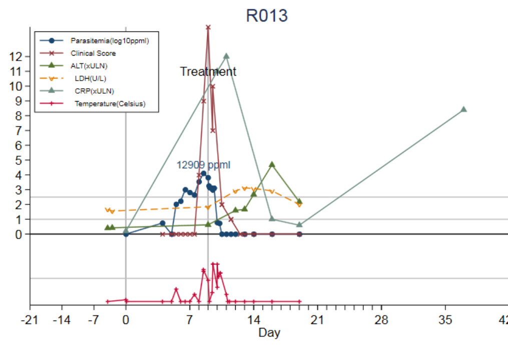
Day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ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
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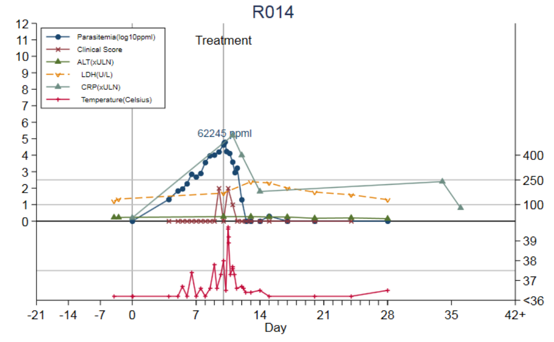
Day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Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
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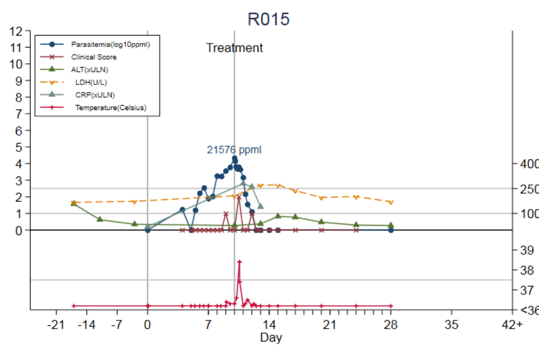
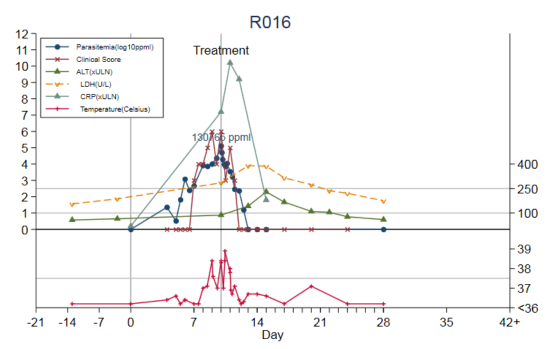
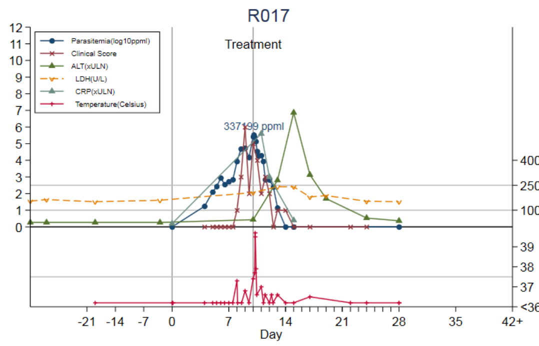
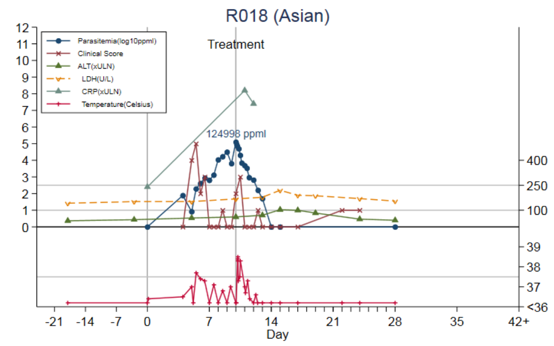
Day


R015
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
21576 ppml
Day


R016
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Day


R017
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Day


R018 (Asian)
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
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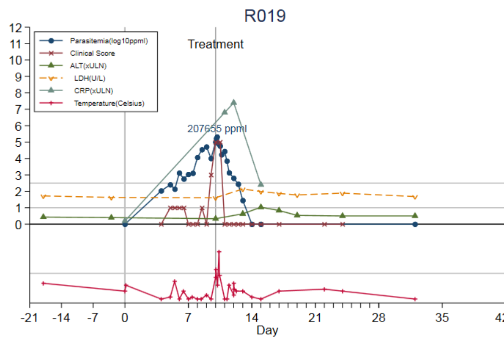
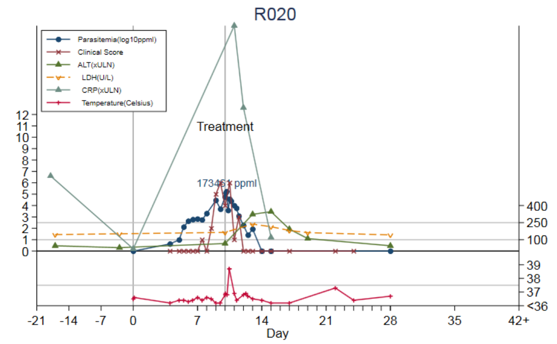
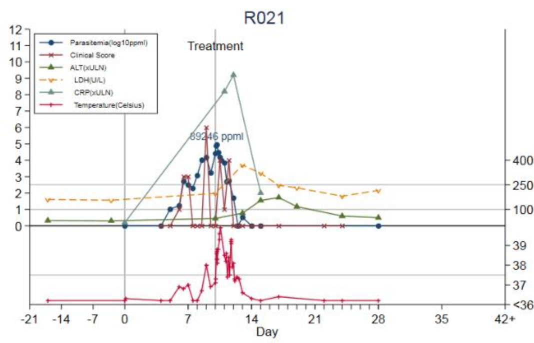
Day


R019
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
207655 ppml
Day


R020
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Day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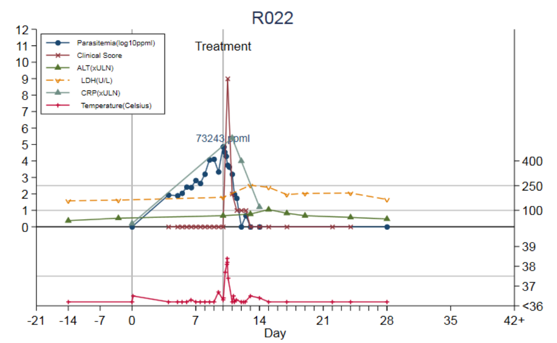
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Day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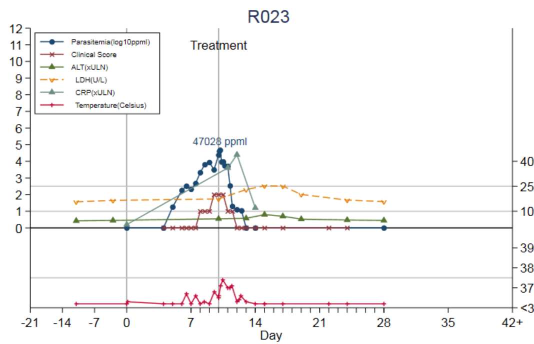
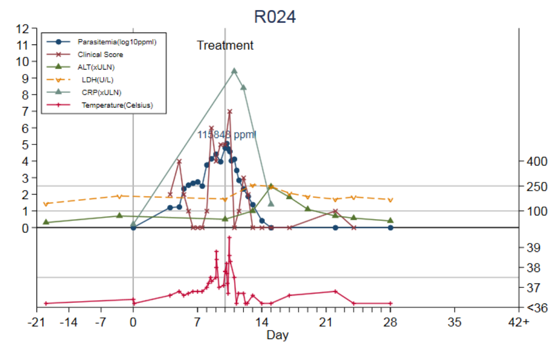
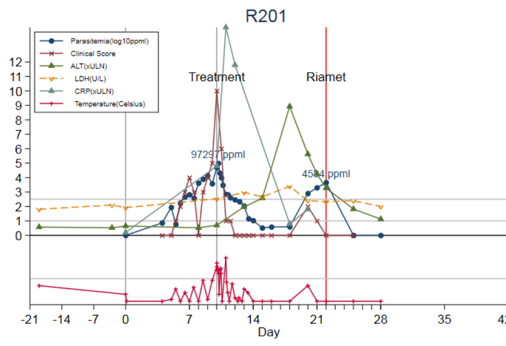
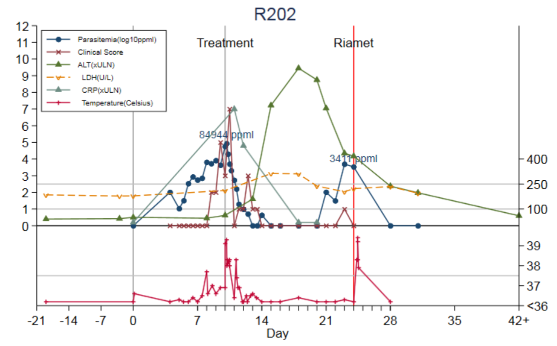
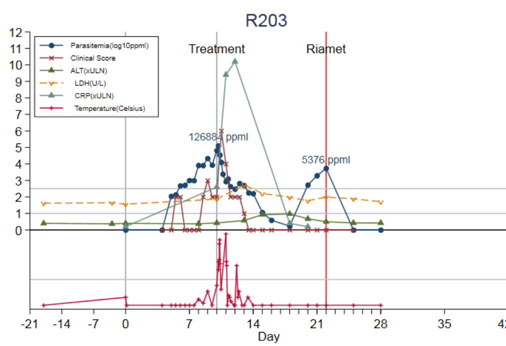
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Day

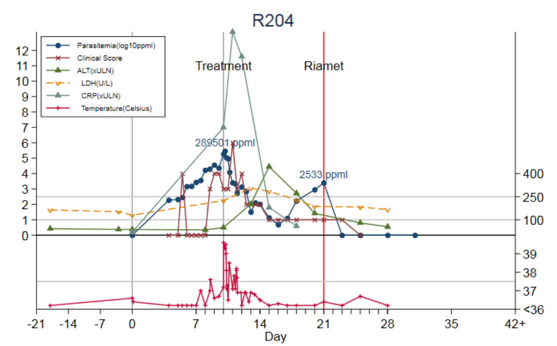

R023
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
47028 ppml
Day

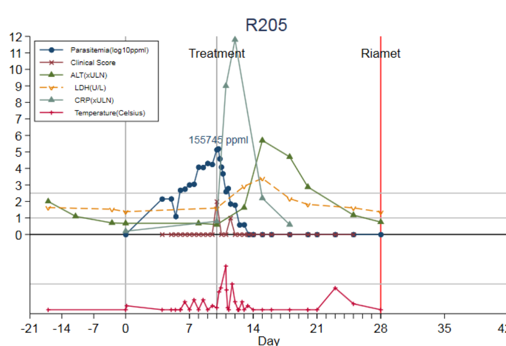

R024
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Day

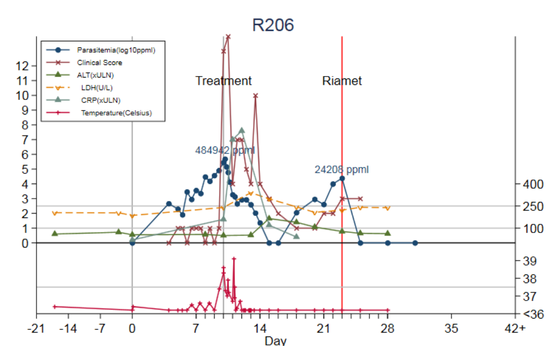

R201
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Riamet
97297 ppml
Day

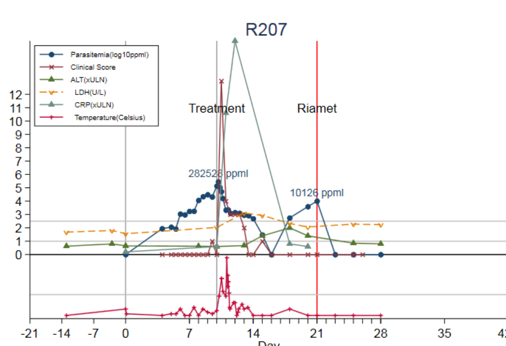

R202
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Riamet
84944 ppml
Day

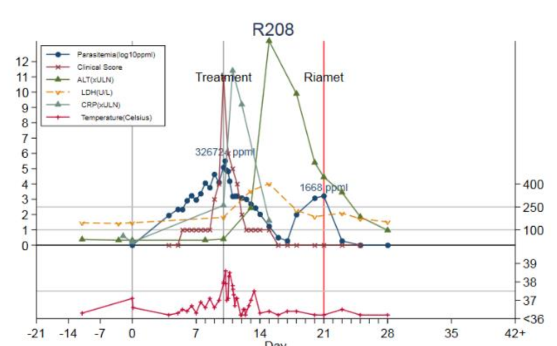

R203
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Riamet
5376 ppml
Day


R204
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Riamet
289501 ppml
2533 ppml
Day


R205
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Riamet
155745 ppml
Day


R206
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Riamet
24208 ppml
Day


R207
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Riamet
282528 ppml
10126 ppml
Day


R208
Parasitemia(log10ppml)
Clinical Score
ALT(xULN)
LDH(U/L)
CRP(xULN)
Temperature(Celsius)
Treatment
Riamet
1668 ppml
Day

ALT: alanine transaminase,; AST: aspartate transaminase; ULN: upper limit of normal; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; LDH: Lactate dehydrogenase; CRP: C-reactive protein.

Graphical representations of parasitaemia (log_{10} scale), clinical score, ALT (× ULN), CRP (× ULN), LDH (U/L) and temperature from screening to end of study for all IBSM subjects. Scales have been manipulated in order to make sure the data could fit on a single graph. ALP, GGT and albumin, were not included as there were no significant rises in any of these parameters. Bilirubin was rarely raised above the ULN, with only two subjects having a bilirubin result at ULN or higher. As such, bilirubin is not included in the graphs. AST behaved similarly to ALT, and is nearly always the lower of the two, hence it was not included. The graphs also include parasitemia on a logarithmic scale as well as documented peak parasitemia. Clinical markers associated with malaria infection such as temperature clinical score and CRP have been included to provide a measure of the level of inflammation from malaria infection. Ethnicity was indicated in those who were non-white.

Subjects with significant ALT abnormalities tended to have a greater number and severity of other malaria related measures and vice versa.

Table 3. Correlation with peak ALT

Clinical measure	Correlation	p-value
Peak AST	**0.99**	**<0.001**
Peak Bilirubin	-0.10	0.57
Peak LDH	**0.58**	**<0.001**
$PCT_{1/2}$	-0.26	0.16
Pre-treatment Peak parasitemia	**0.36**	**0.044**
Overall Peak parasitemia	**0.39**	**0.027**
Burden	**0.58**	**<0.001**
Nadir Neutrophils	-0.09	0.62
Nadir Platelets	-0.26	0.15
Nadir Lymphocytes	**-0.41**	**0.018**
Nadir White cell count	-0.03	0.87
Peak Clinical Score	**0.42**	**0.017**
Maximum Temperature	0.29	0.11
Peak CRP	**0.44**	**0.012**

ALT: alanine transaminase; AST: aspartate transaminase; LDH: lactate dehydrogenase; CRP: C-reactive protein; LDH: lactate dehydrogenase. LFT and

haematology results are in units of overall peak/minimum times ULN or LLN. Significant effects and associations with Pearson's correlation ($p<0.05$) are highlighted in bold.

Table 4. Results of backwards stepwise logistic regression for the risk of elevated peak ALT in induced blood stage malaria with $PCT_{1/2}$ included in all steps of the models

Model	Measure	OR (95% CI)	p
Including cohort effect	**$PCT_{1/2}$**	1.81 (0.57-5.69)	0.31
	Nadir Platelets × LLN^	2.79 (1.12-6.93)	0.027
	Cohort – ref Artefenomel Cohort A2	1	
	Chloroquine Cohort C1	0.16 (0.006-3.89)	0.26
	Chloroquine Cohort C2	0.04 (0.001-1.36)	0.075
	Chloroquine Cohort C3	0.03 (<0.001-1.15)	0.059
No cohort effect	**$PCT_{1/2}$**	0.95 (0.39-2.33)	0.92
	Peak CRP (relative to baseline)	1.06 (1.01-1.12)	0.013

CRP: C-reactive Protein; LLN: lower limit of normal; $PCT_{1/2}$ parasite clearance half-life; OR: odds ratio. ^Effect size (OR and 95% CI) are converted to per 0.1 unit decrease in × LLN. The order of variables removed in the model including cohort effect were parasite clearance burden, white cell count, and then peak CRP relative to baseline. The order of variables removed in the model did not include cohort effect were platelets, parasite clearance burden, and then white cell count.

Table 5. Results of backwards stepwise logistic regression for the risk of elevated peak ALT in induced blood stage malaria with parasite clearance burden included in all steps of the models

Model	Measure	OR (95% CI)	p
Including cohort effect	**PCB (log_{10} scale)**	3.60 (0.19-68.81)	0.40
	Peak CRP (relative to baseline)	1.06 (1.00-1.13)	0.044
	Cohort – ref Artefenomel Cohort C2	1	
	Chloroquine Cohort C1	8.63 (0.026-2889)	0.47
	Chloroquine Cohort C2	2.89 (0.05-153)	0.60
	Chloroquine Cohort C3	1.77 (0.03-114)	0.79
No cohort effect	**PCB (log_{10} scale)**	1.86 (0.42-8.17)	0.41

PCB: parasite clearance burden; CRP: C-reactive protein; OR: odds ratio. ^Effect size (OR and 95% CI) are converted to per 0.1 unit decrease in × LLN. The order of variables removed in the model including cohort effect were $PCT_{1/2}$, platelets and then white cell count. The order of variables removed in the model did not include cohort effect was white cell count, $PCT_{1/2}$ and then platelets.

Table 6. Pharmacokinetic parameters per cohort in induced blood stage malaria

Identifier		Antimalarial	
Cohort	**Statistical measure**	**C_{max} (µg/mL)**	**AUC_{96} (µg/mL × h)**
Artefenomel	Median (IQR)	0.60 (0.43-0.72)	3.86 (3.08-4.66)
Chloroquine C1	Median (IQR)	1.24 (1.11-1.46)	66.28 (59.56-79.36)
Chloroquine C2	Median (IQR)	0.88 (0.73-1.02)	52.88 (41.48-60.37)
Chloroquine C3	Median (IQR)	0.99 (0.82-1.09)	58.24 (48.24-64.48)

C_{max}: maximum concentration of drug; AUC_{96}: area under the curve for drug dose 96 hour after drug administration; IQR: interquartile range.

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