Design, Development and Testing of a Magnetic Haemofilter for Clinical Applications

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Acknowledgements

This is for you.

For my supervisors, who believed in the dream, but kept me in reality,
Who allowed my imagination to wander, but kept my work on track.

For my colleagues, who found that elusive balance of work and play,
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For my collaborators, who gave so much valuable time and expertise,
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For Lise, who has instilled in me a joie de vivre I never thought possible,
Who inspires my hopes, my dreams, my happiness and my love.

You have made me the man I am today.
This is for you.

I, George Charles Martin Frodsham, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

In this thesis I present the magnetic haemofilter - a novel medical device designed to remove magnetic materials directly from a patient's bloodstream. The haemofilter is a high gradient magnetic separator incorporated into an extra-corporeal loop. A patient's blood is constantly circulated through the device, which magnetically captures and retains target agents while the rest of the blood returns to the patient unharmed. Clinical versions of the device were conceived, designed and modelled. Small scale versions, designed to mimic the performance of the clinical designs, were manufactured using 3D printing and tested in benchtop *in vitro* experiments.

Many potential applications for the device are envisioned, and justified with an extensive literary review of magnetic labelling, the process of binding magnetic particles to specific targets to enable their separation. The device has significant potential as a platform technology enabling these varied applications. In this project, however, malaria was chosen as the primary application. Malaria infected red blood cells are paramagnetic, so their separation does not require magnetic labelling - the haemofilter simply exploits their naturally occurring magnetic properties.

The haemofilter was tested using samples of malaria infected blood filtered at a variety of flow rates, with a reduction in parasitaemia observed in every

experiment at throughputs orders of magnitude higher than any previously reported clinical magnetic haemofiltration device. The results demonstrate that even without further optimisation, the clinical version of the device could halve a child's parasitaemia in less than 90 minutes. The flow rates used in the experiments, and those that could be used in the clinical versions, are orders of magnitude higher than any previously reported clinical magnetic haemofilter. Experiments on samples donated by malaria patients demonstrated that no other blood components are affected by the process.

A commercial evaluation of the haemofilter as a medical device to treat malaria was conducted. The market analysis showed a large potential total addressable market, segmented into three principal patient populations. The product development and manufacturing costs were estimated and shown to be reasonable, while a financial analysis showed that a margin could be earned while still saving customers money. A route-to-market and commercialisation strategy is presented.

I conclude that the magnetic haemofilter has the potential to deliver significant clinical benefits to a wide variety of malaria patients, saving lives in serious cases, providing a treatment option for currently untreatable patients, and speeding up recovery in uncomplicated cases, while improving the efficacy and eliminating the side-effects of pharmaceutical drugs.

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Chapter 1

Introduction and Background

1.1 Introduction

1.1.1 Aim

The aim during this PhD was to design and test a magnetic haemofilter which could be used to remove magnetic agents directly from a patient's bloodstream. The idea is that the blood is circulated via an extra-corporeal loop through the device, which captures and retains target agents; the rest of the blood components return to the patient unharmed. Combined with magnetic tagging techniques, using magnetic nanoparticles for example, the device could be used to treat a large number of different medical problems. In these applications, magnetic particles are bound to specific targets, either through the inclusion of a mixing or tagging chamber in the system, or by injecting the magnetic particles directly into the bloodstream prior to filtration. However, the focus of this project has been on the haemofilter's potential use as a novel method for the treatment of malaria, using only the device to exploit the naturally occuring magnetic properties of malaria

infected red blood cells.

Malaria infected erythrocytes produce a paramagnetic waste product called haemozoin, which is stored within the infected cell. The infected cells therefore exhibit paramagnetic properties, enabling them to be magnetically separated from uninfected cells. Passing a malaria patient's blood through a high gradient magnetic separator could therefore remove the infected cells from the bloodstream, reducing a patient's parasite burden without the need for any pharmaceutical drugs, in a dialysis-like process. The blood is flowed continuously until the treatment has reduced a patient's parasite burden, known as parasitaemia, below a target level.

Presented in this thesis are the design of such a device and experiments conducted on a scaled-down version, demonstrating its potential effectiveness. The potential clinical applications and commercialisation plan for the device as a treatment for malaria are also considered.

1.1.2 Summary of device

The device consists of two core parts: an assembly of permanent magnets creating a strong magnetic field, and a separation chamber which is placed within the magnetic assembly and incorporated into a blood circuit connected to a patient. The permanent magnets are 'rare-earth', made from a neodymium iron boron alloy (NdFeB, chemical formula: $Ne_2Fe_{14}B$), the strongest permanent magnetic material currently available. The separation chamber contains a fine ferromagnetic mesh which, when placed within the magnetic field generated by the assembly, generates strong magnetic forces which pull magnetic agents onto the mesh. These are retained, or captured, as long as the magnetic

force exceeds the drag force caused by the flow of bood through the chamber. The magnetic force on an object (discussed in more detail in section 1.3) is given by:

$$\bar{F_m} = \mu_0 \chi V \bar{H} \nabla \bar{H} \tag{1.1}$$

where χ is the magnetic susceptibility of the object, V is the volume of magnetic material, H is the magnetic field (in A/m) and $\nabla \bar{H}$ is the magnetic gradient (in A/m^2). The drag force for a spherical object is given by Stokes' law:

$$F_d = \bar{6} \pi \mu R v \tag{1.2}$$

where μ is the liquid viscosity, R is the object's radius, and v is the velocity of the liquid relative to the object. These two equations underlie the functioning of the device, and are the main drivers behind all the design considerations presented in this thesis.

The blood returns to the patient cleansed of targets, which may include cells, toxins, bacteria, or indeed anything that has or can be given sufficient magnetic susceptibility thanks to magnetic tagging; other blood components are unaffected. The separation chamber is connected to the patient via a venous or central catheter, and the blood is pumped continuously through the circuit at a controlled rate using a peristaltic pump. Anticoagulant is added at the start of the process and, if necessary, saline is added at the end, both at controlled rates. The separation chamber and all of the extra-corporeal circuit - the consumables - are disposable, while the instrument - the magnetic assembly, along with the pump and safety features, amongst others - can be reused throughout its lifetime, which could be many years.

1.1.2.1 A treatment for malaria

Magnetic haemofiltration could have a wide variety of clinical uses, particularly if combined with magnetic nanoparticles (see section 2). Indeed, malaria can be considered as just the primary indication of the device, with further treatments or uses which could become available through future research. Malaria was selected as the initial application and primary focus of this work for the following reasons:

- *The clinical need and social impact is potentially huge*: Malaria is one of humanity's biggest scourges (see section 1.2.3). The continued emergence of drug resistance and the high mortality rate of severe cases demonstrate the clinical need for an innovative and different treatment methodology that could complement conventional techniques as well as fill gaps that currently exist in malaria treatment strategies (see section 5.2).

- *Malaria infected cells are naturally magnetic (see section 1.2.8)* This greatly simplifies testing of the device, since it is not necessary to source or fabricate functionalised magnetic nanoparticles with specific targets. It also removes a variable - if testing reveals that the device does not successfully remove malaria infected cells, it is because the magnetic forces are not overcoming the drag forces, and not, for example, because the magnetic nanoparticles have not successfully bound to the target.

- *Malaria infected cells are a baseline establishing target*: As haemozoin is paramagnetic, its magnetic susceptibility is positive but small, so magnetises less than a superparamagnetic or ferromagnetic particle in the same magnetic field (see section 1.3.3). As such, malaria infected cells will experience smaller magnetic forces com-

pared to a cell or other component labelled with magnetic nanoparticles, unless the total magnetic nanoparticle volume is very small. The advantage therefore in developing the device for the purpose of treating malaria is that if it captures malaria infected cells, it will capture almost anything tagged with magnetic nanoparticles. In other words, if it works for malaria, it will work for everything else.

1.1.2.2 Benefits

Without using any biological or pharmaceutical products, the haemofilter could achieve similar results to the current frontline malaria treatments - elimination of infected cells from the bloodstream - but more rapidly and without the side-effects caused by the circulating debris which results from drug treatments. It could provide a number of clinical benefits, whether utilised as a complement to existing therapies or as a stand-alone treatment. A malaria patient's recovery time and chances of survival are linked to the magnitude of their parasitaemia when they present at hospital: lower parasitaemias mean improved prognosis and patient outcomes. There are also patients who cannot be treated using pharmaceuticals, including pregnant women, the immune defficient, and, most famously, those with drug resistant cases. By removing gametocytes, the sexual form of the parasite responsible for disease transmission, the haemofilter could also help reduce the spread of the disease.

1.1.2.3 Limitations and challenges

The haemofilter does have limitations. Only parasites or infected cells currently circulating in the bloodstream can be passed through the filter, so any within the liver or adhered to blood vessels could not be captured (see section 1.2.2). In addition, the quantity of

magnetic material in an infected cell is dependent on the time since it was invaded by a parasite. As such, only mature cells can be captured - in order to eliminate all of the infected cells, the device will need to either be combined with drug treatments, repeated within 24 hours, or performed continuously.

There are also challenges in terms of adoption. Haemofiltration is a much more complex medical procedure than current malaria treatments, which are usually tablet based, and at worst IV injections. Incorporation of haemofiltration into the current treatment pathway would therefore be disruptive and challenging. It would also, at least initially, require a hospital environment with the supplies and expertise to carry out the procedure. Many malaria patients are in difficult-to-access areas with very limited access to healthcare - these patients could not be treated with the haemofilter without a portable version useable in rural clinics, which would be challenging to achieve. Furthermore, the vast majority of malaria patients are from low income areas, and endemic countries are mostly developing nations. The economics of introducing a new treatment are therefore a significant challenge, and creating a cheap device is essential - a high cost per treatment would probably be a showstopper.

1.1.3 Structure of thesis

During this PhD, both the technical and the commercial feasibility of a magnetic haemofilter to treat malaria were assessed. The results of this research are:

- a design for a medical magnetic haemofilter - Chapter 3
- computational proof-of-principle through magnetic modelling - Chapter 3
- a design and manufacturing process for a scaled down prototype - Chapter 3

- results demonstrating that the device is capable of capturing a high percentage of malaria infected cells from a blood sample - Chapter 4
- evaluation of the potential market for the device - Chapter 5
- a go-to-market strategy and the foundations of a business plan - Chapter 5

In addition, a literature review of high gradient magnetic separation (HGMS), presenting the state of the art of research which relates to potential clinical applications of the haemofilter was completed, and is presented in Chapter 2. The background information presented in section 1.2 details the fundamentals of malaria as a disease and its impact on humanity. Section 1.3 provides the theoretical aspects of magnetism necessary to understand this work. In Chapter 6, it is concluded that the device, as it is designed, is technically feasibile of being used as a medical device to treat malaria. In addition, it is concluded that its commercialisation would be both a worthwhile and viable venture.

1.2 Malaria

1.2.1 Introduction

Malaria is a mosquito-borne infectious disease caused by the protozoan parasite *Plasmodium*. There are six species that affect humans: *Plasmodium falciparum*, *P. vivax*, *P. malariae*, two species of *P. ovale*, and the zoonotic species *P. knowlesi* which causes malaria in monkeys[8]. *P. falciparum* and *P. vivax* account for the vast majority of infections[2]. Malaria has been eradicated from most of the developed world and endemic countries are predominently in Africa, South America and South East Asia (Fig.

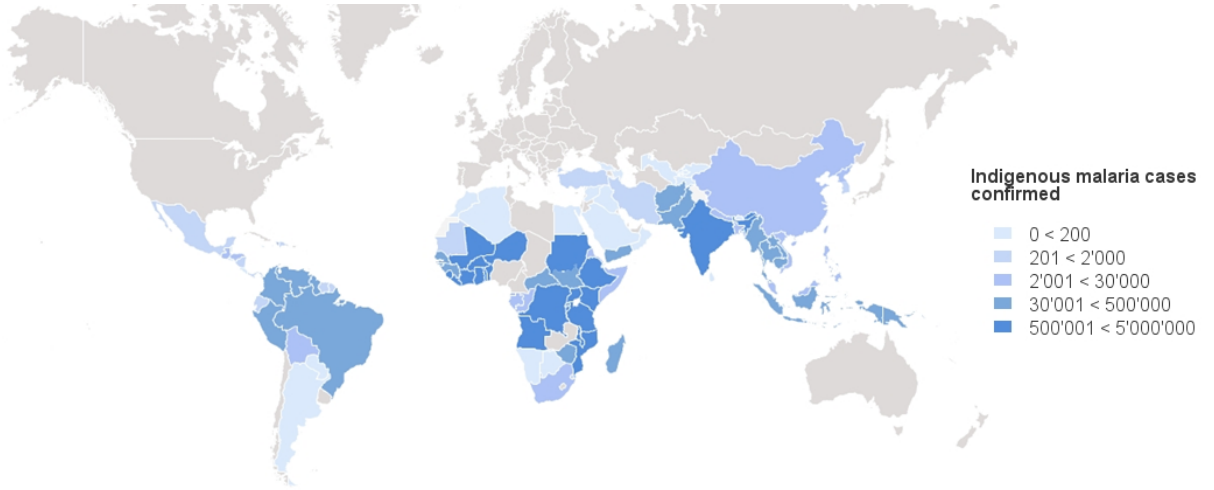


Figure 1.1: Map of global malaria incidence, created using Global Malaria Mapper[1]

1.1). Tropical regions tend to have a high incidence due to favourable conditions for the *Anopheles* mosquito, the females of which are the primary malaria vector.

While it is both preventable and curable, malaria continues to cause huge suffering to hundreds of millions of people, significantly impacting quality of life and economic development (section 1.2.3). It is a disease that is inexorably linked to poverty. Firstly, poverty causes malaria: not only are poorer patients less able to afford treatment, but they are also more likely to live in environments that favour mosquito reproduction (e.g. hot and humid rural conditions, poorly drained land, etc.), and have lifestyles which increase the likelihood of mosquito bites (e.g. rudimentary housing, no door screens or mosquito nets, majority of time spent outside, etc.). Secondly, malaria causes poverty, severely impacting economic growth for example by incapacitating workers during the harvest season[9, 10].

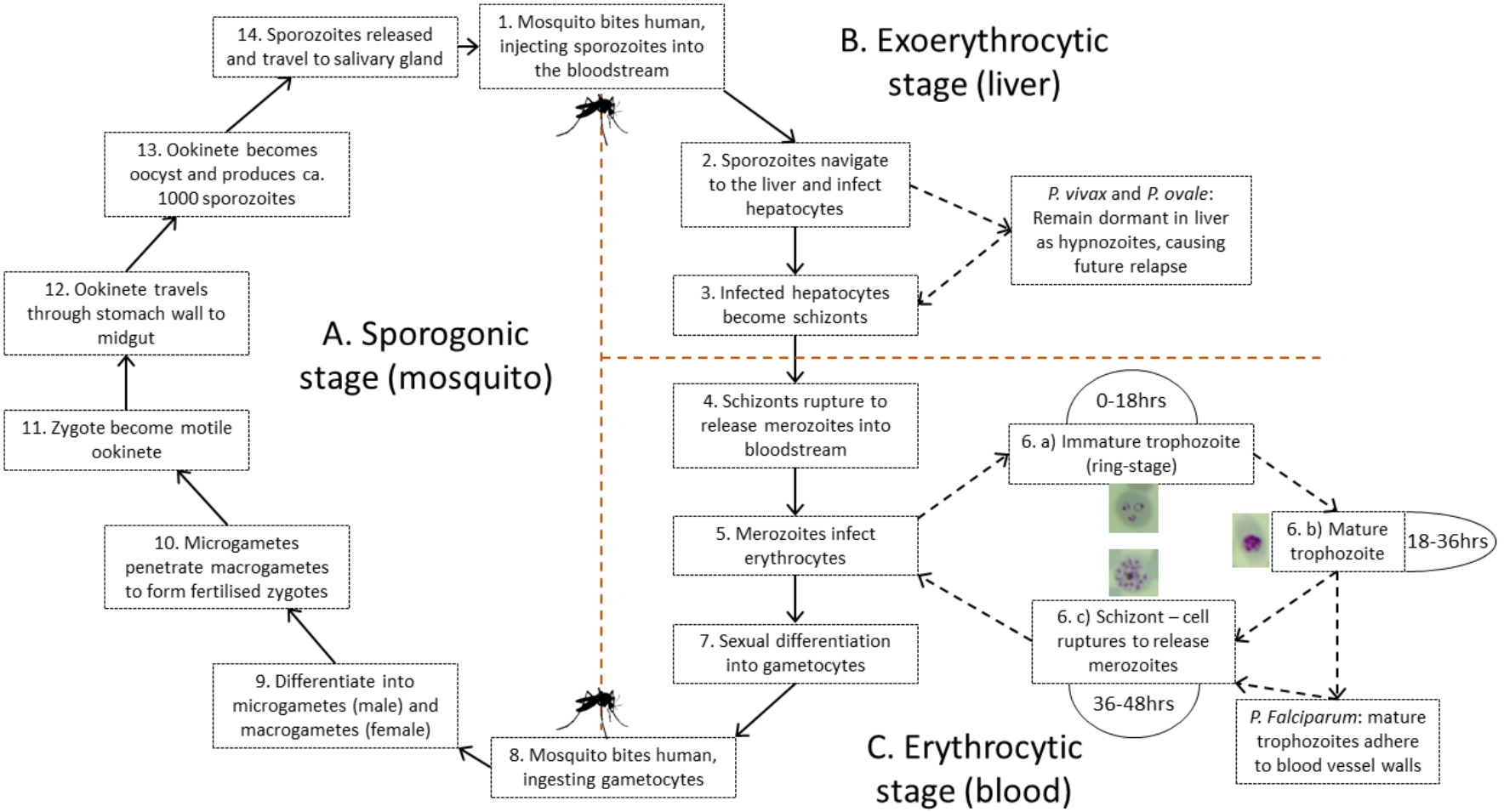


Figure 1.2: Life-cycle of malaria parasite. Note that the haemofilter device is concerned with infected cells in the erythrocytic stage, particularly targeting mature trohpzoites and schizonts. Note also the variations in the life-cycles between different species, notably the sequestration of schizonts to blood vessel walls in cases of *P. falciparum* (see section 1.2.4.3)[2, 3]

1.2.2 Life cycle

The malaria parasite has three distinct stages: sporogonic (mosquito stage), exoerythrocytic (liver stage) and erythrocytic (blood stage) (Fig. 1.2). The exoerythrocytic stage begins when a female anopheles mosquito carrying the malaria parasite bites a human, injecting sporozoites from its salivary gland into the bloodstream. The sporozoites navigate to the liver where they infect hepatocytes. In *P. vivax* and *P. ovale*, the parasites can differentiate into hypnozoites and remain dormant in the liver for significant periods of time before resuming the life-cycle, causing patient relapses sometimes decades after first disease indication[2].

Infected hepatocytes differentiate into schizonts each containing from 10,000 to 30,000 merozoites, which are released into the bloodstream when the cells rupture, beginning the erythrocytic stage[2]. Each merozoite infects an erythrocyte and begins to consume the cell's haemoglobin, producing haemozoin which is stored in a food vacuole within the cell (section 1.2.8). The parasite grows from ring-stage to mature trophozoite, finally differentiating into a schizont, at which point it divides into several merozoites. When the cell ruptures, the merozoites immediately each infect a new erythrocyte. According to Warrell *et al.*, there are on average 16 merozoites in each *P. vivax* and *P. falciparum* erythrocytic schizont when it ruptures, 8-16 in *P. ovale* schizonts, and 10 in *P. malariae* and *P. knowlesi*[2]. Furthermore, they state that the erythrocytic stage for *P. vivax*, *P. falciparum* and *P. ovale* lasts 48 hours, while it lasts 72 hours for *P. malariae* and 24 hours for *P. knowlesi.*

During the erythrocytic stage, *P. falciparum* infected erythrocytes adhere to blood vessel walls in a phenomena known as cytoadherence. One of the modifications to the cell

that the parasite causes, which include decreased deformability and the formation of a tubovesicular membrane network which delivers nutrients to the parasite, is the formation of 'knobs' on the membrane of the infected erythrocyte. These knobs cause infected erythrocytes to adhere to the endothelium of blood vessels and capillaries and accumulate within organs, resulting in reduced circulation and causing some of the symptoms of severe malaria (see section 1.2.4.3)[11, 12, 13, 14, 15].

While still at the ring-stage, the parasite can differentiate into a gametocyte, its sexual form responsible for retransmission of the disease. These are ingested by female anopheles mosquitoes upon taking a blood meal, beginning the sporogonic cycle. The gametocytes first differentiate into the male and female forms, the microgametes and macrogametes respectively. While still in the stomach, the microgametes penetrate the macrogametes to form a fertilised zygote. The zygote differentiates into a motile ookinete which navigates through the stomach wall to the midgut and develops into an oocyst, which produces about 1,000 sporozoites. These are then released and travel to the mosquito's salivary gland, ready to reinfect a new human upon the next blood meal[2, 3].

1.2.3 Global impact

1.2.3.1 Human impact

With half the world's population at risk, malaria is one of the biggest healthcare challenges facing humanity. According to the World Health Organization (WHO), in 2012 there were 207 million cases and 626,000 deaths (Table 1.1). 77% of global deaths, and 82% of deaths in Africa, are children under 5 years old[4, 2]. Some believe that the WHO underestimates the numbers, due to the unreliability of the data they use. This is generally provided by

Region	Africa	The Americas	Eastern Mediterranean	Western Pacific	South East Asia	World
Cases '000	165,000	800	13,000	1,000	27,000	206,800
Deaths	562,000	800	18,000	3,500	42,000	626,300
P. falciparum	99%	35%	72%	84%	53%	91%
P. vivax	1%	65%	28%	16%	47%	9%

Table 1.1: The global burden of malaria[4]

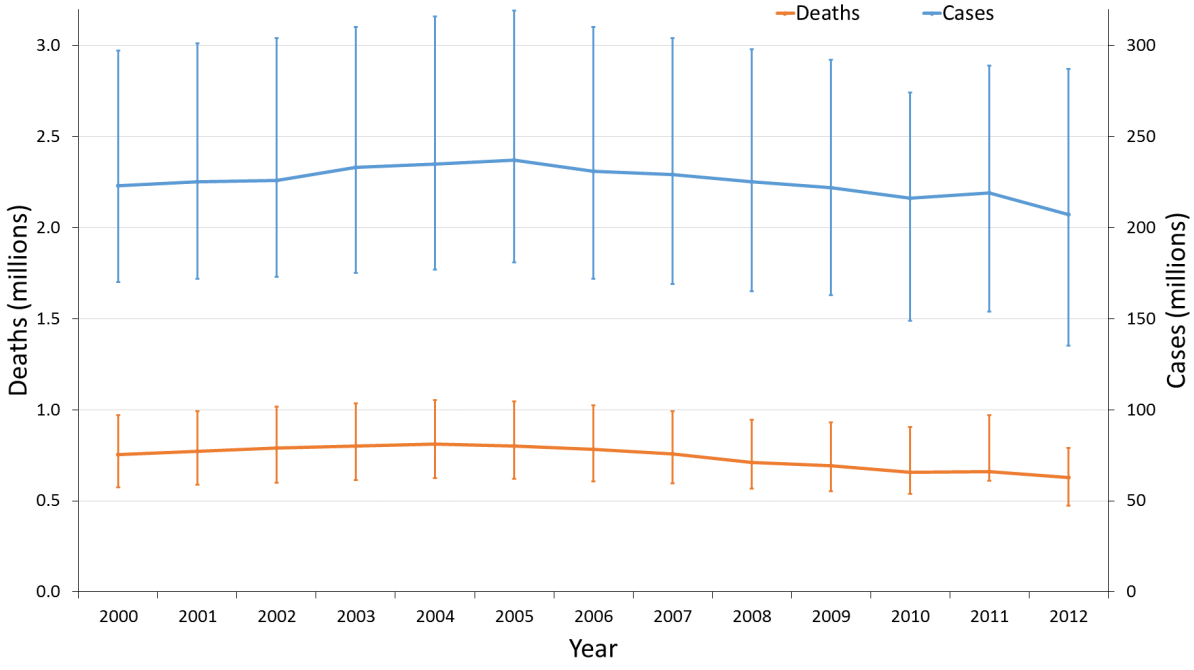


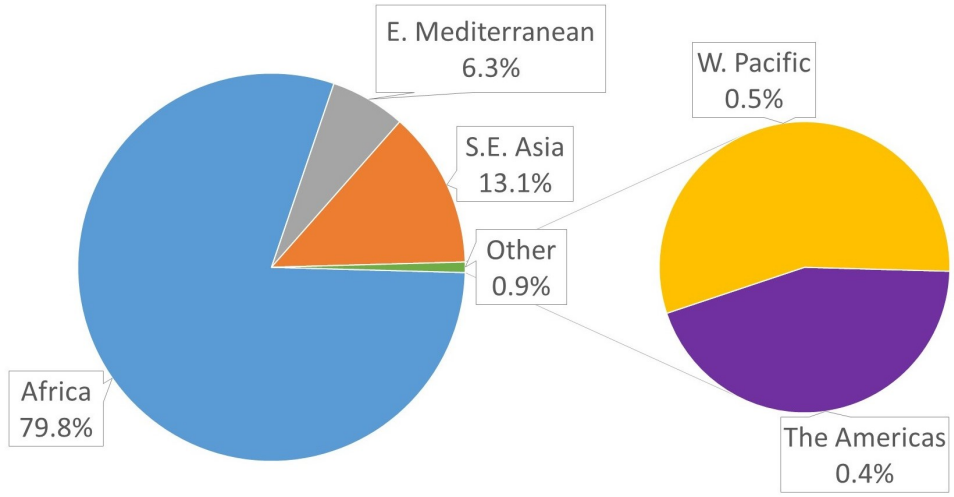
Figure 1.3: Evolution of malaria cases and deaths and margin of error in WHO estimates[4]

governments who collect numbers from patients who present at clinics, ignoring those who remain in rural villages. Furthermore, bias from those reporting the figures, bias from governments wishing to show that their efforts to tackle malaria are having an impact, and non-inclusion of patients who remain undiagnosed, all suggest that the real burden of malaria is even higher[16, 17, 18, 19].

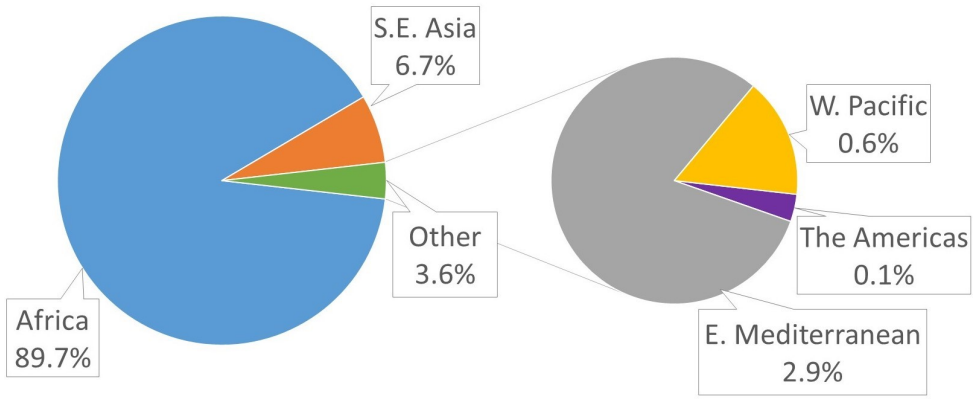
While global incidence and mortality has decreased over the past decade (see Fig. 1.3), climate change is forecast to cause an expansion in the size of endemic regions and increase the population at risk[20, 21]. However, other factors such as drug resistance, migration and net spending on treatment and prevention will probably play more significant roles[22, 23].

The geographical spread of malaria is unequal (Fig. 1.1). Africa accounts for 80% of the cases, and 90% of the deaths, while much of the developed world has eradicated malaria completely. Outside of Africa, the majority of cases and deaths are in South East Asia, which accounts for 13% of global cases, just under twice the remaining regions combined (Fig. 1.4a). The distribution of malaria cases is primarily driven by environmental factors, with tropical countries seeing high incidence due to conditions favourable to mosquitoes. However, the economic situation of a country and its population also play a major role, with wealthier countries more able to instigate control and treatment programmes. In poorer regions, however, limited access to, affordibility and the low quality of treatments promotes transmission, while prevention programmes rely on international donations.

In terms of the number of deaths, the two main factors are poverty, with its resultant effect of limited access to and poor quality of treatment, and local parasite species, with the predominant malaria species in the region being *P. falciparum*, which has significantly



(a) Global distribution of malaria cases



(b) Global distribution of malaria deaths

Figure 1.4: Global distribution of malaria burden[4]

higher mortality rates than *P. vivax*. The effect of *P. falciparum* on mortality can be seen in Table 1.1: in the Americas, where *P. vivax* dominates, there is only one death per thousand cases. In Africa and Western Pacific however, where *P. falciparum* dominates, there are respectively 3.4 and 3.5 deaths per thousand cases. The higher mortality rate of *P. falciparum* is partly due to cytoadherence (which is discussed in sections 1.2 and 1.2.4).

1.2.3.2 Economic impact

Malaria is a disease indelibly linked to poverty, significantly hindering the economic development of the most severely affected countries. The map in Fig. 1.1 highlights the countries with the highest levels of malaria incidence, but could also be a map highlighting the countries with lowest GDP. There has been some debate as to whether malaria is a cause or consequence of poverty; in reality, it is both.

The environments which favour high mosquito populations - swamps and wetlands - also hinder economic development by limiting the ability to grow crops or build infrastructure. As with any disease which can affect a high proportion of the population, controlling and treating it requires significant resources - richer countries have been able to eradicate malaria, whereas poorer ones cannot (see section 1.2.6). So clearly, malaria is to some degree a consequence of poverty, although it is important to note that is not a direct result of poor living conditions or sanitation, like tuberculosis for example.

However, several studies have indicated that high malaria incidence is also a cause of poverty, not least due to the loss of life and man hours - an afflicted individual can be bedridden for days, and is thus unable to perform his normal job or tend to crops. A much cited study by Gallup and Sachs estimates that, after accounting for factors such

as environment, colonial history and geographical location, countries with severe malaria incidence had income levels in 1995 of only 33% of those free of malaria, regardless of whether they were in Africa or elsewhere[10]. Another study estimated that in 2009, the annual costs of treating childhood malaria in Ghana, Kenya and Tanzania were $38 million, $109 millon and $132 million respectively[24]. USA's Centers for Disease Control and Prevention (CDC) estimates that the global direct costs of malaria (including for example treatment and premature death) are $12 billion and that "the cost in lost economic growth is many times more than that"[25].

1.2.3.3 Cost of fighting malaria

Over the past decade, a drastic increase in the funding to fight malaria has had a significant impact on malaria incidence and deaths. Indeed, seven endemic countries, including for example Algeria, are categorised by the WHO as being in the elimination phase, and 12 in the pre-elimination phase. The number of both cases and deaths has been declining, respectively by 13% and 22% between 2005 and 2012 (Fig. 1.3). Incidence reduction and movements towards elimination are overwhelmingly due to total spending on control and treatment, and therefore economic growth, although recent progress is largely due to the impact of international aid from organisations such as the Global Fund to Fight AIDS, Tuberculosis and Malaria, the Medicines for Malaria Venture (MMV) and the Bill and Melinda Gates Foundation. Much of the money is spent on insecticide treated nets (ITN) - the Global Fund has already distributed over 400 million nets worldwide - but global investment also covers other prevention methods as well as research into new treatment methods.

Global investment in malaria has increased from $100 million in 2000 to $2 billion in

2013; the Global Fund accounts for 50% of this amount[4]. The investment comes largely from governments - particularly USA and UK - as well as philanthropic foundations (such as the Gates Foundation) and non-governmental organisations (such as the World Bank). Despite the large increase in investment, global commitments remain less than half of the estimated requirements. The Roll Back Malaria Partnership estimates that $5.1 billion will be required every year to 2020[26].

1.2.4 Symptoms and complications

1.2.4.1 Uncomplicated malaria

Symptoms of malaria are driven by the body's immune response. The most common symptoms are fevers and headaches - in a survey of 46 malaria patients conducted for this PhD, the proportion of respondents who declared that they had a fever and headache was 98% and 74% respectively (see Appendix B). Other symptoms include exhaustion, vomiting, joint pains, shortness of breath, chest pains, and feeling unusually cold. Symptoms can vary widely from patient to patient, but most are bedridden and unable to operate normally. Some describe malaria as "the worst I have ever felt".

Patients are typically unwell for about a week, but symptoms can persist for over a month, particularly if the administered treatment is ineffective. In the survey, 83% of respondants took time off work or school, with an average of 8.8 days missed (range 1-42). The time between starting treatment and symptom elimination also varies significantly, from less than 24 hours to over a week.

The majority of patients exhibit some of the above symptoms before recovering either due to successful tablet based treatments or thanks to the patient's immune system.

Sporozoites induce an antibody response in patients, leading to the production of circumsporozoite proteins (CS). CS occur in a large proportion, perhaps over 90%, of the adult population in endemic areas, and were shown to inhibit sporozoite development in mice over 30 years ago[27, 28]. This natural immunity results in malaria being a much more severe disease for children than adults in endemic regions, which is the main reason why such a high proportion of the deaths are in children under five (section 1.2.3.1) - those who survive their first bout tend to have the natural defences required to overcome subsequent cases.

1.2.4.2 Severe malaria

While uncomplicated cases undoubtedly cause significant suffering (and economic damage), they do not require hospitalisation, and are treated (if at all) with relatively simple tablet courses. Survival rates are high. Severe, or complicated, malaria, however, carries much greater risks and requires more serious interventions in the form of parenteral treatments. The WHO classifies a malaria case as severe when there is "clinical or laboratory evidence of vital organ dysfunction". The criteria for defining a case as severe are well defined and include imparied consciousness, prostration, convulsions, respiratory distress and acute renal failure, amongst others[29].

Several of these symptoms can be exhibited simultaneously. Survival rates amongst severe malaria patients are much lower than uncomplicated malaria, and can occur within hours of complications developing. The causes of escalation from uncomplicated to severe malaria vary, including amongst others old age, pregnancy, immune defficiency and delayed treatment; patients who originate from non-endemic countries (and thus have no immunity) are particularly strongly at risk[30, 31, 32, 33].

1.2.4.3 Cytoadherence

Most, though not all, severe malaria cases are caused by *P. falciparum*. Some have suggested that this is caused by the phenomenon of cytoadherence (or sequestration), in which trophozoites and schizonts stick to blood vessel walls, which occurs exclusively in *P. falciparum*. The cells no longer circulate in the bloodstream, and their accumulation at adherence sites can cause blockages, which in turn cause complications. This is particularly the case for cerebral malaria, in which patients lapse into a coma which can last several days; mortality can be as high as 50%, although most survivors are free of residual neurological problems[30, 34]. Cerebral malaria is frequently associated with clumps of schizonts within the blood vessels of the brain. Indeed, these and other vascular obstructions are a common clinical feature of severe malaria[34, 35].

The mechanisms of cytoadherence are not completely understood, and are not relevant to this project, so will not be discussed. However, the phenomenon could have serious implications for haemofiltration as a malaria treatment, since sequestered erythrocytes do not circulate in the bloodstream and cannot be captured by the device. Peripheral parasitaemia levels can be artificially low. However, in severe cases (where the haemofilter would be most useful - see section 5.2), schizonts and trophozoites can still be seen in the bloodstream, and parasitaemia levels can still be high. Furthermore, a high mature stage parasitaemia has been shown to be a strong indicator of fatality[36, 37].

Cytoadherence is likely to limit the haemofilter's effectiveness in treating patients with *P. falciparum*. It is however probable that it could still provide patient benefits, by removing the erythrocytes containing haemozoin that are still circulating, as well as any circulating haemozoin from infected cells that have recently ruptured, either naturally or

as a result of pharmaceutical treatment. Furthermore, some research groups are working on products that inhibit cytoadhesion - combining these with the haemofilter could be very effective. In any case, in cases of *P. falciparum*, in order to be effective, the haemofilter will likely need to be combined with some form of pharmaceutical injected into the bloodstream[38, 39, 40].

1.2.5 Treatment

Malaria is typically treated with anti-malarial tablets, with the objective of eliminating the infection in uncomplicated cases, and preventing death in severe cases. One of the oldest traditional treatments used bark from South American cinchona trees, effective because of the naturally occuring quinine found in the bark. Quinine was extracted from the bark as early as 1820 to become one of the first pure chemical compounds used to treat a disease. The anti-malarial qualities of quinine are renowned as being at the origin of the popularity of gin and tonic, used by British colonialists to counteract the bitterness of cinchona bark - originally, tonic was simply quinine mixed with soda water. Quinine is still used to treat malaria, but is no longer recommended by the WHO[41, 42, 43].

Another traditional herbal remedy that has inspired modern treatments is the qinghao plant from China, the source of artemisinin, today's frontline malaria drug. Records of the plant's antimalarial properties date back 1,000 years - Shen Gua (also spelt Shen Kuo) mentions the benefits of *Artemisia annua* in his Dream Pool Essays of 1086. Artemisinin was the frontline malaria treatment for many years, and still is for *P. vivax*. However, because of emerging resistance by *P. falciparum* to artemisinin and its derivatives (see section 1.2.7), the WHO now recommends that *P. falciparum* be treated with

artemisinin combined with drugs of a different class (such as mefloquine). These are known as artemisinin combination therapies (ACT), and are currently considered the best anti-malarial treatments[44, 42, 41, 45, 46].

Arguably the most successful antimalarial of modern times is the synthetic compound chloroquine, which has been widely used since the 1940s. It functions by inhibiting the production of haemozoin, preventing the parasite from avoiding the toxicity of free heme groups (see section 1.2.8), and is still used widely today, particularly as a treatment for *P. vivax*. Chloroquine was used to treat all malaria species until the 1980s, when *P. falciparum* began developing widespread resistance; more recently, resistance has also been observed in *P. vivax* (see section 1.2.7), and is likely to lead to a reduction in the use of the drug[42, 41, 47].

The WHO recommends using chloroquine in combination with primaquine, a synthetic drug that prevents relapse by eliminating hypnozoites (see section 1.2.2); primaquine is currently the only licensed drug able to target hypnozoites and prevent relapse. It is worth noting that primaquine should not be used on patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency, a genetic defect linked with natural malaria resistance, as it can cause severe haemolysis[42, 48, 2, 49, 50].

1.2.6 Prevention

1.2.6.1 Historical vector control

Some prevention methods focus on mosquitoes rather than humans, with mixed results. For example, the National Malaria Eradication Programme (NMEP) launched by the USA in 1947 successfully eradicated malaria within four years, primarily with the ex-

tensive use of the insecticide dichloro-diphenyl-trichloroethane (DDT). Inspired by the NMEP's success, the Global Malaria Eradication Programme (GMEP) was launched by the WHO in 1955. The methods were essentially the same, combining DDT with the destruction of mosquito breeding habitats, but the results were not. During the programme, treatment with anti-malarials, emerging resistance to chloroquine and other methods of malaria prevention were overlooked, as were the logistical challenges of implementing the programme in remote, rural regions. While malaria was eliminated from some areas, others, particularly in Africa, experienced severe resurgences in the 1960s, some of epidemic proportions. While the GMEP succeeded in eliminating malaria from Europe, North America, the Caribbean and parts of Asia and South-Central America, it failed to have a significant impact in sub-saharan Africa, and the programme was eventually dropped in 1969[51, 52, 53].

1.2.6.2 Mosquito nets

Most of the resources spent fighting malaria go towards prevention, particularly the distribution of ITN in endemic regions. Unprotected individuals can be bitten on average *ca.* 250 times in a single night - a significant reduction in this number could clearly have an impact on transmission rates[10]. Indeed, ITN have been estimated to reduce malaria cases by up to 50% and save 5.5 lives per year for every 1,000 children protected[54]. ITN are currently the most popular malaria prevention measure, and arguably the most cost-effective method of saving lives, and can even provide additional economic benefits when manufactured in endemic countries - Tanzanian based A to Z Textile Mills Ltd., Africa's largest ITN manufacturer, produces 30 million ITN annually and employs over 7,000 people[55]. Globally, ITN are estimated to have saved the lives of 250,000 children

between 2000 and 2008[56].

Their large-scale introduction has not been without controversies - some estimate that fewer than 20% of the nets are regularly used. In her TED talk in 2013, investigative journalist Sonia Shah compares giving African families ITN with distributing free face masks to everyone in Europe and the USA during flu season: lives would be saved, but people simply wouldn't use them[57]. A further problem is that mosquitoes develop a resistance to the insecticides - the WHO claims that resistance has been identified in 64 endemic countries and estimates that widespread resistance to pyrethroids, the most common insecticide, would prevent 120,000 deaths from being averted every year[58, 59]. Other issues include the nets' fragility, toxicity, and effect on the natural immunity of populations in endemic countries to malaria[60]. Despite their indisputable beneficial impact, ITN can only ever be one part of a wider anti-malaria effort, and some have argued that there is a danger in over-focussing malaria control efforts on ITN.

1.2.6.3 Vaccines

Efforts to develop a malaria vaccine have been made for decades, but no candidate has managed to successfully get to market. There is still a lot of research attempting to develop a viable and effective malaria vaccine, and a lot of the research funding for malaria goes towards these efforts. The Bill and Melinda Gates Foundation, for example, have committed over $200 million to support their development[61, 62].

The most promising candidate to date is the RTS,S/AS01 vaccine developed by Glaxo-SmithKline (GSK, Brentford, UK). Results from Phase 3 clinical trials showed that the vaccine efficiency was 46% against clinical malaria and 34% against severe malaria. The results were not as good for infants, with a vaccine efficiency of 27% against clinical

malaria, and no effect against severe malaria. The results from the trial, which enrolled over 15 thousand children in Africa, were slightly worse than had previously been hoped[63, 64, 65, 66].

1.2.7 Drug resistance

The ability of malaria parasites to develop a resistance to pharmaceutical anti-malarials has always been a problem - the WHO first defined drug resistance of malaria in 1967[67]. For decades chloroquine was the frontline drug against all malaria strains; it still is for *P. vivax*. *P. falciparum* resistance to chloroquine emerged in the 1980s, causing significant increases in incidence and mortality[68]. In the Mlomp district of Senegal, for example, mortality rates in children under 5 increased 14-fold in the ten years to 1995, as resistance to chloroquine rose from 0% to 44%[69]. The WHO reports that chloroquine resistance is high or extremely high in every endemic country except Honduras, Malawi and Nicaragua[67].

P. vivax has also developed a resistance to chloroquine, although it is speading at a much slower rate than *P. falciparum* resistance. A recent review by Price *et al.* found that some level of chloroquine resistance is present in most endemic countries[70]. Indonesia is particularly badly affected - several trials have reported early treatment failure (parasitaemia above 0 less than three days after treatment) in over 20% of cases, and relapse after a month in over 70% of cases. This has prompted some to call for chloroquine to be replaced by ACT as the frontline *P. vivax* treatment; the WHO currently recommends ACT combined with primaquine in resistant cases[42, 71, 70, 72, 50].

P. falciparum resistance to artemisinin has recently been observed in the Greater

Mekong region, particularly along the Cambodia-Thailand border, and the number of resistant parasite populations has been increasing[73, 68, 74, 75, 76]. New drug resistance has often first been observed in Cambodia before spreading to other areas of the world[77]. In order to combat the spread of resistance, the WHO recommends ACT, combining several drugs in order to reduce the chances of resistance being developed to one of them. However, their uptake remains insufficient, not least due to their higher cost[44]. The Roll Back Malaria partnership are putting a lot of effort into fighting this emerging resistance, and have launched the Global Plan for Artemisinin Resistance Containment (GPARC)[78].

There are two main reasons for the high level of concern regarding resistance to artemisinin and its derivatives: the destructive effects of resistance to previous drugs, and the lack of alternatives to artemisinin therapies in the pipeline[67, 78]. Currently, the resistance has not spread to Africa, but it is likely to do so despite GPARC's efforts[79]. While the haemofilter presented in this thesis is envisaged to hold benefits for patients beyond those with drug resistant malaria, the recent re-emergence of resistance adds urgency and relevancy to the device (see section 5.2.1).

1.2.8 Haemozoin and magnetic properties

During the erythrocytic stage of the life-cycle, the malaria parasite breaks down the haemoglobin of the cell, consuming the released amino acids in order to reproduce asexually within the cell. During this process, toxic free haem is produced as a by-product, and synthesised into haemozoin, also known as the malaria pigment, to avoid the destruction of the cell and parasite by the free haem. Haemozoin, which is paramagnetic, is stored in a food vacuole within the infected cell, giving malaria infected cells their magnetic

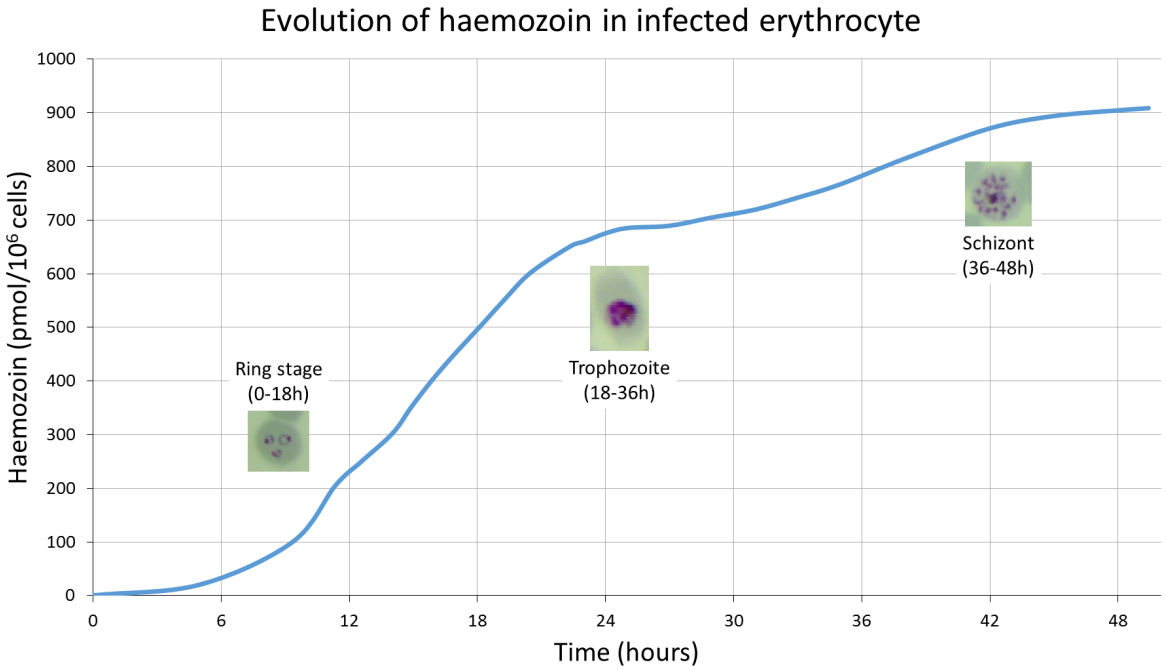


Figure 1.5: Evolution of quantity of haemozoin in infected erythrocytes from moment of infection with parasite[5]

properties[47, 80, 81, 82, 83, 84].

The quantity of haemozoin within an infected erythrocyte depends on the time since infection by the parasite (see Fig. 1.5, reproduced with data from [5]). In the presence of a magnetic field, this haemozoin will magnetise with a linear dependence on the magnetic field (see section 1.3). Thus the magnetic moment of an infected cell within a magnetic field will depend on the quantity of haemozoin in a cell as well as the magnetic field. Haemozoin has the same crystal structure as β-haematin - indeed, the latter is frequently used in experiments to demonstrate the effectiveness of new drugs - with an iron environment single spin of $s = 5/2$[85, 86, 87, 88, 89].

The molar magnetic susceptibility of haemozoin iron has been found to be $\chi_{mol} = 11 \times 10^{-3} ml/mol$[90]. To convert this to the dimensionless magnetic susceptibility χ from Eq. 1.1, the following relationship can be used:

$$\chi_{mol} N_{mol} = \chi V \tag{1.3}$$

where N_{mol} is the number of moles of haemozoin. So Eq. 1.1 for an infected erythrocyte becomes:

$$\bar{F_m} = \mu_0 \chi_{mol} N_{mol} \bar{H} \nabla H \tag{1.4}$$

Some groups have attempted to measure the volumetric magnetic susceptibility χ of malaria infected erythrocytes, which were first isolated using HGMS in 1981[91]. This is a challenging task due to the small size of the cells and high variation in haemozoin content (and hence susceptibility). χ in Eq. 1.4 is really the difference between the susceptibility of an infected cell and its environment (i.e. whole blood), and several studies have reported values ca. $1.5 - 2 \times 10^{-6}$ for mature infected erythrocytes (i.e. schizonts)[5, 90, 92].

Recent research using HGMS to isolate malaria infected erythrocytes is discussed in section 2.2.1.

1.3 Theory of magnetism

1.3.1 Origins of magnetism

The spin magnetic moment, $\bar{\mu_s}$, of electrons within a material depend on the electrons' spin angular momenta, $\hbar\bar{s}$:

$$\bar{\mu_s} = -g\mu_B \bar{s} \tag{1.5}$$

where μ_B is the Bohr magneton, equal to 0.927×10^{-20}emu, and g, a constant known as the g-value, is equal to 2.0023. Electrons are therefore magnetic dipoles[93].

In atoms, the angular momentum of electrons also creates a magnetic field in the same way a current flowing through a wire does - this is known as orbital angular momentum. For atoms, the total magnetic moment (ignoring contribution from the nucleus) therefore depends on both the sum of the orbital angular momentum and the sum of the spin angular momentum. In atoms with completely filled orbital shells, these angular momenta sum to zero. In atoms with incomplete shells however, the two combine to give the atom a net magnetic moment[93].

The magnetic moment of a material or object is equal to the vector sum of all the atoms' individual magnetic dipole moments. In non-permanent magnetic materials, the dipoles are randomly oriented, giving a net-zero magnetic moment. When exposed to a magnetic field, however, the internal dipoles align, magnetising the material. Magnetised objects create their own magnetic fields, and can have magnetic forces exerted upon them.

1.3.2 Magnetic fields and susceptibility

Magnetic fields are expressed as either the H-field, which has units of $\mathrm{A{\cdot}m^{-1}}$, or the B-field, more commonly known as magnetic induction, which is measured in teslas (T). They are related by the permeability of free space: $\mu_0 = 4\pi \times 10^{-7}\mathrm{m{\cdot}kg{\cdot}s^{-2}{\cdot}A^{-2}}$ and magnetisation of the space[94]:

$$\bar{B} = \mu_0(\bar{H} + \bar{M}) \tag{1.6}$$

In free space, $\bar{M} = 0$, so the two are linearly connected - any difference between them

is purely in terms of units. However, within a magnetically responsive material, $\bar{B}$ is related to $\bar{H}$ via the degree to which the material is magnetised by $\bar{H}$. This is defined using the dimensionless magnetic susceptibility, χ, which determines its magnetisation, or magnetic moment, $\bar{M}$ in a given magnetic field:

$$\chi = \frac{\bar{M}}{\bar{H}} \tag{1.7}$$

$$\therefore \bar{B} = \mu_0 \bar{H}(1 + \chi) \tag{1.8}$$

$$\bar{B} = \mu_r \mu_0 \bar{H} \tag{1.9}$$

where $\mu_r = (1 + \chi)$ is known as the relative magnetic permeability of a material[94].

1.3.3 Forms of magnetism

The class of magnetism to which a material belongs is determined by its relative magnetic permeability (and therefore susceptibility), which is in essence a measure of how favourably a magnetic field passes through a material. Materials with susceptibility less than or equal to 0 are diamagnetic. With the notable exception of superconductors, which will not be discussed here, their susceptibilities are very small and their magnetic responsiveness is almost imperceptible. As these materials do not apparently respond to permanent magnets they are generally considered to be 'non-magnetic'; in fact, they are weakly repelled by magnets (see Eq. 1.10).

The way in which a material's internal dipoles respond to a magnetic field determ-

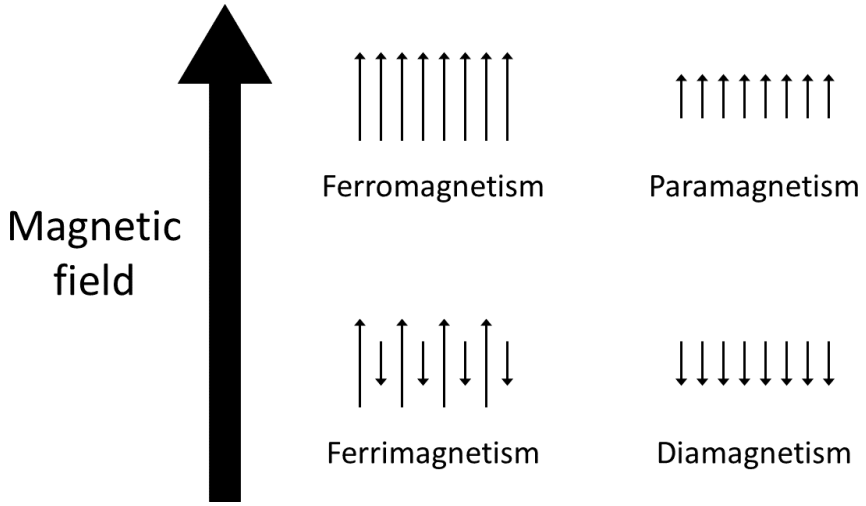


Figure 1.6: Magnetic dipole alignment for different magnetic material classes

ines the magnetic class to which it belongs (Fig. 1.6). For diamagnetic materials, the dipoles align in the opposite direction to the field. For materials with positive magnetic susceptibilities, the dipoles align in the same direction as the field and the object magnetises. For non-ferromagnetic materials, when the magnetic field is removed, the dipole orientations randomise, and the object demagnetises. Ferromagnetic materials have the ability to maintain their magnetisation while below their Curie temperature, the point above which thermal energy causes the magnetic ordering to be lost.

Paramagnetic materials have small, positive magnetic susceptibilities. They are considered 'weakly' magnetic, and their response can be difficult to detect.

Ferromagnetic materials have large, positive susceptibilities. They are 'strongly' magnetic, and comprise the materials most frequently labelled 'magnetic', most of which are iron based, such as steel.

Superparamagnetic materials are paramagnetic below certain temperatures

Other forms of magnetism, such as ferrimagnetism and antiferromagnetism, are not relevant to this project and will not be discussed.

1.3.4 Magnetic forces

As discussed in section 1.3.2, magnetic fields magnetise objects, giving them a magnetic moment, $\bar{M}$. In the case of paramagnetic materials, such as haemozoin, the effect of this acquired magnetic moment is that, in the presence of an inhomogeneous magnetic field, the object will be attracted in the direction of the highest magnetic field. This phenomenon is the result of the magnetic force exerted on an object, which is given by Eq. 1.1[95]. Combining Eq. 1.1 with Eq. 1.7 and Eq. 1.6 gives the equation in terms of the B-field:

$$\bar{F_m} = \mu_0 \chi V \bar{H} \nabla \bar{H}$$

$$\bar{B} = \mu_0 \bar{H}$$

$$\therefore \bar{F_m} = \frac{\chi V}{\mu_0} B \nabla B \tag{1.10}$$

where V is the volume of magnetic material, and ∇B is the field gradient.

So the magnetic force on an object depends on both its magnetic moment and the local variance in the magnetic field. The consequence of Eq. 1.10 is that to generate a magnetic force on an object, the field in which it is located must be inhomogenous, and the object will move towards higher fields. When trying to generate high magnetic forces, the objective should therefore be to maximise not only the magnitude of the field, but also the field gradient in the direction of the desired force. Indeed, priority is typically given to the generation of a high magnetic gradient, as higher magnetic fields cease to

have an effect on the force on an object once the object's saturation magnetisation has been reached.

Chapter 2

Clinical Applications of High Gradient Magnetic Separation

2.1 Introduction

The focus of this thesis is the design and development of a magnetic haemofilter for the treatment of malaria. However, as mentioned in section 1.1.2, the device is theoretically capable of removing any magnetic material from the bloodstream, giving rise to a host of potential applications. In the case of malaria, the intrinsic magnetic properties of the infected cells are exploited; any other agent in the blood with paramagnetic, superparamagnetic or ferromagnetic properties can also be captured as long as the magnetic force on the agent is greater than the drag force it experiences within the separation chamber.

Biological entities with positive magnetic susceptibilities, such as magnetotactic bacteria or the cells that give rise to magnetoreception, are uncommon and very rarely present

in the bloodstream[96, 97, 98]. However, a large number of different agents can be 'magnetically labelled' using magnetic beads or nanoparticles[95, 99, 100]. The ability to functionalise these magnetic carriers, for example by conjugating antibodies to their surface, enables their binding to specific agents, which can then be magnetically actuated.

In these applications, functionalised magnetic particles are mixed with blood, either through prior injection into the patient, or within the extra-corporeal loop. In the latter, the loop would include a mixing chamber in which a 'swarm' of magnetic particles is created, causing a high number of collisions between the particles and targets. The particles bind specifically to the target agent that needs to be removed - this could be an 'undesirable' agent, such as a toxin, or a 'desirable' agent, such as a stem cell that can then be used in research or a treatment. These magnetic particles, which are usually iron oxides, need to be biocompatible, able to survive in the bloodstream, exhibit high magnetic susceptibilities, and bind to their targets with high efficiency and specificity. The specificity is particularly important to avoid false positive capture, while the biocompatibility and non-toxicity are paramount: the magnetic particles must not cause any harm to the patient.

Thus combined with magnetic labelling techniques, the magnetic haemofilter could be used to remove from the bloodstream any agent that can be labelled. This gives rise to a large number of potential applications for the device presented in this thesis. Many research groups are already working on magnetic nanoparticles (MNP) or beads which target specific blood-borne agents in order to deliver clinical benefits; some have designed their own magnetic separators, including one group working on a malaria treatment similar to the one in this thesis, albeit with a very different haemofilter design (see section 2.2.1).

Others are working on magnetic particles for diagnostic or bench-top purification purposes (a well established field), sometimes without considering their clinical applications.

This Chapter provides an overview of the state of the art of magnetic particle research as it could be applied to the magnetic haemofilter, outlining some of the many possible future clinical applications of the device presented in this thesis. To facilitate this analysis, the Chapter has been broken down into three main sections which constitute potential applications of the haemofilter:

1. *Cells*: The separation of undesirable cells from the bloodstream for treatment or diagnosis of a disease, or the separation of desirable cells for re-use in treatment or research.

2. *Sepsis*: Magnetic haemofiltration as a treatment for sepsis, through the removal of bacteria, fungi and other sepsis-causing pathogens.

3. *Detoxification*: The detoxification of blood, for the treatment of kidney disease and renal failure, drug overdoses or radiation exposure.

4. *Viruses*: The diagnosis, treatment or control of viral infections through direct magnetic extraction of circulating virions.

This Chapter should not be taken as a comprehensive review of the applications of magnetic particles, or of the potential biomedical applications of HGMS - these topics have been written about in great detail in many reviews and books[95, 99, 100, 101, 102, 103, 104, 105]. One of the core strengths of the magnetic haemofilter is that its applications are limited only by what can be usefully magnetically labelled. MNP have been used to target an incredible number of different agents, including cells, DNA, viruses and bacteria,

and hold great promise in terms of unlocking future therapeutic applications.

Table 2.1: Summary of clinical applications of HGMS presented in chapter

Target	**Targeting moiety**	**Applications**	**Ref.**
Malarial erythrocytes	N/A (naturally magnetic)	Treatment for malaria Purification for diagnosis and research	[106, 92, 107, 108]
Malaria gametocytes	N/A (naturally magnetic)	Reduce malaria transmission Purification for diagnosis and research	[107, 108, 109, 110, 111]
Colon CTC	BER-EP4	Improved detection of CTC for diagnosis	[112]
Prostate CTC	BER-EP4	Improved detection of CTC for diagnosis	[113, 114, 115]
Kidney CTC	BER-EP4	Improved detection of CTC for diagnosis	[113, 114]
Lung CTC	BER-EP4 EpCAM	Improved detection of CTC for diagnosis Lab-on-a-chip diagnosis	[116, 117, 118]
Melanoma CTC	Ig-G	Improved detection of CTC for diagnosis	[119]
Leukaemia CTC	CD3	Improved detection of CTC for diagnosis	[115]
Breast CTC	EpCAM	Lab-on-a-chip diagnosis	[117, 120, 118]

Target	**Targeting moiety**	**Applications**	**Ref.**
Circulating epithelial cells	EpCAM	Improved detection of CTC for diagnosis	[121]
Fetal cells	CD71 GPA CD14 and CD45 (depletion)	Diagnose pre-natal diseases	[122, 123, 124]
Hematopoietic stem cells	CD34 CD133	Stem cell transplantation	[125, 126, 127]
Sepsis causing pathogens	Mannose binding lectin	Treatment	[128, 129]
Sepsis related cytokines	Cytokine antibodies	Treatment	[130, 131, 132]
Homocysteine P-cresol	Bovine serum albumin	Improve treatment of kidney disease	[133, 134, 135]
Caesium-137	Prussian Blue	Blood detoxification (radioactive toxins)	[136]
Horseradish peroxidase	Streptavidin	Blood detoxification	[137]

Target	**Targeting moiety**	**Applications**	**Ref.**
Uranyl ions	Biophosphonate	Blood detoxification (radioactive toxins)	[138]
Digoxin	Digoxin antibodies	Blood detoxification (drugs)	[139]
Thrombin	DNA polymers	Blood detoxification (drugs)	[140]
Doxorubicin	DNA polymers	Blood detoxification (drugs)	[140]
Lead ions	Iminodiacetic acid	Blood detoxification (heavy metals)	[139]
Cadmium ions	Phenylazanediyl diacetic acid	Blood detoxification (heavy metals)	[141]
HIV virions	CD44	Lab-on-a-chip diagnosis	[142]
Avian flu virus	Anionic polymer Monoclonal viral antibodies	Improved detection for diagnosis	[143, 144, 145]

Target	**Targeting moiety**	**Applications**	**Ref.**
Hepatitis virus	Poly(ethylene imine) Protamine hydrochloride Hepatitis antibodies Lectin	Improved detection for diagnosis	[146, 147, 148, 149]

2.2 Cells

2.2.1 Malaria

As was discussed in section 1.2.8, the magnetic properties of malaria infected cells have been known since 1981, when Paul *et al.* first performed magnetic separation of malarial blood samples[91]. Since then, bench-top magnetic separation columns (for example the MACS columns available from Miltenyi Biotec, Germany) have been used to purify malaria infected cells for research[83, 84, 108]. A couple of research teams have taken this further, suggesting exploiting the magnetic properties of haemozoin to improve existing diagnosis techniques or as a potential treatment.

Gandini *et al.* have attempted to produce a device similar to the one presented in this thesis: a magnetic haemofilter for the treatment of malaria[92, 106]. Their device, like many magnetic separators presented in this section, functions in a very different manner to the haemofilter presented in Chapter 3, relying on separation by fractionation within microfluidic channels. They have performed computational simulations of infected cells passing through the device.

The group's device consists of a $200\mu m$ diameter microfluidic channel across which a magnetic field gradient is created using a permanent magnet and series of ferromagnetic wires, placed adjacent to the channel. The infected cells are pulled to one side of the channel and, thanks to laminar flow, separated into a capture chamber. In their simulations, only the infected cells entering the channel on the side of the permanent magnet were captured within a channel length of 80mm. This could be resolved by increasing channel length or magnetic force, or by having ferromagnetic elements on both sides of

the channel, rather than on one side only. Their simulations were conducted at flow rates of 1ml/min and 5ml/min in a Newtonian fluid (note they do not specify the viscosity used in their simulations).

Gandini's group has not published any further work. The patent is assigned to AccelDX, a recent start-up that does not appear to be working on the malaria device. CEO and lead author Gandini, was quoted in a blog in 2012 as saying "I developed a blood purification device, however the project was too costly and we couldn't get funding"[150]. In August 2012, AccelDX was awarded a $350k grant from the US National Institutes of Health for a project titled "Blood Filtration System for the Treatment of Severe Malaria Patients"[151]. However, the project was due to finish on 28 February 2013, and no results were published, and AccelDX have not mentioned the project on their website.

Karl *et al.* have used magnetic separation in order to purify both malaria infected erythrocytes and gametocytes, the sexual form of the malaria parasite (see section 1.2.2), from a blood sample[109, 110, 111, 107]. They compared their *in vitro* magnetic separation method with the established methods of light microscopy and reverse transcription-polymerase chain reaction (RT-PCR), which have detection thresholds of 16 and 0.13 per μL of blood respectively; the magnetic separation method was expected to have a detection threshold of 0.05 per μL. They found that the magnetic separation method was as effective as RT-PCR in detecting gametocyte positivity and quantitative density, and was also easier to carry out in-the-field, requiring less specialised equipment and training[109].

The magnetic separator used by Karl *et al.* is similar to the MACS column sold by Miltenyi Biotec, but with significantly lower costs. It consists of a modified plastic pipette filled with $420\mu m$ diameter S110 steel shot spheres which is placed between two NdFeB

magnets. These are connected with an iron yoke, creating a maximum field of 1T in the area in which the column was placed. Using this arrangement, the group extracted nearly 100% of gametocytes at a flow rate of 0.28mL/min[111].

Other suggestions for exploiting the magnetic properties of haemozoin for malaria diagnosis include improving the detection of parasites through magnetic rotation of the haemozoin crystal[152, 153], a magneto-optic finger probe for non-invasive *in-vivo* diagnosis[154], and improving sensitivity of detection using MNP[155].

The existing research demonstrates the potential of exploiting the magnetic properties of haemozoin in several areas of malaria, from basic research through diagnosis to potential treatments. However, no group has successfully developed a magnetic haemofilter appropriate for the treatment of patients, due to the difficulty of achieving high separation efficiencies at low cost. The literature not only supports the idea of the magnetic haemofilter presented in this thesis being valuable as a novel treatment, but also suggests that it could form part of a cheaper and more effective diagnosis technique. Furthermore, the work of Karl *et al.* indicates that the magnetic haemofilter may have an effect on malaria transmission rates through the removal of gametocytes, which should be easier to magnetically capture than infected cells.

2.2.2 Circulating tumour cells

Cancer spreads from its primary site to other areas of the body through metastasis, during which circulating tumour cells (CTC) can be found in the bloodstream. These settle in other areas of the body, forming new cancers and leading to extreme complications. Many groups have demonstrated the possibility of magnetically labelling CTC using magnetic

particles. One of the first was Enis *et al.* in 1997. They used MNP functionalised with monoclonal antibodies to purify CTC from colon cancer cell lines, facilitating detection with RT-PCR[112].

Zigeuner *et al.* performed a similar study with prostate and kidney cancer cells, using commercial magnetic beads (Dynabeads®, Dynal AS, Norway) with a diameter of $2.8\mu m$. These were functionalised with antibodies specific to endothelial cells (BER-EP4) for positive selection of the cancer cells from mononuclear cells isolated from healthy volunteers. For samples with only one CTC per million mononuclear cells (an accepted CTC concentration for early-stage patients), the group increased positive detection of CTC from 23% to 93% of cases[113, 114]. Kularatne *et al.* used the same antibody to magnetically target lung cancer cells[116]. Georgieva *et al.* used a similar method but different antibodies to purify melanoma cells, using immunoglobulin-G coated magnetic beads[119].

More recently, Song *et al.* developed MNP able to target several different cancer cell types, functionalising Fe_2O_3 MNP with anti-CD3 monoclonal antibodies to target leukaemia cells, and prostate specific membrane antigen antibodies to target prostate cells. The combined cocktail of MNP were able to magnetically label up to 96% of cancer cells after a 15 minute incubation time. The group was able to detect the presence of CTC at a minimum concentration of one cancer cell per 10,000 (0.01%) healthy cells[115].

Other groups have focussed on the use of microfluidics in combination with these MNP technologies in an attempt to provide 'lab on a chip' cancer diagnostic tests. Ingber *et al.* used $2.8\mu m$ microbeads conjugated with epithelial cell adhesion molecule (EpCAM) antibodies to extract breast cancer cells from whole blood flowed through their device.

They were able to isolate nearly 90% of cancer cells from an initial concentration as low as 2 cells per ml of blood. The group also successfully used their method to extract CTC from blood samples of mice with breast cancer, with high specificity (only 0.4% of the captured cells were leukocytes)[120]. Kim *et al.* have reported a very similar system, also using EpCAM functionalised magnetic nanobeads and a microfluidic magnetic separator to isolate CTC from blood taken from patients with lung and breast cancer[117].

Neither Ingber *et al.* nor Kim *et al.* suggest incorporating their device into an extra-corporeal loop as a potential treatment for cancer or method to prevent its spread through the body. Indeed, with flow rates of 1.6ml/hour and 5ml/hour respectively, their devices would not be appropriate for such an application. Chang *et al.* have reported a similar device capable of significantly higher flow rates (up to 4.3ml/min), also using EpCAM antibodies to capture breast and lung cancer cells, but like the other groups, propose a benchtop device to purify samples taken from patients, not incorporated into an extra-corporeal loop[118].

The rarity of CTC, which can be as low as 1-10 CTC per ml of blood, which would also contain 10^6 leukocytes and 10^9 erythrocytes, makes their detection (and thus the detection of metastasis) very challenging, so the focus on their purification from samples of blood from cancer patients in understandable[156]. This section barely scratches the surface of the research going into new methods for the purification of CTC from whole blood - the subject has recently been the subject of entire theses - but serves to give an idea of the volume and type of research being carried out and demonstrate the state of the art of the selective magnetic labelling of CTC[156, 157, 158, 159, 160].

The question remains whether extra-corporeal magnetic haemofiltration using MNP

specific to CTC could provide any clinical or diagnostic benefit. One could imagine that continuous filtration 24 hours a day, using a discreet wearable magnetic filter, could reduce the risk of cancer spreading. It could also have the additional advantage of facilitating the detection of metastasis through analysis of the captured cells when the 'separation cartridge' is changed. However, it is doubtful that such a method could prevent cancer spreading - only one cell is required to settle at a new site for a new tumour to form. That being said, the huge human and economic toll exerted by cancer, and the significant worsening of patient prognosis caused by metastasis, could justify investigation into such a device, which could be valuable even if it only slows, rather than prevents, the spread of the disease[161, 162, 163].

2.2.3 Other rare cells

CTC are one of a class of 'rare cells' that it could be beneficial to isolate from whole blood. Others include circulating endothelial cells, fetal cells, viral or parasitic cells, progenitor cells and stem cells[157, 160, 164]. Circulating endothelial cells, similarly to CTC, are used for disease prognosis and personalised cancer treatment. They can be isolated using similar methods, for example using EpCAM antibodies conjugated to magnetic particles[121]. Fetal cells are used to diagnose prenatal diseases such as sickle cell anaemia or to perform genetic analyses[122]. Their benchtop magnetic separation has been possible for two decades - initial techniques required the depletion of other cells to ensure specificity, but 'one-step' techniques have also been reported[123, 124].

While CTC and fetal cells are extracted from peripheral blood for diagnostic purposes, progenitor cells and hematopoietic stem cells (HSC) are used in various treatments. HSC,

for example, are extracted for the purposes of stem cell transplantation (SCT), principally as a treatment for leukaemia. This may include autologous SCT, wherein the patient's own stem cells are used. These can be extracted from the bone marrow or from peripheral blood, but are extremely rare in both cases[164].

Magnetic separation of HSC sometimes involves the depletion of other cells, but markers such as CD34 and CD133 have been used to positively extract them; negative depletion is not a feasible option within an extra-corporeal loop. MNP functionalised with anti-CD34 antibodies could be used to extract HSC directly from donors with high purity[164, 157, 165, 125, 126, 127]. Similar methods have been used to isolate more differentiated progenitor cells, which also have valuable clinical applications[166, 167].

Mesenchymal stem cells also have significant clinical potential, and can be isolated with the use of MNP[168, 169]. However, recent research suggests that they are not found within the bloodstream[170]. The same is true of embryonic stem cells, particularly valuable as they can differentiate into any cell type. While they can be magnetically labelled, their magnetic separation will always remain a benchtop technique and is not appropriate for extra-corporeal magnetic haemofiltration[126, 171].

2.3 Sepsis

Sepsis is caused by the body's immune response to pathogens in the bloodstream[172]. It is one of the most common deadly diseases and one of the leading causes of death in the developed world, exerting a huge human and economic toll. The mortality rate is 36%. In the UK, there are over 100,000 cases and 37,000 deaths annually; in the US, there are over a million cases and approximately 250,000 deaths. With an annual cost

of $20.3 billion in 2011 (up from $4.4 billion in 1997), it is the most expensive condition in the US, making up 5.2% of total healthcare spending. Direct costs to the NHS in the UK are over £2.5 billion. Sepsis incidence has been increasing rapidly, by about 10% in the decade to 2013. Incidence is 50% greater than myocardial infarction (heart attacks) and 33% greater than stroke. Severity and mortality is strongly linked to the number of pathogens in the bloodstream[161, 162, 173, 174, 175, 176].

2.3.1 Haemofiltration for the treatment of sepsis

Recently, Ingber *et al.*, the same group mentioned in section 2.2.2, presented a microfluidic magnetic separation device designed to remove pathogens and toxins directly from the bloodstream using functionalised MNP[128, 129]. They used genetically engineered mannose-binding-lectin (MBL) as a targeting agent, binding it to the surface of 128nm diameter MNP via immunoglobulin-G and streptavidin. MBL is able to bind a wide range of pathogens and bacteria, so is ideal for MNP functionalisation[177]. They have designed a microfluidic extra-corporeal magnetic separator so that the MNP mix with the blood within the blood loop and do not need to be injected into the bloodstream.

In their early study, the group used functionalised magnetic microbeads to extract 80% of *Candida albicans* fungi, a prominent sepsis pathogen. In a more recent *in vitro* study, they group simutaneously removed from whole blood 98% of anaerobic and 80% of aerobic bacteria in a single pass through the device, and over 90% of *Staphylococcus aureus*, *Candida albicans* and *Escherichia coli* after five passes. Initially, their separation device only removed 80% of the MNP, but this was increased to nearly 100% by adding $1\mu m$ diameter magnetic microbeads to capture the MNP. In these experiments, the MNP

were added to the blood in a constant flow, so binding took place during the process. The experiments were conducted at a flow rate of only 10ml/hour, but it should be noted that the factor limiting the flow rate is the binding of the MNP to the targets, rather than the magnetic extraction of the pathogen-bound MNP. 90-99% separation efficiencies were achieved at 535ml/hour for 'pre-bound' MNP[128].

The group then tested their device on septic rats, achieving similar results to their *in vitro* experiments: 90% of *S. aureus* and *E. coli* were removed from the rat's bloodstream in one hour. Furthermore, significant reductions of pathogens and $CD45^+$ inflammatory cells in the lung, spleen and kidney were achieved. After injection with a lethal dose of lipopolysaccharide, rats being treated with the device had significantly less of the endotoxin in those organs, and saw improved survivability when compared to untreated rats. The device appears to alleviate or prevent the symptoms of sepsis - the treated rats not exhibiting any signs of clinical distress while untreated rats did[128].

The separation device used by Ingber *et al.* consists of 16 microfluidic channels with a height of $16\mu m$. Each channel has two sections - the blood containing pathogens bound to MNP flows through the bottom channel, while saline is flowed through the top channel. NdFeB magnets placed above the device pull the MNP through small holes out of the blood flow and into the top channel, which is then discarded. In terms of magnetic separation efficiency, their device is quite effective, able to capture 90% of MNP from blood at a flow rate of 9ml/min, and 60% at 17ml/min. However, its potential flow rate is severely limited by the binding of the MNP to the target pathogens, which required a flow rate of 10ml/hour. In order to filter the total blood volume of a typical adult at this flow rate, 500 hours would be required. While the group could use several of

their devices in series, it would still take a long time to effectively clear the patient of pathogens (especially because filtering only total blood volume is insufficient - see section 3.1.2). Even if the MNP were injected into the patient so that the device could run at 9ml/min, 10 hours would still be required to filter an adult's total blood volume, and 40 hours would be required to achieve a 90% pathogen reduction.

2.3.2 Bacteria and cytokines

While Ingber *et al.* are the only group to have attempted the development of a magnetic haemofilter combined with MNP for the treatment of sepsis, other groups have used functionalised MNP to target bacteria or cytokines responsible for sepsis, which can be caused by a wide variety of pathogens. Weber and Falkenhagen, for example, used iron oxide MNP functionalised with appropriate antibodies to target interleukin 1β and tumor necrosis factor α, two major sepsis causing cytokines[178, 179]. They successfully removed 80-90% of the pathogens from blood plasma[130]. Herrmann *et al.*, another research group developing an extra-corporeal magnetic separator (see section 2.4.3), also used antibody functionalised MNP to target interleukin-6β, another inflammatory mediator linked to sepsis, reducing concentation in blood by 38% in a single pass through their device[131, 132, 180].

Other research groups have focussed on the purification of bacteria to increase detection sensitivity with mass spectrometers. This could lead to applications in food quality control or water treatment[181, 182]. Bacteria targeted with functionalised MNP include *Salmonella*, *Bacillus*, *Staphylococcus*, *E. coli*, *Bifidobacterium longum, Listeria monocytogenes* and many others[183, 184]. Others have used non-magnetic functionalise nano-

particles such as immunoglobulin coated platinum to target various bacteria[185]. Others target bacterial nucleic acids such as DNA and RNA[186, 187].

In conclusion, the extensive use of functionalised MNP to target a wide range of bacteria and other pathogens responsible for sepsis indicate that the treatment of sepsis could be a very promising application for magnetic haemofiltration. The work of Ingber *et al.* has created the link between magnetic labelling and sepsis therapy, and their *in vivo* results are particularly promising; their magnetic separation device however needs improvement before becoming a feasible medical device, particularly in terms of flow rate.

One significant challenge will be the development of MNP able to target an extremely wide range of sepsis-causing pathogens while remaining unharmful to other blood components. As sepsis can be caused by almost any infection with bacteria or fungi, one solution may be to produce a 'cocktail' of different MNP, each targeting a different common pathogen known to cause sepsis. Together, the MNP could be designed to target over 99% of pathogens commonly associated with sepsis. This cocktail, along with an appropriate magnetic haemofilter, could then be administered to any patient that succombs to an infection in a hospital intensive care unit (ICU), even before the cause of the infection is identified. It is feasible that such a method, if it were cost-effective (particularly in terms of the MNP cost) could become common-place in ICU throughout the developed world, helping to reduce deaths caused by one of the developed world's biggest killers.

2.4 Detoxification

2.4.1 Kidney disease

Stamopoulos *et al.* have used MNP alongside dialysis in a technique they call magnetically assisted haemodialysis (MAHD). Their intention is to deliver clinical benefits to late stage chronic kidney disease and end stage renal disease patients by injecting functionalised MNP into the patient in order to target toxic substances circulating in the bloodstream. The MNP and targets are removed by adding a magnetic filter to traditional dialysis circuits, removing toxins with higher efficiency and specificity than existing dialysis membranes, which rely on diffusion and convection through micro- or nano-sized pores[188, 133, 134, 135].

The group advocates the use of iron oxides (Fe_2O_3 and Fe_3O_4) as the MNP, principally because of their biocompatibility. The group studied the effect that unfunctionalised Fe_3O_4 MNP and Fe_3O_4 functionalised with bovine serum albumin (BSA) conjugates had on blood cells. They did not find any interference between the MNP and white or red blood cells, even when concentrations were high or when the cells were matured in the presence of MNP[188, 189]. The group used homocysteine and p-cresol as simulant targets *in vitro*, both of which occur in humans and are known to cause clinical symptoms. Elevated homocysteine levels in the bloodstream can cause cadiovascular disease and arteriosclerosis, while p-cresol has an impact on the metabolism. Binding efficiencies of approximately 40% and 20% were reported for homocysteine and p-cresol respectively[135]. The group claims that their method increases removal of homocysteine by 60% when compared to normal dialysis[134].

Interestingly, the group talks of the possibility of producing MNP functionalised to bind with multiple targets: "creatinine, urea, homocysteine, β-2-microglobulin etc."[135]. The separation of β-2-microglobulin could be particularly valuable - its inefficient removal is a cause of hemodialysis-associated amyloidosis, which causes joint problems ranging from carpal tunnel syndrome to paraplgeia, and can be fatal[190, 191, 192]. Indeed, other groups have investigated novel, non-magnetic methods to remove it and prevent amyloidosis[193]. Stamopoulos *et al.* target β-2-microglobulin with MNP functionalised with BSA, reporting rapid and efficient adsorption[133].

The magnetic separator used by Stamopoulos *et al.* was simply a disc NdFeB magnet placed below the tubing of the dialyser. The MNP are retained on the walls of the tubing. With this design, the group were able to capture 80% of unfunctionalised MNP from saline in a single pass. However, 15-20 passes of the BSA functionalised MNP were required to achieve a similar separation efficiency[135]. The flow rates used by the group varied from 80ml/min to 250ml/min. Separation ceased to be efficient above about 150ml/min[133]. The group's work demonstrates that their MNP technology has significant potential in terms of binding toxins and delivering clinical benefits to patients with kidney disease, but their current magnetic separator is inadequate. The group has only analysed the separation in saline, and previous research has demonstrated the difficulty in achieving magnetic separations at higher viscosities (i.e. blood rather than saline)[194].

2.4.2 Radionuclides

MNP have been functionalised in order to treat nuclear waste by tageting radionuclides or actinides[195, 196, 197, 198]. Kaminski, who has worked on magnetic microspheres

to target actinides since 1996, has for over ten years been collaborating with Rosengart, Chen and other colleagues on projects looking at using these MNP in the domain of blood detoxification, with a focus on radionuclides and blood-borne toxins. Their objective is to develop "a magnetically based detoxification system as a therapeutic tool for the selective and rapid removal of biohazards, i.e. chemicals and radioactive substances, from human blood"[194]. Their research has been partly funded by the Defense Advanced Research Program Agency (DARPA), the research arm of the US military, as the researchers believe that a portable version of their device could provide a convenient 'in-the-field' treatment to troops exposed to biological or chemical weapons.

Most of the group's work has focussed on the magnetic separator, which consists of several narrow tubes or capillaries placed between two ferromagnetic wires. A strong external magnetic field is applied, creating local field gradients close to the wires, pulling magnetic materials onto the surface of the tubes, where they are isolated. This design has been modelled extensively, showing a potential 90% MNP capture efficiency (although the design needs to be improved to be as effective for viscous fluids)[199].

The group has suggested some MNP which could be used, including MNP to separate caesium-137, a radioactive isotope of casesium. These are magnetite nanoparticles encapsulated in a poly(ethylene)glycolic acid matrix, coated in long-chain poly(ethylene)glycol (PEG), which is known to prolong lifetime in the blood by preventing clearance by the spleen, and finally the surface functionalised with Prussian Blue, a chelating agent that binds strongly to caesium[136]. They have also functionalised commercial magnetic latex particles with streptavidin, demonstrating a binding affinity to horseradish peroxidase conjugated with biotin which was used as a model toxin. Successful binding was demon-

strated via magnetic separation, although not under constant flow conditions using the magnetic separator described above[137].

One research group looked at the parameters which affected the separation of commercial $1.7\mu m$ magnetic spheres, consisting of magnetite within a polystyrene matrix (Micromod, Germany). The main variables were viscosity, flow rate and tubing thickness. The group concluded that it was possible to remove 90% of spheres from blood only if they had 3,000 individual tubes of polyetheretherketone tubing with an inner diameter of 0.5mm and length of 15mm. They suggest this could be improved by optimising their design, increasing the magnetic field and magnetic, and using particles with higher magnetic content[194].

Kaminski and Rosengart are clearly hopeful about the potential of magnetic haemofiltration as a detoxification technology and, similarly to this thesis, have focussed largely on the magnetic separator. Indeed, in 2006, they published a patent proposing using magnetic particles to remove any "deleterious substance" and detailing their magnetic separator[200]. The potential applications mentioned in their research are wide-ranging: "for selective and rapid decorporation of biological, chemical, and radioactive biohazards from humans"[201], or simply "decorporation of blood-borne toxins from humans"[136]. They have not developed any novel MNP, and have not yet successfully extracted a clinically relevant target from blood using their haemofilter. There have been no publications related to this research since 2010.

Wang *et al.* have also removed radionuclides from blood, targeting uranyl ions, suggesting that the same method could be used to remove a number of other radioactive metal toxins from blood[138]. The group used Fe_3O_4 MNP functionalised with a novel

conjugate of dopamine and bisphosphonate. Using a simple *in vitro* process, in which they spiked water and blood with uranyl ions and their MNP, they removed 99% of the uranyl from water and 69% from blood by dipping a bar magnet into the solutions. The lower efficiency on blood is almost certainly due to their basic magnetic separation technique, as the 99% removal from water indicates that the binding efficiency of the MNP to the uranyl is high[138].

One limitation of Kaminski and Rosengart's device may be the need to have a large series of essentially microfluidic channels (500-750μm). This could make each separation chamber very expensive to manufacture. The group have been trying to design a portable magnetic separator, that can be strapped to the arm, and filters 6L of blood in 30 minutes. This creates extreme engineering challenges, requiring a flow rate of 200ml/min through microfluidic channels without removing too much blood from the body at any one time. Their 2007 design used channels 15cm long and with inner diameters of 0.5mm, but required 3,000 channels to achieve their desired separation efficiency of 90%[194]. Furthermore, it is questionable whether a 90% separation efficiency of the magnetic particles would be sufficient.

In conclusion, the magnetic haemofilter design of Kaminski, Rosengart *et al.* will need significant improvement before it is able to deliver the potential benefits, which the researchers clearly believe are broad and significant. The group's work over five years demonstrates not only the potential value of such a device, should an appropriate one ever be produced, but also the difficulty in designing one which can achieve the required flow rates and separation efficiencies. Their work, combined with that of Wang *et al.* and those who have tageting radionuclides with MNP for the purposes of water purification,

show that this could be a very promising technique for removing such particles from exposed humans.

2.4.3 Digoxin and other drugs

Herrmann and colleagues have worked on a magnetic separator in which, similarly to Ingber *et al.*, the MNP are never introduced to the system - they are introduced within the extra-corporeal loop, binding with the targets before entering the magnetic separation chamber[128, 131, 132, 139, 202]. Their ambition is for this technique to be used to remove any targetable blood-borne toxin. They use several model toxins to demonstrate the potential of their method, one of which is digoxin. Digoxin is a natural glycoside extracted from foxglove plants; it is the active ingredient for many drugs used in the treatment of several heart conditions[203]. Digoxin is toxic, and the accumulation of high levels in the bloodstream can be lethal[204].

Herrmann and colleagues used carbon-encapsulated platinum-spiked iron carbide MNP, 20-40nm in diameter. For the removal of digoxin, antibodies were conjugated to their surface via cross-linking with polyethylene-glycol (PEG) to render them specific to digoxin molecules. In their most recent study, published in 2013, they successfully reduced digoxin concentrations in rats by 50% in 10 minutes and 75% in 40 minutes. MNP concentrations were reduced from 0.5mg/ml to below the detection limit of $1\mu g/ml$[139].

The magnetic separator used by Herrmann *et al.* was built according to the design by Bockenfeld *et al.* (a group including Kaminski and Rosengart from section 2.4.2), in which the tubing is placed between four ferromagnetic wires magnetised by external permanent magnets[199]. The group does not specify if their design varied for each of their studies.

Magnetic materials are retained on or close to the walls of the tubing, which was 3mm in diameter. The blood is passed through the separator four times in order to achivee the required separation. The flow rate through the magnetic separator was 1.5ml/min during the rat experiments[131, 139].

The work of Herrmann and colleagues is particularly promising in that it avoids the problems associated with introducing MNP into the patient and could lower treatment costs due to efficient use of MNP. Whether this system will work for different target agents, such as cancer cells or bacteria, as claimed by the authors, remains to be seen. The lower contact time between the MNP and the target agents may limit capture efficiency and flow rates, causing unacceptably long treatment times.

Their magnetic separator has limitations in terms of flow rates - a 1.5ml/min flow rate would need 55 hours to address the typical total blood volume of an adult - and the fact that the blood needs to be circulated throught the device four times. However, the group may yet detail a scaled-up design more appropriate for human use, and their current method certainly seems successful in terms of removing MNP (although MNP are much easier to separate than, for example, a mangetically labelled cell). In any case, their work, which focusses on the MNP rather than on the separator, clearly demonstrates the potential of magnetic haemofiltration to remove disease causing toxins from the blood. The extra-corporeal magnetic labelling that they advocate is particularly interesting, avoiding the need to inject MNP into a patient.

Several other groups have demonstrated the binding of nanoparticles to specific drugs for which overdoses are common[205]. Wang *et al.*, for example, have functionalised MNP with DNA polymers in order to target thrombin and doxorubicin, used as models of

large and small molecules respectively. They successfully bound 70% of thrombin and 93% of doxorubicin[140]. In his review, Leroux details a number of nanocarriers being investigated to target different drugs, although most are not MNP based[206]. However, the volume of research indicates that many different drugs could be targeted with MNP, using many different binding methods.

2.4.4 Heavy metal ions

Herrmann *et al.* also used their device to remove lead ions (Pb^{2+}). Using the same platinum iron carbide MNP as in section 2.4.3, they functionalised the MNP with physisorbed poly(ethylene imine) (PEI) and iminodiacetic acid to target Pb^{2+}, reducing concentations in rats by 50% in 10 minutes and 75% in 40 minutes, the same results as they achieved with digoxin. This demonstrates that if a given MNP can extract a certain percentage of one toxin, it can probably extract the same percentage of another toxin that it binds to[139].

Jin *et al.* have similarly used PEI coated MNP to target cadmium ions. They synthesise their own iron oxide MNP, with a diameter of about 50nm, and use them to remove 98% of cadmium ions from water, as well as 50% of copper ions, demonstrating a high binding efficiency. After 10 minutes of incubation in cadmium ion spiked blood, the removal rate was 80%. These were achieved by simply placing a NdFeB magnet next to the sample. They also demonstrate that their particles do not cause any haemolysis in blood, even at very high concentations. The group also coat the MNP with 2,20-(phenyl azanediyl) diacetic acid and PEG to both improve dispersion and reduce uptake by the reticuloendothelial system[141].

2.5 Viruses

2.5.1 Human immunodeficiency virus

HIV type 1 virions have successfully been purified *in vitro* using MNP[142]. The purpose of the method was to provide a novel diagnostic tool as a replacement for centrifugation or RT-PCR, purifying the virus from samples of whole blood. The idea is to use 'lab-on-a-chip' magnetic separation to enable easy and cheap HIV diagnosis even from samples with very low viral concentrations. However, if the MNP are biocompatible and can survive within the bloodstream for a sufficient length of time, they could be used with the magnetic haemofilter described in this thesis to extract HIV directly from a patient's bloodstream.

It is not clear whether this would provide any clinical benefits, or what those would be, but it is feasible that such a method could be used to clear the virus from the body. HIV infection severity is linked to the viral load, or the concentration of virions in the blood[207]. Targeting these circulating virions could prevent further spread of the infection, and over time possibly help clear the virus from the body sufficiently to trigger remission. However, it would likely require very long treatment times, not least because only viruses that have not infected a cell would be separable, and achieving the required specificity may be a significant challenge.

Chen *et al.* used commercial anti-CD44 superparamagnetic particles with 50nm diameter (purchased from Miltenyi Biotech, USA). Their magnetic separator was a microfluidic device which first mixed a sample of blood spiked with HIV, and was then followed by a magnet placed above a 5x4x0.12mm separation chamber. The maximum viral sep-

atation efficiency achieved was 78%, achieved at a flow rate of $50\mu L/min$ and when the MNP and viruses were mixed in advance and not within the mixing device. While they report that 100% binding between viruses and MNP is possible within a test tube (albeit with a one hour incubation time), the mixing efficiency within their microfluidic device is only 79%. Thus the separation efficiency when mixing within the device dropped to approximately 50%[142].

The research by Chen *et al.* shows that magnetic capture of HIV virions is possible using functionalised MNP. Combining these or similar MNP with the magnetic haemofilter in this thesis could theoretically reduce a patient's viral load. However, the group's difficulty in achieving high binding between the MNP and the virus (1 hour incubation in one experiment, and 250 passes through their microfluidic mixer in another) demonstrates that this will be a significant challenge in an extra-corporeal loop device.

It is therefore likely that the MNP would need to be injected into the bloodstream in order to have sufficient time to magnetically label the viruses. They would thus have to survive within the bloodstream for that period. Furthermore, investigations into the consequences of a tagged virus infecting a T-cell would have to be investigated. The cell may become magnetically labelled and also be captured, or the MNP could prevent the cell from being infected, both of which would be positive results. However, it is also possible that the infection would leave the MNP bound to neither the virus nor the cell, and that as a result neither would be captured.

In conclusion, there does appear to be a potential clinical application for the magnetic haemofilter in terms of treatment of HIV. However, it is a particularly complicated area with questionable clinical benefits. Furthermore, the MNP required for such a treatment

are likely to incur a significant cost (particularly if they are conjugated with antibodies such as anti-CD44). As most of the regions in which the HIV/AIDS burden is highest are also poor[208], the economic viability of such a treatment would be a significant challenge.

2.5.2 Influenza

Influenza can be a highly dangerous virus, as demonstrated recently by the global concern at the bird flu and swine flu outbreaks in 2004 and 2009 respectively, and historically by the Spanish flu pandemic which caused the death of 27 million people[209]. Furthermore, the flu is estimated by the WHO to kill 250,000-500,000 people worldwide every year, out of 3-5 million severe cases[210].

Various groups have successfully functionalised MNP with influenza viral antibodies, for the purpose of diagnosis by viral enrichment from blood samples - a sample is taken from the patient, incubated with functionalised MNP, and enriched through bench-top magnetic separation. Current diagnosis techniques, which include RT-PCR and antibody-based assays, are insufficiently sensitive, unable to differentiate between particular strains and cumbersome.

Sakudo *et al.* used 300nm diameter ferrite MNP functionalised with the anionic polymer poly(methyl vinyl ether-maleic anhydride) to magnetically label the avian flu viruses H5N1 and H5N2[143]. Chou *et al.* used *ca.* 100nm diameter Fe_3O_4 MNP functionalised with subtype specific monoclonal antibodies, which successfully conjugated to H5N2 (but not H5N1). The group further suggests that hemagglutinin and nuraminidase, both abundant glycoproteins on the surface of influenza viruses, could be used as general influenza targets[144]. More recently, Hung *et al.* used 100nm manganese ferrite MNP

functionalised with anti-influenza A nucleoprotein monoclonal antibodies to sucessfully label H1N1[145].

This research conclusively demonstrates that antibody functionalised MNP can be used to target the influenza virus, and it's removal from a patient should result in significant clinical benefits, particularly if combined with antiviral pharmaceutical treatments. Specific subtypes can be targeted, but for the purposes of virus removal from the bloodstream, a general MNP which targets all subtypes, for example by binding to hemagglutinin, would be preferable. In conclusion, the magnetic removal of influenza viruses from the bloodstream could be a very promising application of the haemofilter.

2.5.3 Hepatitis

Hepatitis (Hep) viral infections are responsible for over a million annual deaths from liver disease cause by the virus; there are hundreds of millions of chronic hepatitis infections globally[211]. Seveal research groups have investigated the use of functionalised MNP to target the virus, usually to provide a new highly sensitive diagnostic tool.

One of the main studies, by Uchida *et al.*, targets Hep A, B and C with MNP functionalised with polyethyleneimine (PEI)[146]. PEI conjugated MNP have in fact been reported to bind to several viruses (see section 2.5.4)[212]. Uchida's group demonstrated that the PEI-MNP successfully bound to and concentrated Hep A and Hep C viruses, but was less effective against Hep B; concentration was however successfully achieved by adding an anti-Hep B immunoglobulin-M antibody. Meanwhile, Yassin *et al.* have successfully targeted Hep C using MNP coated with protamine hydrochloride, claiming a 100% binding efficiency[147]. Arkhis *et al.* achieved similar results on Hep B and G

using magnetic latex microparticles[213]. Leary *et al.* have used magnetic microparticles functionalised with specific monoclonal antibodies to target and purify Hep C[148], while Ko *et al.* avoided the use of expensive antibodies by using lectin bound MNP to target Hep A[149]. Several other groups have successfully targeted hepatitis DNA and RNA using MNP functionalised with specific antibodies or streptavidin[214, 215, 216].

The possibility of targeting hepatitis viruses with functionalised MNP has been conclusively demonstrated for the purposes of purification. It should be feasible to use some of these methods to extract viruses directly from the bloodstream. It should be noted that PEI-MNP may not be suitable for this application due to the lack of specificity and PEI's toxicity[217], but several groups have demonstrated that the method works just as well using antibodies (although it is likely more expensive).

No group has suggested that hepatitis patients could be treated by extra-corporeal removal of viruses. In order to be effective, the technique would face similar challenges to the magnetic separation of HIV from the bloodstream (see section 2.5.1). However, if it could be both clinically and economically viable, it is one of the applications with the highest potential human impact, treating millions and saving the lives of hundreds of thousands.

2.5.4 Other viruses

In addition to HIV and influenza, a large number of other viruses have been targeted using MNP, including yellow fever, dengue and herpes[215, 218, 219]. Indeed, thanks to the ability to conjugate specific antibodies to MNP as well as the possibility of binding specific DNA or RNA, almost any virus could be targeted using MNP. It may even be

possible to synthesise generic MNP which target many different viruses, although its lack of specificity could cause problems due to the unwanted separation of non-viral bodies from the bloodstream.

These techniques raise the possibility of using magnetic haemofiltration as a method to treat all viral infections, possibly using the haemofilter in combination with anti-viral medication. The advantages of this technique are the same as in other applications: a drug-free treatment prevents the evolution of drug resistance, and avoids or minimises unwanted side-effects. There is also the possibility of treating patients whose virus can be targeted, but against which there is no drug treatment.

There would be significant challenges in developing such a treatment, and many unanswered questions:

- How many virions are circulating freely in the bloodstream, available for separation?
- How many of them would need to be removed to achieve a clinical benefit?
- Considering that it would be impossible to remove 100% of the infection, would the disease always relapse?
- Bearing in mind the significant cost of MNP and antibodies, could any of these methods ever be cost-effective?

Despite the significant difficulties that need to be overcome, the treatment of viral infections through MNP haemofiltraion could be a valuable new therapeutic tool in the long term. The chaos caused by the recent re-emergence of viruses such as SARS, bird flu, swine flu and ebola underlines the potential need for radical new methods of fighting viruses. Generic MNP could be developed, approved and appropriate for functionalisation

with any antibody. This would enable the very rapid development of novel treatments as a response to a new viral outbreak.

2.6 Conclusions

This Chapter provides a small snapshot of the wealth of research that is going into the magnetic labelling of agents using MNP or magnetic beads. Magnetic separation is already an invaluable clinical technique, used principally for the benchtop purification of agents to improve the sensitivity of detection methods and improve diagnoses. However, there is clearly also a much wider future for magnetic separation for novel treatment and diagnostic techniques.

In some areas, such as rare cells and viruses, the research is focussed almost exclusively on diagnosis. However, several research groups have already proposed specific and general therapeutic applications for magnetic haemofiltration within an extra-corporeal loop, namely in the areas of malaria, kidney disease, blood detoxification and sepsis. All of these applications, should they be realised, could save a huge number of lives throughout the world. In most cases, haemofiltration is proposed as a complement to existing therapies: antimalarials, dialysis or antibacterials. However, all also promise something new, such as the ability to treat drug resistance or remove radioactive particles.

The most promising application is arguably as a treatment for sepsis. It is a disease which affects millions and kills hundreds of thousands throughout the world. It's impact in the developed world is high and growing, demonstrating not only the need for new treatments, but also the commercial potential of those treatments should they be viable. Magnetic haemofiltration holds a number of potential advantages over antibacterials, as

it does not require diagnosis of the sepsis-causing pathogen, and is immune to the resistance that can plague antibacterials. It is no understatement to say that a cocktail of biocompatible MNP able to target 99% of sepsis-causing pathogens, combined with an effective and efficient magnetic haemofilter, could revolutionise healthcare.

Despite this potential, there is no extra-corporeal HGMS currently in use or commercially available. This could be due to a number of factors, not least the difficulty in developing the MNP or beads appropriate for extra-corporeal use: these need to be effective, approved by the relevant bodies (e.g. FDA), and affordable; each is a significant challenge. Those who have proposed extra-corporeal magnetic haemofilters have focussed on microfluidic devices, which have their own complications. While they can be effective separators, they can be very expensive to manufacture and difficult to scale up. Flow rates are particularly challenging - these applications will have to be capapble of filtering many litres of blood within an acceptable time-frame.

Magnetic haemofiltration is clearly a technology with a lot of potential far beyond malaria. It is impossible to predict which, if any, of the potential applications presented in this Chapter will be clinically successful, or what new applications may be developed in the future. There is still a lot of work to be done, most of which will focus on the development and approval of appropriate magnetic particles, but some of which must focus on the development of the haemofilter technology. The development of specific haemofitlers for each application would be an inefficient expenditure of time and resources. Ideally, there will be a single, flexible haemofilter appropriate for use with any magnetic particles and in any application, enabling these and other new clinical techniques to reach the bedside as quickly as possible. The aim of this project is to create that haemofilter.

Chapter 3

Design and Construction of Haemofilter

3.1 Introduction

This section describes the process and results of designing and manufacturing the prototypes that were used to establish whether a magnetic haemofilter for the treatment of malaria was feasible. The objective was to design a prototype which could be manufactured in-house and used on a variety of different malaria infected blood samples. The experiments (described in section 4) required an apparatus that was able to filter a variety of different sample volumes at controllable flow rates.

From the start of the project, the intention was to design a device that was usable and effective in a clinical environment. Any experiments that were conducted therefore needed to be representative of a clinical scale version of the device, avoiding a scenario in which a scaled-down version functioned correctly but was impossible to scale up. Furthermore, a clinical scale version has requirements beyond simply 'able to remove malaria infected red blood cells'. While it must be efficient and fit for use, it must also be easy to use and

function quickly and safely. As the device will be used in malaria endemic countries, its design must also be simplified as much as possible so that it is cheap to manufacture and use.

In order to ensure that any testing or experiments were conducted on a prototype representative of a clinical scale version, the latter was designed first, and then scaled down to enable benchtop testing. The scale down was necessary due to the challenges in obtaining large volumes of malaria infected blood - a clinical scale version may have a total volume of 100ml, which would have been logistically, economically and ethically unsuitable for a benchtop version - there is no reason to ask malaria patients for donations of several hundred ml of blood, when much smaller sample sizes are sufficient to investigate whether the device works. The benchtop experiments were to be conducted using samples of 10ml and above, so the scaled down prototype needed to be small enough for the sample to be passed through the separation chamber to enable a measurement of the final parasitaemia to be made.

For simplicity, this section presents the design of the separation chambers and magnetic assembly as if the chambers were designed first, and the magnetic assembly designed to fit around them. In reality, both were designed simultaneously in order to integrate well together. At the start of the design process, some features had already been decided on:

- The separation chambers would be manufactured in plastic, to enable the 3D printing of prototypes for experiments.
- The ferromagnetic mesh within the chambers would be SS430 stainless steel, with wire diameter of $50\mu m$ and aperture size $250\mu m$. This was the finest ferromagnetic mesh that could be affordably sourced within the UK in small volumes.

- The magnetic assembly would consist of an arrangement of block neodymium iron boron (NdFeB) magnets. This was considered ideal because permanent magnets generate very strong magnetic fields without using any power. Furthermore, block magnets are generally cheaper than other shapes since they are easier to manufacture.

- The separation chamber would be placed as close as possible to the surface of the magnets in order to be in the area of highest field strength.

- The magnetic assembly constructed in order to test the device would be as close as possible a representation of the final clinical version, but the separation chambers would be scaled down to allow testing on small volumes.

Finally, it was accepted that it was probably not possible to find the optimal separation chamber dimensions. These were therefore designed to be easily modifiable, so that it would be easy to tweak the dimensions and design while maintaining the overall idea.

For applications beyond malaria (such as those mentioned in section 2), a magnetic haemofilter would need to include a magnetic particle mixing chamber as well as a HGMS. In this chamber, a high number of collision events between the magnetic particles and the targets would be achieved through the actuation of the particles, for example by creating a 'magnetic swarm', wherein the particles are agitated in all directions using an external magnetic field, or causing the formation of rapidly rotating chains of magnetic particles. One way of achieving this would be through a rotating array of permanent magnets, or a series of electromagnets activated in turn similarly to a motor. Since the focus of this thesis is on malaria, which does not require a mixing chamber, potential designs for these are not included in this chapter.

3.1.1 Required specifications

The haemofilter needs to be effective, safe, and usable if it is to be a viable medical device. It must be as simple to manufacture and use as possible, and the design should therefore avoid any unnecessary complexities. The identified key specifications were:

- High efficiency (>90%), capturing the maximum number of haemozoin containing infected cells that pass through the separation chamber.
- High specificity (>90%), ensuring that only infected cells are captured while healthy cells and other blood components pass through unaffected.
- High throughput, so that total time to reduce the parasitaemia to the target value is as short as possible.
- Able to incorporate different chamber types into the instrument (e.g. small, medium, large), to allow consumables intended for infants, children or adults to be used on any instrument.
- Controlled, consistent flow rates that can be varied for different patients or chamber sizes.
- No harm caused to other blood components and risks to patient minimised to conform with regulatory requirements.
- Intuitive and easy-to-use user interface (UI) with an automatic mode, in which only the chamber type and patient weight needs to be inputted, and a manual mode, in which the clinician can control parameters such as the flow and total volume addressed.

- Consumables cheap to manufacture, keeping the cost per treatment to a minimum.

Many of these specifications are required for a final product, but not for a proof-of-concept prototype. Therefore, the design of the separation chamber is much more important than the design of the instrument. In short, the separation chamber provides the performance, whereas the instrument provides the functionality. The separation chamber, combined with the magnetic assembly, determines whether infected cells are captured, the efficiency, and the throughput. It will also be responsible for any negative effects on other blood components. The design process therefore focussed on the separation chamber and magnetic assembly, with the intention of including features such as the UI once a proof-of-concept had been produced.

3.1.2 Volumes and flow rates

One critical requirement of the device is its ability to treat a patient within an acceptable time-frame (i.e. a few hours). The total treatment time depends on three key factors:

1. The flow rate
2. The separation efficiency
3. The desired total reduction in parasitaemia

It is important to remember that if the separation efficiency of the device is 90%, more than the total blood volume (TBV) of the patient needs to be filtered in order to achieve a total parasitaemia reduction of 90%. This is because as the treatment progresses and the patient's parasitaemia decreases, the device is removing fewer infected cells per minute. This effect is illustrated in Fig. 3.1, which shows that for two adults with a TBV of 5l,

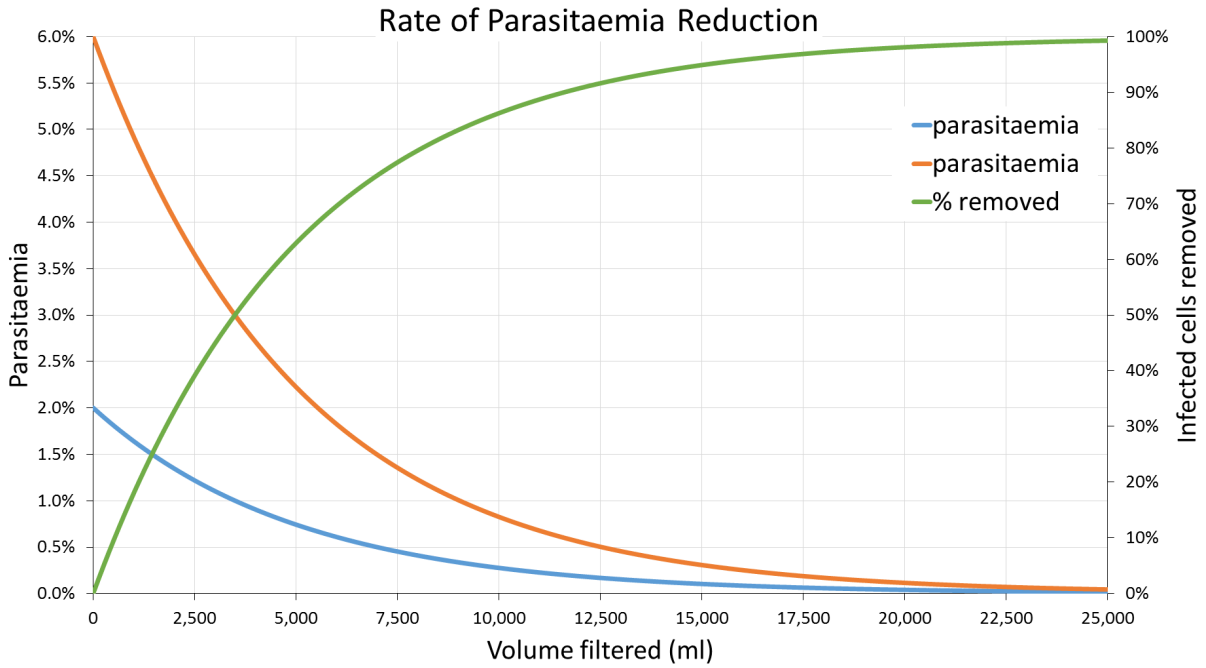


Figure 3.1: Parasitaemia reduction by device assuming a separation efficiency of 90% in a single pass, 2% initial parasitaemia, and 5l TBV

with initial (removable) parasitaemias of 2% and 8%, and treated with a device with a separation efficiency of 90%, 12.8l of blood need to be filtered to reduce their parasitaemia by 90%. Note that the percentage reduction in parasitaemia occurs at the same rate for both patients, regardless of initial parasitaemia (although naturally the patient with a higher initial parasitaemia will need to have more blood filtered in order to reach the same final parasitaemia). To reach a parasitaemia reduction of 99%, 25l of blood need to be filtered. If the device's efficiency is 99%, 11.6l need to be filtered to get a 90% reduction in parasitaemia, and 23.2l need to be filtered to get a 99% reduction.

It should be noted that the model for Fig. 3.1 is simplified - it calculates the reduction in steps of 25ml, assuming a constant separation efficiency and assuming that the parasitaemia reduction is immediately spread throughout the body (i.e. each incremental step has a slightly lower parasitaemia). In reality, the reduction may be faster, or in-

deed slower, if the separation efficiency has a strong dependence on initial parasitaemia. However, it serves to communicate two important messages:

1. Much more than TBV needs to be filtered for the total parasitaemia to be reduced by the same percentage as the separation efficiency.

2. The separation efficiency of the device has a limited effect on total treatment time. If the target parasitaemia reduction is 90%, increasing the separation efficiency from 90% to 99% only reduces the treatment time by 6% (11.6l filtered vs. 12.8l).

This underlines the importance of the flow rate in terms of achieving results within an acceptable time-frame. It is expected that a higher flow rate will lower the separation efficiency by increasing the drag force (see Eq. 1.2), so the right balance between the two will need to be found. However, priority should be given to the flow rate, and this can easily be illustrated with the model used in Fig. 3.1.

Fig. 3.2 shows the rate of parasitaemia reduction from an adult (initial parasitaemia 2%) for two theoretical filters: one with a flow rate of 120ml/min and separation efficiency of 80%, and one with a flow rate of 60ml/min and a separation efficiency of 99%. The model assumes that the device captures 80% or 99% of the infected cells that pass through device and subtracts the captured cells from the total number of infected cells, moving in steps of 25ml. So in the case of the the first filter, 80% of the infected cells in 25ml of blood are removed from the patient in each step, with each step corresponding to 12.5 seconds. For the second filter, 99% of the infected cells in 25ml of blood are removed per step, with each step corresponding to 25 seconds.

The first filter is superior, achieving a 90% reduction in overall parasitaemia within two hours. Fig. 3.2 underlines why the low flow rates from the studies discussed in Chapter 2

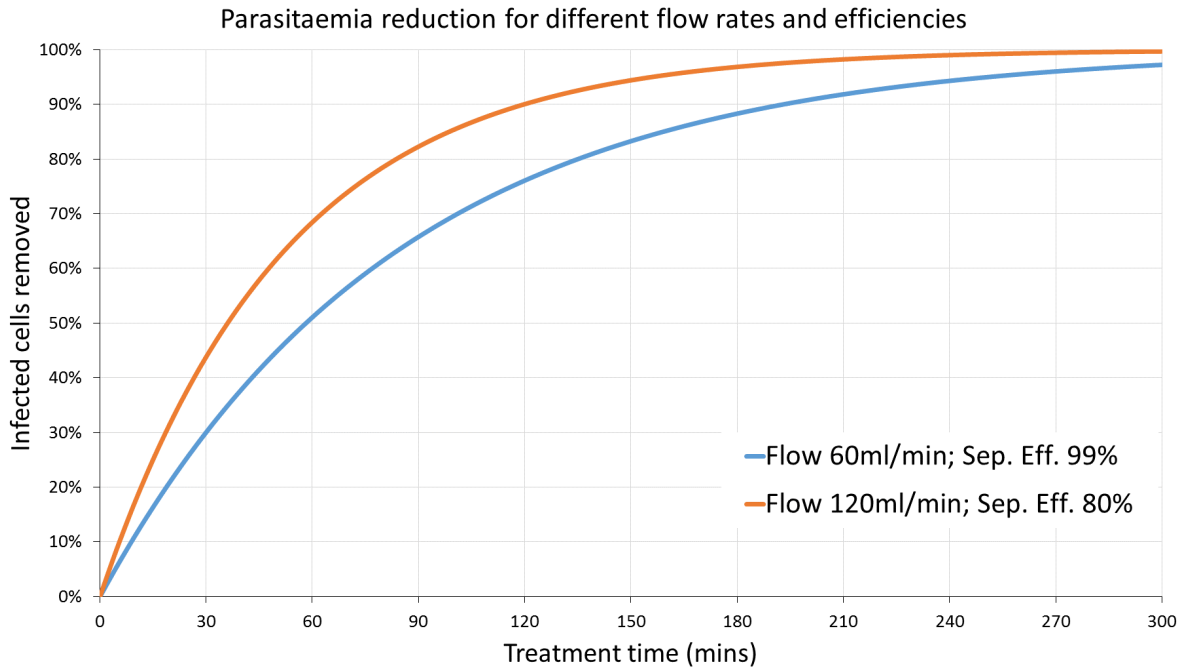


Figure 3.2: Reduction in parasitaemia for two theoretical filters: one operating at 60ml/min with a separation efficiency of 99%, and one operating at 120ml/min with a separation efficiency of 80%

are crucial to increase in order to create a viable medical device. A core design objective of the haemofilter is therefore to achieve acceptable separation efficiencies at high flow rates, prioritising the latter over the former.

3.1.3 Design process

The process followed or order to produce the prototypes was the following:

1. Establish the maximum total volume that a clinical scale device could have, by looking at existing dialysis circuits and other extra-corporeal loops.

2. Create a separation chamber design with a large surface area, enabling maximum flow rates with low drag forces.

3. Ensure the design could achieve reasonably homogeneous flow across its cross-

section.

4. Design an assembly of permanent magnets to create the maximum possible magnetic field throughout the device, while ensuring that it is cheap and easy to build by avoiding complex magnet arrangements.

5. Model the magnetic field and forces within the device, to provide an estimate of the flow rates that could be utilised.

6. Source a fine ferromagnetic mesh appropriate for use within the separation chamber.

7. Scale down the clinical design to a smaller volume, while maintaining the magnetic forces and chamber length (i.e. on average a cell travels the same distance in the small scale chamber as in the larger scale).

8. Construct the magnetic assembly at clinical scale, so that the magnetic field that is used in the experiments is the same as would be used in a clinical device.

9. Devise a cost-effective manufacturing process using 3D printing, to give the capability to make the number of chambers required for the experiments.

The result of this process was an apparatus suitable for bench-top testing. The magnetic forces in the small scale prototypes would be the same as in the clinical scale design, while the flow rates could be controlled so that the drag forces would also be the same as in the clinical version. The theory is that the separation efficiency depends overwhelmingly on these two forces (see section 1.1.2) so the results using the small scale prototypes should be representative of what could be achieved using the clinical scale design. The prototypes are essentially 'cut-outs' of the clinical version, with the volumetric flow rate adjusted to achieve the same linear flow rate (i.e. drag force) as the clinical scale version.

3.1.4 Magnetic modelling

In order to assess the quality of the designs, the magnetic modelling software Opera Vector Fields V16 (Cobham Technical Services, Oxford, UK) was used. This software is able to calculate the magnitude of magnetic fields across a 3D space. A CAD model is first built and meshed in the modeller; this includes all objects and their magnetic properties, provided through BH curves (see section 1.3 for information on BH curves). The model is then processed using the TOSCA analysis for static models, which calculates the fields generated by any permanent magnets and any distortions caused by magnetic material within the model. The magnetic field data can then be visualised within the post-processor.

Opera Vector Fields was used to model the magnetic fields generated by different arrangements of permanent magnets in order to design the magnetic array. This enabled not only the best magnetic arrangement, but also an analysis of any weak areas in the field generated, and the degree to which the field varied within the assembly. The software can also be used to model the effect that the ferromagnetic mesh has on the field.

One complication is that the mesh is orders of magnitudes smaller than the magnetic array - the different array designs had dimensions of tens of millimetres, whereas the area of interest with respect to the mesh is of the order of microns. The software was unable to manage such a degree of complexity and variance in size within a single model. The data from the magnetic array was therefore inputted into a second model as a constant background field, so that the effect of the mesh could be investigated. The results could then be used to estimate the magnitude of the magnetic force on an infected cell using Eq. 1.10.

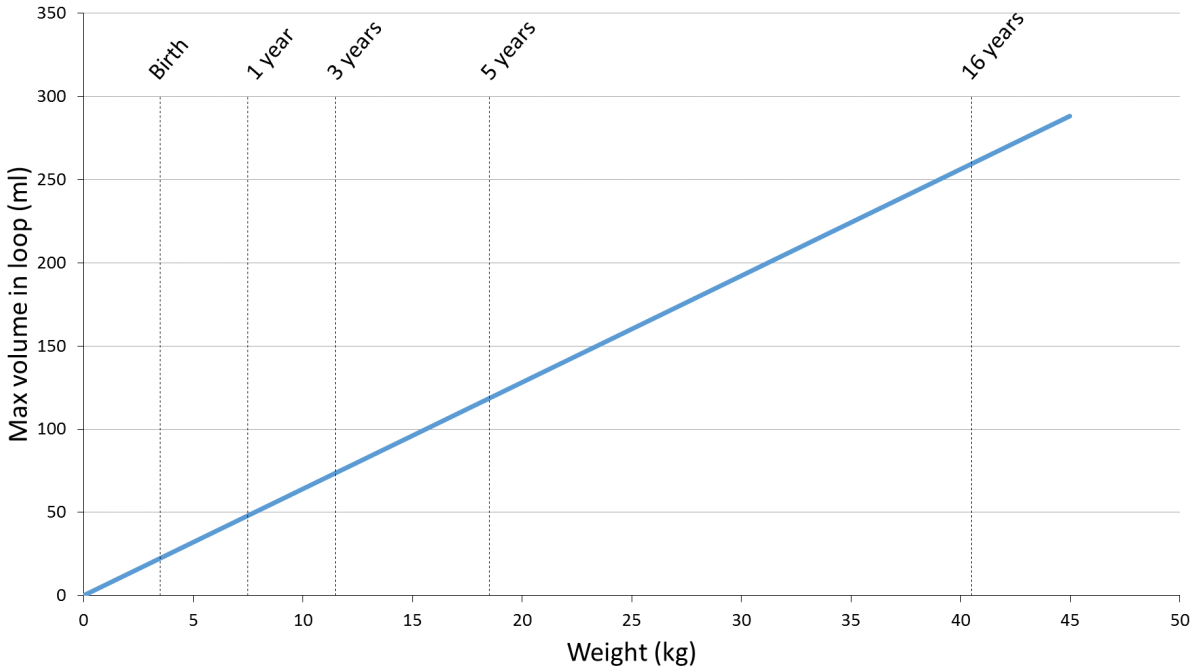


Figure 3.3: Approximate maximum blood volume in extra-corporeal loop for different weights and ages[6, 7]

3.2 Design of the consumables

3.2.1 Volume

The primary design constraint for the separation chamber is the volume of blood that can be taken out of a patient without causing any harm or discomfort. This is well established in the field of nephrology at 8% total blood volume[6]. Total bood volume is typically taken to be 80ml/kg, so the average adult (65kg) will have about 5 litres of blood. This gives a maximum volume within the blood circuit of 400ml. However, a more sensible limit is probably not more than 200ml, particularly considering the fact that malaria patients frequently suffer from anaemia. In any case, 200ml is likely to be easily sufficient to achieve the aims of the haemofilter.

For children, who in the case of malaria are likely to make up the majority of patients, the situation is more complicated. Fig. 3.3 shows a graph of approximate maximum

blood volumes that can be taken out of a patient with different total blood volumes and ages. This shows that for an infant under 12 months of age, the total extra-corporeal loop volume may need to be as low as 30ml. This increases to 45ml for a one year old, 70ml for a three year old, and 115ml for a five year old.

As a result, several different separation chamber volumes will be required in the final product. Assuming the tubing occupies a total volume of 10ml, the separation chambers will need to range from 'infant' (20ml), through 'small' (60ml) and 'medium' (100ml), to 'adult' (180ml). Occasionally, several of these chambers could be used in parallel to afford further flexibility to clinicians. As the chambers will be filled with stainless steel mesh, a certain proportion of the volume will be occupied by the mesh - up to approximately one third. As such, a chamber designed for 60ml of blood may actually have a total volume of 90ml.

3.2.2 Surface area

It is desirable for the flow rate achievable through the device to be as high as possible, in order to achieve rapid parasitaemia reduction and to minimise the treatment time. The flow rate is dictated primarily by the drag force described in Eq. 1.2, since it is not possible to control the magnetic force. Higher flow rates can therefore be increased by maximising the surface area across which the blood flows - a higher flow rate across a higher surface area corresponds to the same drag force.

As such, given a rectangular chamber, for example, the direction of flow will determine the linear flow rate and thus the drag force. Consider, for example, the 20x10x5cm box shown in Fig. 3.4. It has a volume of 1 litre, so if we flow 1 litre of liquid through it

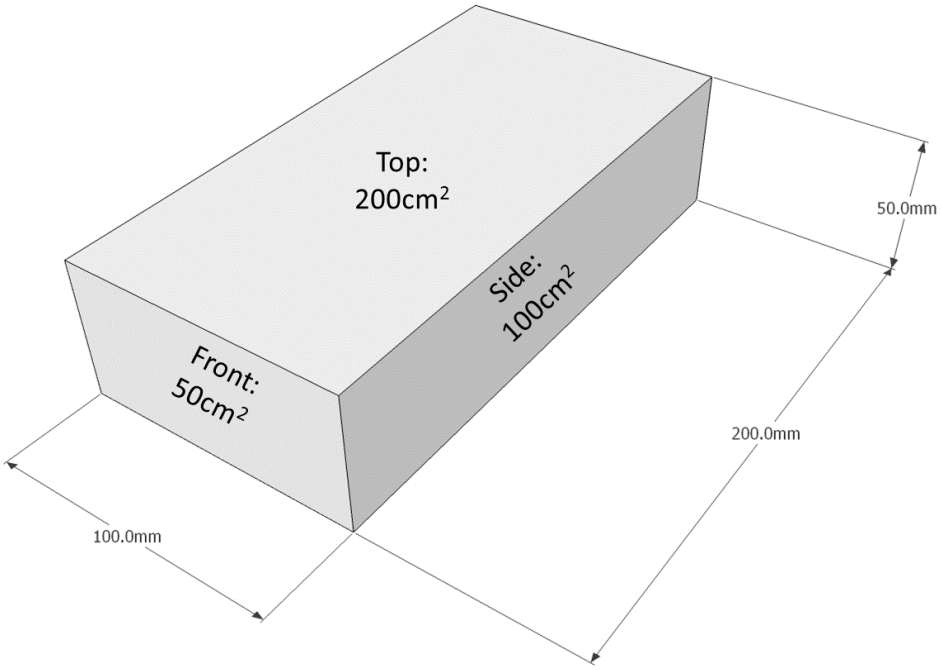


Figure 3.4: Example box to demonstrate effect of flow direction on drag force

at 100ml/min, it will take 10 minutes for the liquid to pass through, regardless of the direction of flow. If the liquid is flowed through from the front, the linear flow rate will be:

$$f_{linear} = \frac{f_{volume}}{A}$$

$$f_{linear} = \frac{100}{50} = 2cm/min$$

However, if the liquid is flowed from the bottom up through the top, the area A is four times larger, so the linear flow rate (and hence the drag force) is four time lower. Hence despite fixed chamber dimensions and volumetric flow rates, the drag force can be minimised by changing the direction of flow. A consequence of this is that if one was to design a chamber with a volume of 125ml, for example, the optimal dimensions would

not be 50x50x50mm, but rather something like 125x10x10mm, with the direction of flow through the 125x100mm surface.

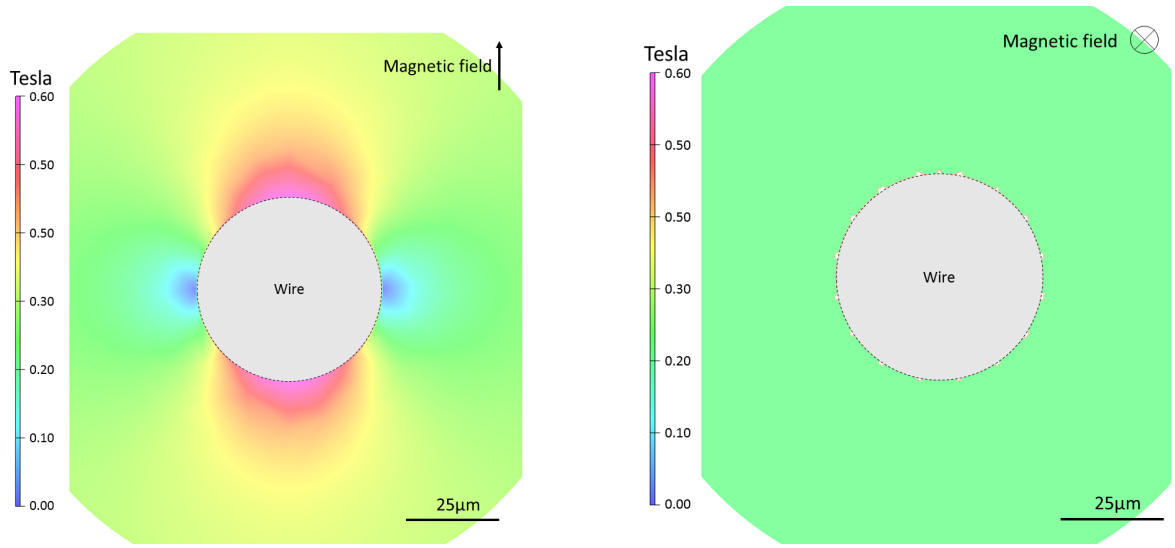
One caveat to this is that this method reduces the 'flow path length', or in other words the number of layers of ferromagnetic mesh through which a cell travels. In our example, it is reduced from 50mm to 10mm. When designing the chambers, the intention was thus to minimise the drag force while maintaining a sufficient flow path length. This was chosen to be at least 100 layers of mesh, corresponding to approximately 30mm.

3.2.3 Orientation of mesh

The orientation of the mesh within the separation chamber is an important consideration for two reasons:

1. Ferromagnetic wires magnetise differently depending on their angle with respect to the magnetic field. If the wire is aligned in the same direction as the field, it will become magnetised along its length, and not generate the desired local field gradient. However, if it is at 90° to the field direction, it magnetises across its axis, creating the strong local field. This is demonstrated in Fig. 3.5, which shows how a $50\mu m$ wire affects a background magnetic field of 250,000A/m (equal to 0.31T, using Eq. 1.6). The force generated when the field is parallel to the wire (Fig. 3.5b) is essentially zero as there is no gradient. However, when the field is perpendicular to the wire (Fig. 3.5a), the field is accentuated close to the wire, increasing to nearly 0.6T on the wire's surface (Fig. 3.6).

2. It is desirable for the blood to flow through the apertures as much as possible. This ensures that each cell is forced to pass close to the magnetised wires at every single



(a) Field perpendicular to wire **(b)** Field parallel to wire

Figure 3.5: Effect of $50\mu m$ SS430 stainless steel wire on a 0.3T background magnetic field (note that the magnetic field within the wire has been hidden for clarity; it has no effect on the magnetic force on an object near the wire)

mesh layer, increasing the likelihood of capture. If the direction of flow tends to be between the layers of mesh, rather than through them, escape channels are more likely to form.

The conclusion from these points is that the mesh should be layered perpendicular to both the magnetic field and the direction of flow, which should therefore be in the direction of the magnetic field. The field created by such an arrangement is shown in Fig. 3.7. If the field was parallel to the mesh, one set of wires would still be perpendicular, but the other half would behave as in Fig. 3.5b, thus reducing the capture area by half.

One final option is to have the field parallel to the mesh, but diagonal to both sets of wires. However, as shown in Fig. 3.8, the wires do not magnetise diagonally but along their length, due to magnetic anisotropy: the wires prefer to be magnetised along their length due to the favourable interaction of the internal magnetic dipoles. This effect is demonstrated by the vectors displayed along the wires.

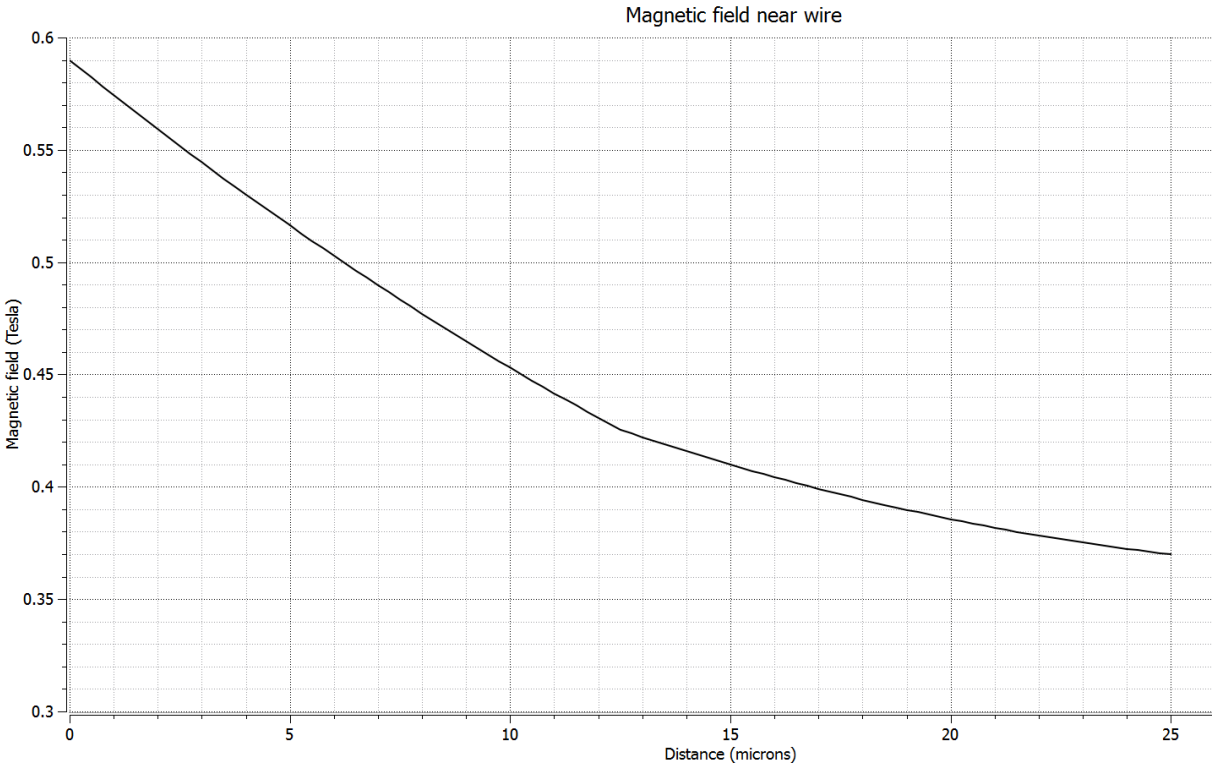
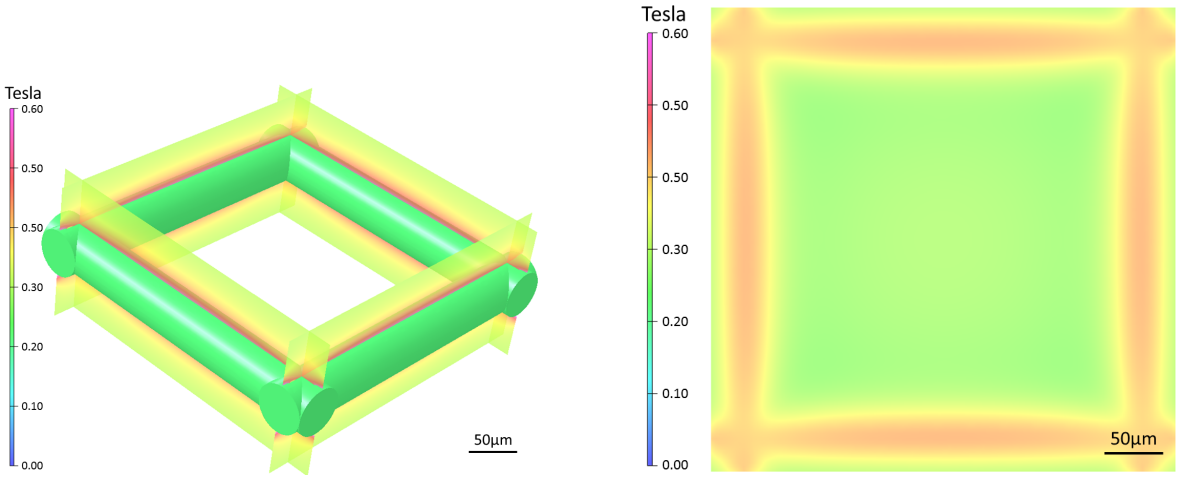


Figure 3.6: Magnetic field from the surface of one of the wires of the ferromagnetic mesh, in the direction of the magnetic field (i.e. perpendicular to the wire)



(a) Field variation above and below constituent wires

(b) Field 10μm above mesh

Figure 3.7: Magnetic field perpendicular to stainless steel mesh, with background field of 0.3T

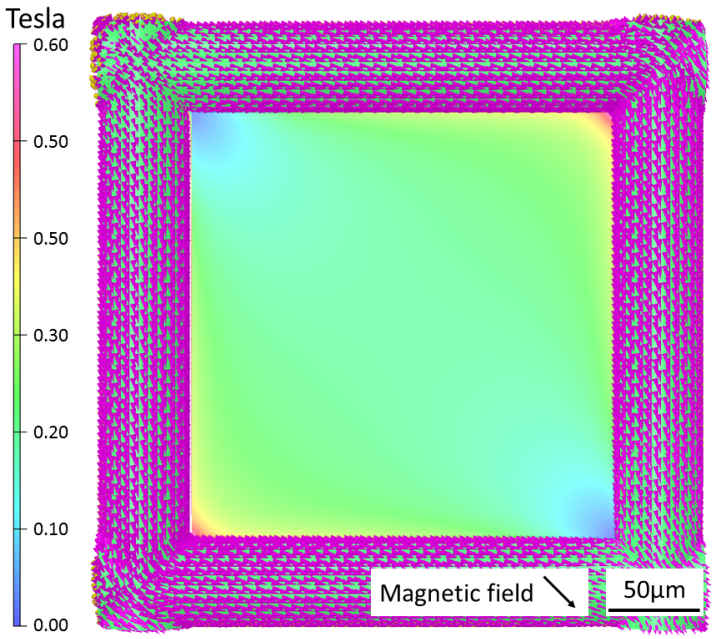


Figure 3.8: Magnetisation and magnetic field generated with background field at 45^{o} to mesh

Thus the arrangement in Fig. 3.7 generates the largest overall 'capture areas' as well as the highest magnetic force. Eq. 1.10 shows that for a given magnetic agent, the force depends on the field times the field gradient. A simple calculation using the data in Fig. 3.6 gives:

$$B\nabla B = 0.516\,(Tesla) * 1.43 \times 10^{4}\,(Tesla/m)$$

$$= 7.38 \times 10^{3}\,\left(T^{2}/m\right)$$

for $B\nabla B$ $5\mu m$ from the wire in the case of a perpendicular field. As a comparison, the field generated by a a single 50x25x10mm NdFeB block magnet, capable of holding 32.2kg of steel (according to the supplier, First4Magnets, Tuxford, UK) is shown later in Fig. 3.19. $B\nabla B$ 1mm from the magnet in this case is:

$$B\nabla B = 0.215\,(T) * 11.11\,(Tesla/m)$$

$$= 2.39\left(T^2/m\right)$$

In other words, the magnetic force on a given malaria infected cell at a distance of $5\mu m$ from one of the wires in Fig. 3.7 is 3,088 times greater than at a distance of 1mm from what would be considered a very powerful permanent magnet.

As a final point, having the mesh stacked at a right angle to the field creates 'vertical' capture areas above and below the wires. These areas, as they fill up with captured cells, avoid the higher drag forces that are present within the aperture.

3.2.4 Design of chamber

The instinct when designing a flow chamber is to design a cylindrical shape. The main reason for this is that corners have a tendency to form dead zones in which there is no flow. However, in this case, it was decided that a rectangular box shape was more appropriate for one primary reason: the ferromagnetic mesh needed to be cut to size. Not only is the cutting of the mesh laboursome - over 100 are required for each chamber - but the resulting pieces need to be consistently the correct size. This is particularly challenging when cutting circles. A hole punch was designed and fabricated for this purpose, but was not successful in cutting through the stainless steel. However, the mesh could relatively easily be cut into rectangles using scissors or a paper guillotine.

Furthermore, the choice of having the direction of flow and magnetic field in the same

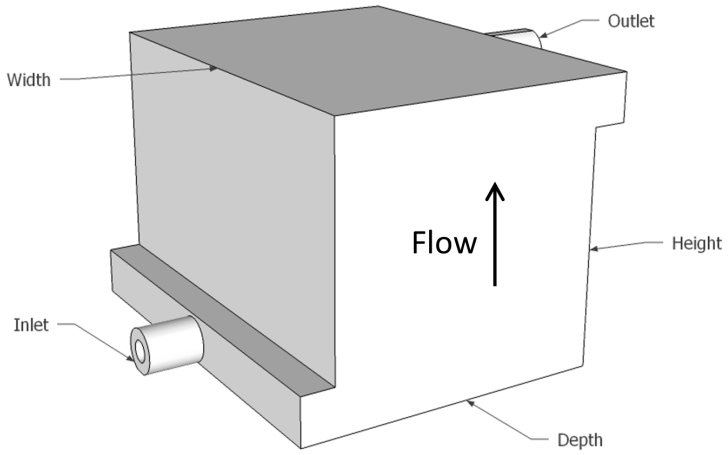
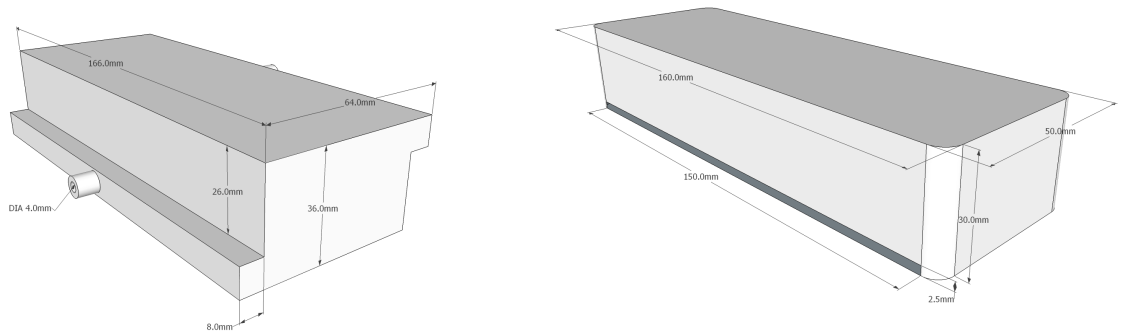


Figure 3.9: Basic chamber design (dimensions variable depending on patient's TBV)

direction, both perpendicular to the layers of mesh, presents a design problem when considering the inlet and exit. Assuming the chamber will be placed between two or more magnets, the direction of flow will be from one magnet towards the other. But the inlet and exit cannot be 'through' the magnets for a variety of reasons, not least that one cannot drill holes through block magnets. The inlet and exit therefore need to be on opposite sides of the chamber, at the top and bottom respectively, encouraging the desired 'bottom to top' flow. This solution has the advantage of simplicity, enabling the chamber to be easily inserted between magnets, and should provide approximately the desired flow profile. However, it is inevitable that there is a sacrifice in terms of flow homogeneity throughout the chamber. This design is illustrated in Fig. 3.9.

With this basic design in place, the dimensions were worked out based on the different chamber volumes from section 3.2.1. The height was set at 30mm, which should allow for over 100 layers of mesh to be be stacked - four layers of mesh were measured to be approximately one mm in height. The walls were 3mm thick, giving a total height of 36mm. For the 'adult' version, a total volume of 180ml was deemed appropriate; incorporating the fact that one third of the volume inside the separation chambers is



(a) External **(b)** Internal (walls invisible)

Figure 3.10: Design of adult (180ml) separation chamber

taken up by the mesh, a total chamber volume of 240ml was required. With a height of 30mm, that leaves an area of 80cm^2. The depth and width were chosen to be 50mm and 160mm respectively (Fig. 3.10).

Fig. 3.10a shows the chamber as it would appear, whereas Fig. 3.10b shows the shape of the internal chamber (i.e. without the walls). The most significant difference is that the inlet and outlet are circular on the outside of the device, but long thin slits with a height of 2.5mm on the inside. The idea is that, between the inlet and the slit, the flow is spread homogeneously across the whole width of the chamber (as described in section 3.2.5)

The dimensions of the design in Fig. 3.10 can easily be changed to change the chamber volume. Using the chamber volumes from section 3.2.1, the following chamber dimensions (width x depth x height) were used:

- *'Medium' chamber (blood volume 100ml)*: 100x50x30mm (volume before mesh: 150ml)
- *'Small' chamber (blood volume 60ml)*: 75x40x30mm (volume before mesh: 90ml)
- *'Infant' chamber (blood volume 20ml)*: 40x25x30mm (volume before mesh: 30ml)

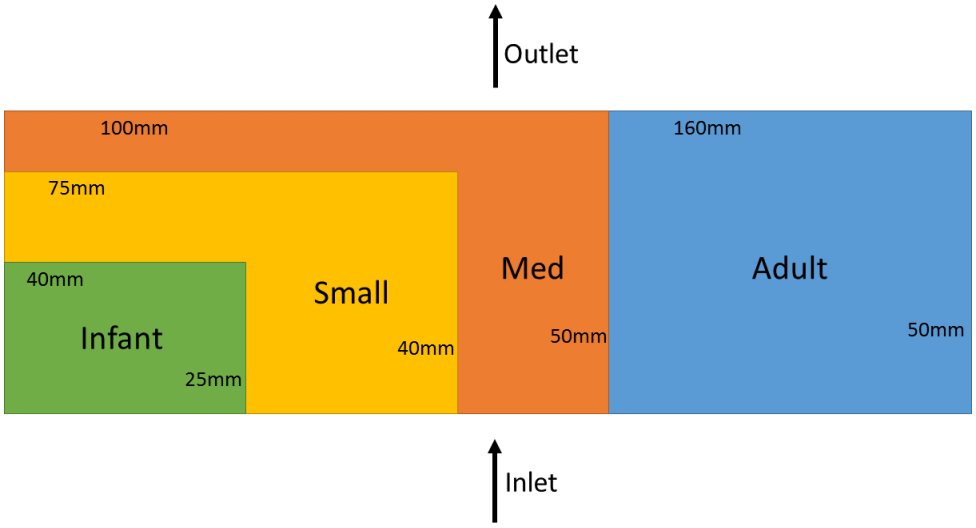


Figure 3.11: Cross-sections of four chamber designs

All of these designs use the same height, and their respective cross-sections (across which the blood flows) are shown in Fig. 3.11. The areas of the cross-sections are 10cm^2, 30cm^2, 50cm^2 and 80cm^2 respectively. This means that, in order for the drag force to be the same in all the chambers (and assuming that the flow is homogeneous throughout all the chambers), the volumetric flow rate will need to be eight times slower in the infant version than in the adult version, and three time slower than in the small version (and so on). This should not have significant implications in terms of treatment times - a patient using the adult chamber is projected to have about 20 times the total blood volume of an infant. Indeed, despite the lower flow rates, a child will likely be cleared of their infection much faster than an adult.

3.2.5 Homogenising flow

The biggest challenge of the designs based on Fig. 3.10 is to achieve a homogeneous flow across both the width and the depth of the chamber, particularly for the adult version. To investigate this, flow dynamics experts from The Fluid Group were consulted, and

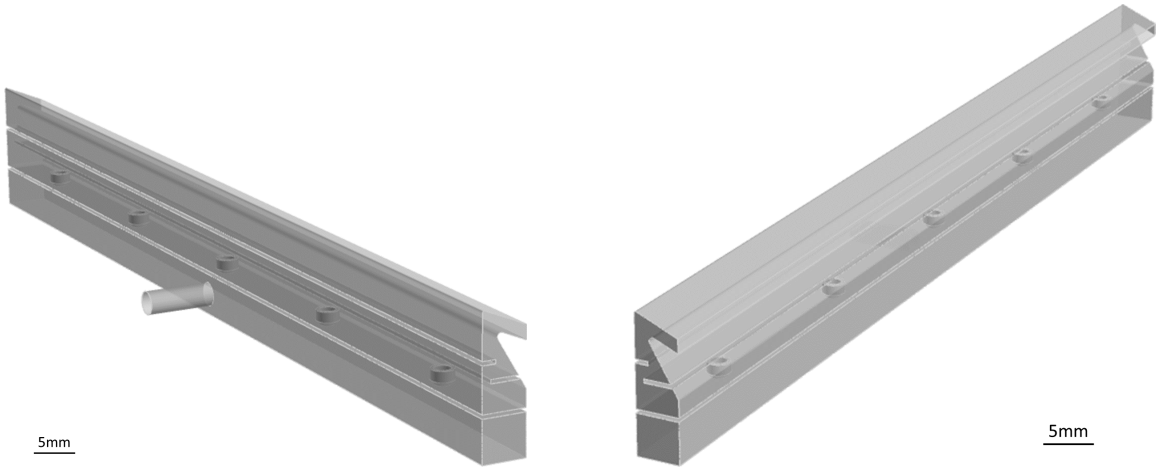
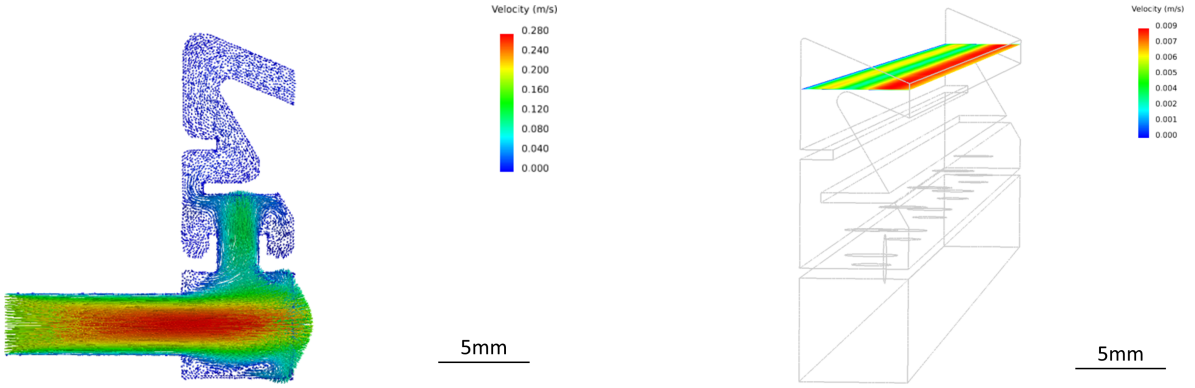


Figure 3.12: Design of flow homogeniser (produced by The Fluid Group)



(a) Cross-section showing flow profile

(b) Linear flow rate at exit

Figure 3.13: Flow of liquid through flow homogeniser (produced by The Fluid Group)

suggested that a flow homogeniser could be used to split the flow evenly from a tube to the 2.5mm slit. Jim Wicks from The Fluid Group (Oxford, UK) proposed the design shown in Fig. 3.12, which he has graciously donated to this project and is reproduced with their permission. Their computational flow dynamics (CFD) modelling shows that the fluid is evenly distributed across the width of the slit upon exit (Fig. 3.13).

In terms of homogenising flow across the depth of the chamber, this is achieved in large part thanks to the mesh. Upon entry into the chamber, there is a 2-3mm space in which there is no mesh. The pressure required to travel through the mesh encourages the liquid to flow deeper into the chamber before flowing upwards. In addition to this, having

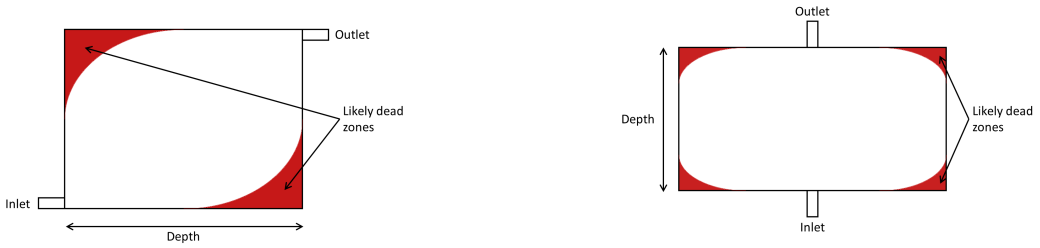


Figure 3.14: Probable dead zones in chambers

the outlet on the opposite side of the chamber encourages a more even flow across the width.

Despite these steps, some dead zones within the chamber are inevitable, and complete flow homogeneity will probably be impossible to achieve, particularly across the depth of the chamber (Fig. 3.14). The important consideration concerning these dead zones is the increased likelihood of blood clotting in these areas, and the difficulty in successfully priming the chambers to ensure there is no air within them.

3.2.6 Scaled-down version

In order to test the design, small scale prototypes were designed, able to process volumes as small as 10ml. This design followed the same principle as those in section 3.2.4, maintaining the same height and simply scaling down the width and depth. The dimensions of the scaled down version were 21x18x30mm, giving a total volume of 11.34ml, with inlet and outlet internal diameters of 4mm and outer diameter of 8mm. The walls were 3mm thick. They were designed in two parts, a chamber and a cap - the chamber could then be loaded with the mesh and sealed with the cap. The design is shown in Fig. 3.15. Because of the small size of the chamber, there are unlikely to be many problems in terms of flow inhomogeneity. Despite this, gradual smoothing of the tube into a slit 'letterbox' shape

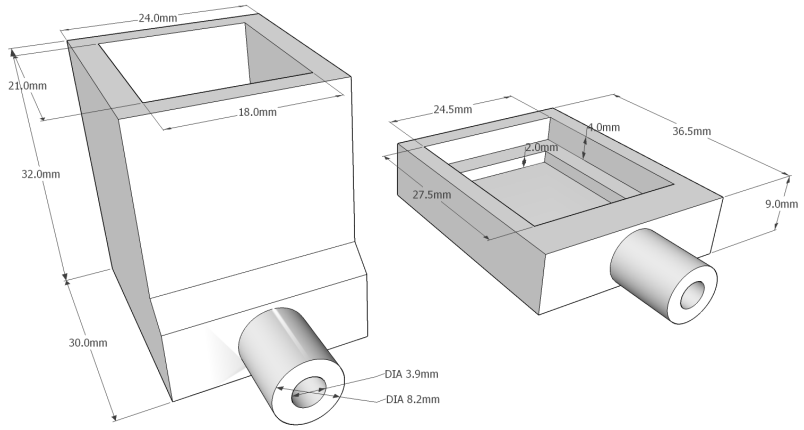
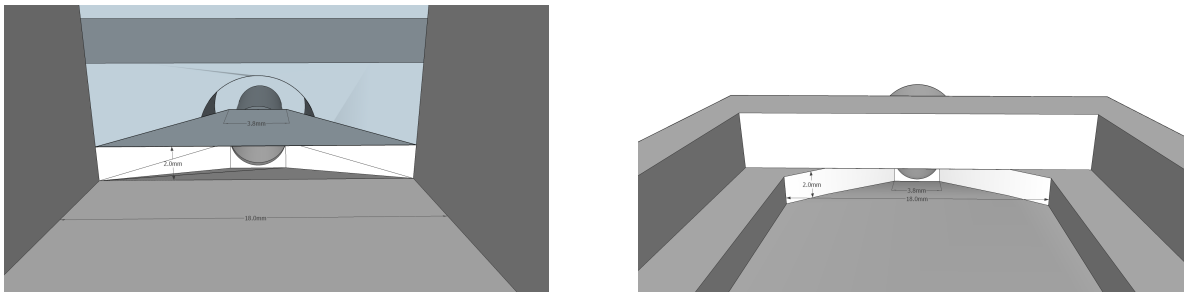


Figure 3.15: Scaled-down chamber for use in experiments



(a) Inlet, with some walls of the model hidden to illustrate structure

(b) Outlet in cap

Figure 3.16: Homogenising flow in scaled-down chamber

was designed to even out the flow across the width, as shown in Fig. 3.16.

The scaled-down chambers were designed to be 3D printed, so that a new one could easily be manufactured for each experiment. Their manufacture is described in section 3.5.

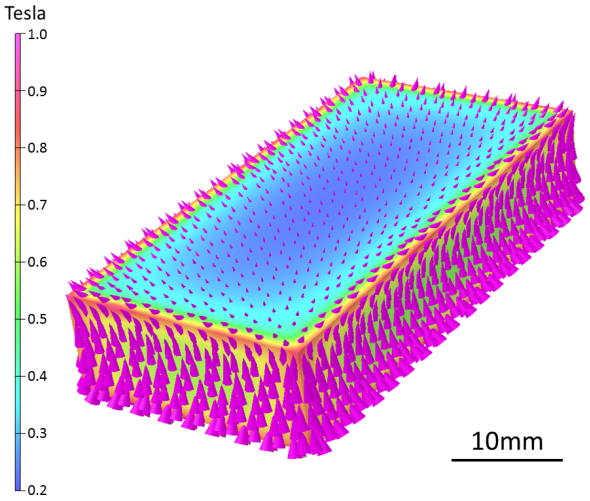


Figure 3.17: Magnetic field on surface of 50x25x10mm NdFeB magnet

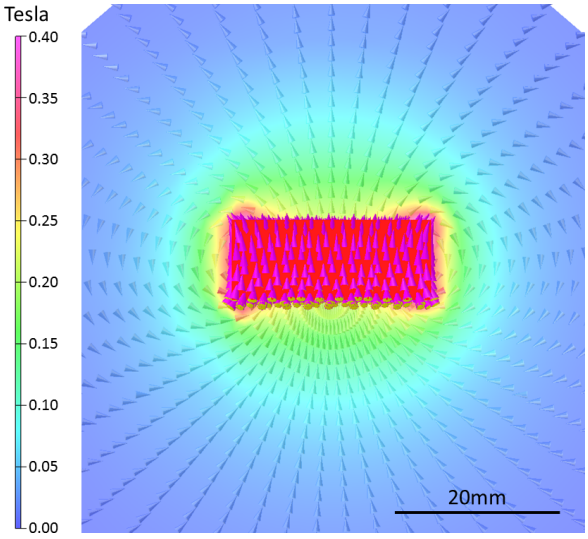


Figure 3.18: Magnetic field generated by 50x25x10mm NdFeB magnet

3.3 Magnetic assembly

3.3.1 Design

In order to create a strong magnetic field at an affordable price, large commercial NdFeB magnets were sourced from a commercial supplier (First4Magnets, Tuxfod, UK). The objective was to find magnets that could achieve a high magnetic field over a large area, with the highest possible strength to price ratio. The magnets that were chosen were N42 50x25x10mm rectangular blocks, magnetised in the direction of the 10mm axis, with a pull of 32.2kg, costing £12.50 each.

Before they were purchased, the field generated by the magnets was modelled in order to find the most effective magnetic arrangement, generating a strong localised magnetic field that could accommodate the separation chamber. The magnetic field generated by a single block magnet is shown in Fig. 3.17 and 3.18. It generates a magnetic field of 225mT close to its surface, with the field strength dropping to 22mT 30mm away (Fig. 3.19).

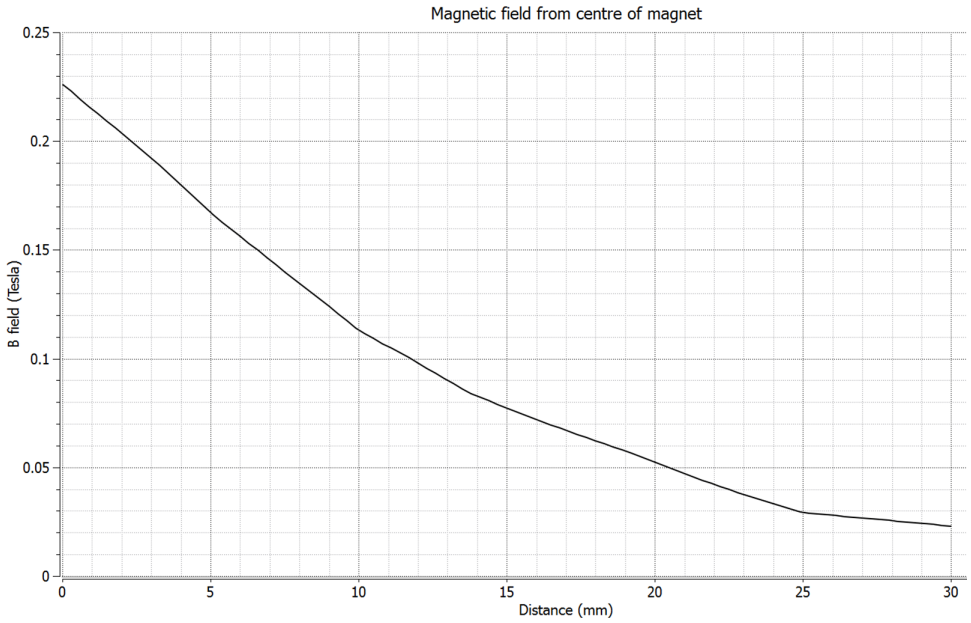


Figure 3.19: Magnetic field generated by 50x25x10mm NdFeB magnet

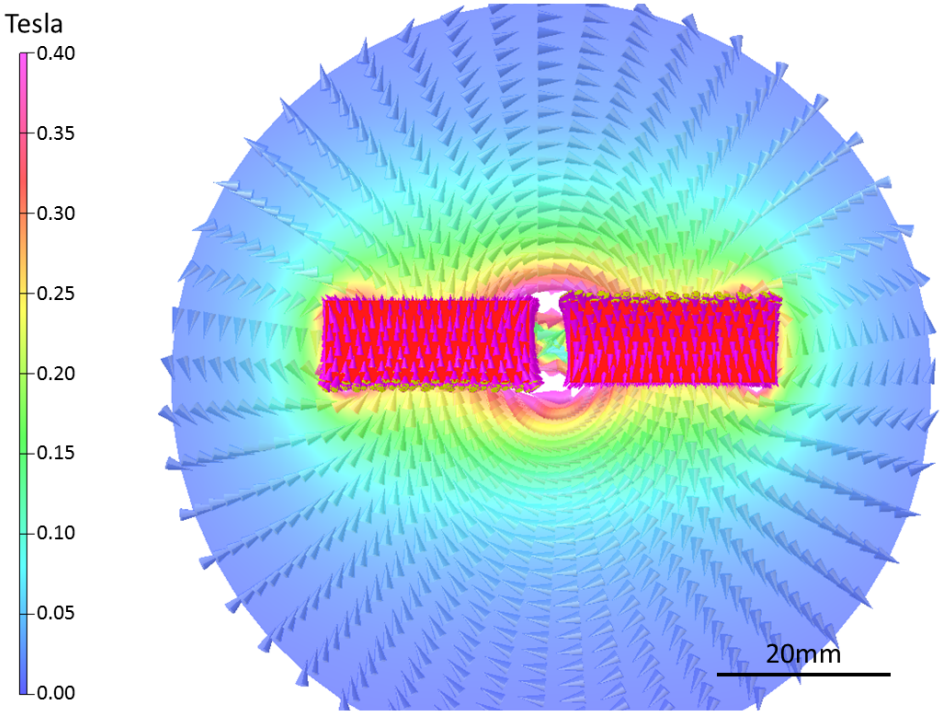


Figure 3.20: Magnetic field generated by two 50x25x10mm NdFeB magnets

Placing two magnets side by side, 5mm apart and with opposing polar orientations (Fig. 3.20) naturally increases the overall field strength, particularly close to and between the magnets where it is above 400mT. However, this arrangement is not suitable for the magnetic assembly for two reasons:

1. *The arrangement is not stable*: the magnets attract each other very strongly, and would need to be tightly held in position.

2. *Stray fields*: the magnetic field is just as strong both sides of the magnets. For safety reasons, it is desirable to 'focus' the magnetic field only within the working area (i.e. the area in which the magnetic separation chamber is placed)

To overcome these problems, the magnets were placed on a steel back plate. Not only does this hold the magnets in position by nature of their stronger attraction to the plate than each other, but the plate also acts as a 'magnetic conductor', as shown in Fig. 3.21, due to the high magnetic permeability of steel. The magnetic field behind the plate is eliminated, while the field in front of the magnets remains the same, or is even slightly increased (Fig. 3.22).

Fig. 3.23 shows the magnetic field in a circle around two blocks both with and without a steel back plate. The circle on which the field is measured is centred between the blocks and has a radius of 35mm. 0° is therefore 'behind' the magnets, 30mm from their surface, while 180° is 30mm 'in front' of the magnets (Fig. 3.23a and 3.23c). Fig. 3.23b shows that the field of 25mT is the same at the front of the magnets as it is at the back, as would be expected. Fig. 3.23d however shows that adding a steel plate causes the field to drop to close to 0T at the back. Meanwhile, it is increased by 15mT to 40mT at the front. The steel plate 'focusses' the magnetic field to the front, removing it from the back, an ideal

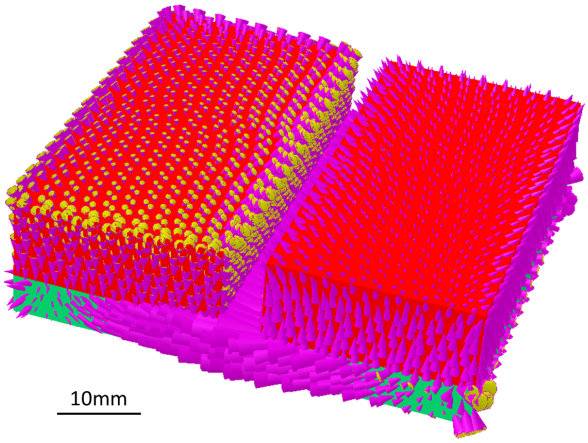


Figure 3.21: Magnetic field flow through steel back plate

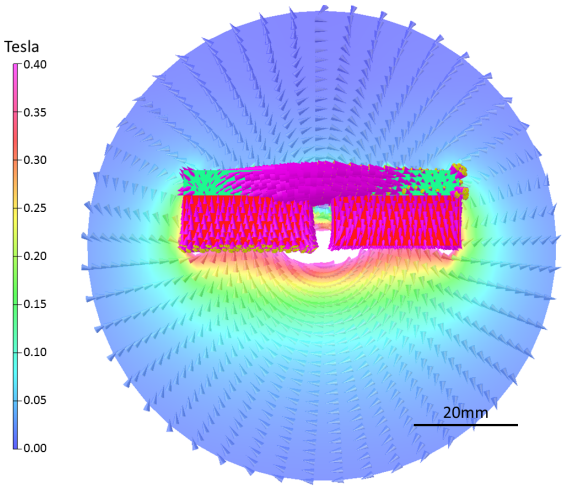
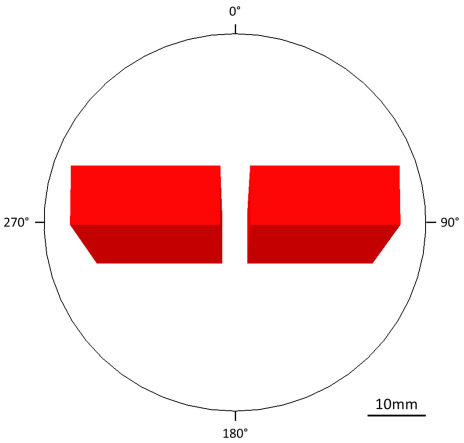


Figure 3.22: Magnetic field generated by two magnets with steel back plate

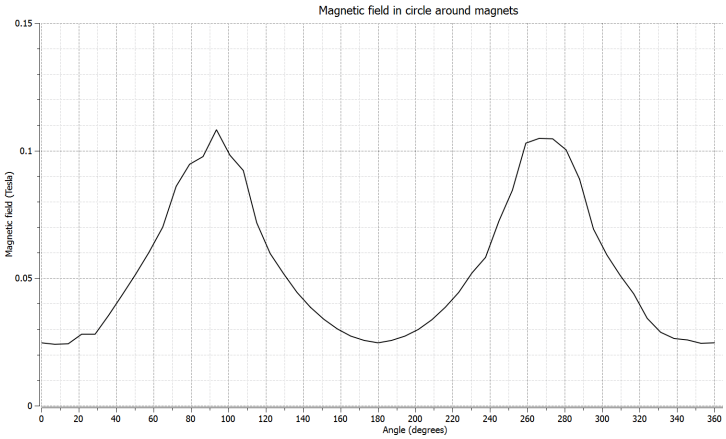
result for the magnetic assembly where strong fields are required where the chambers will be, with as few stray fields as possible (Fig. 3.24).

Putting two of these pairs of magnets with steel backs opposite each other so that they are attracting each other, as shown in Fig. 3.25 and 3.26 further increases the magnetic field while keeping it confined largely to the space between the two pairs of magnets. The spacing between them can be varied to increase or decrease the magnetic field, with a minimum field value at the halfway point. When 30mm apart, the magnetic field half-way between the two pairs was calculated to be 220mT at its peak, but 0T at its minimum (Fig. 3.28). This compares to a maximum and minimum of 126mT and 57mT respectively for a single pair (Fig. 3.27).

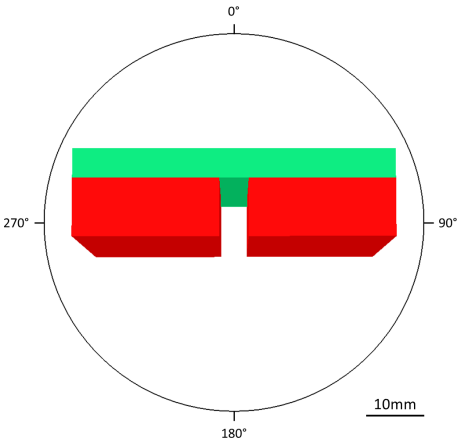
The four magnet arrangement thus has a magnetic field with higher peaks, but much lower troughs where the field is close to or at 0T. However, closer to the surface of the magnets, the magnetic field becomes high throughout. Indeed, the central area with very low field halfway between the two pairs (i.e. 15mm from the surface of both) is actually the area with the strongest field 4mm from the surface in the same arrangement (Fig. 3.29).



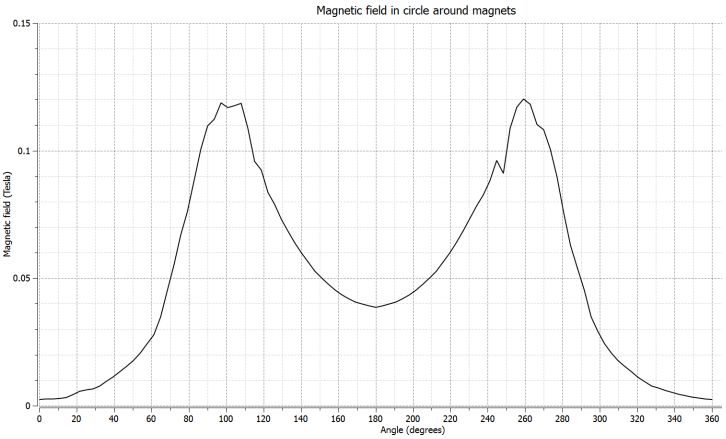
(a) Line on which field was calculated



(b) Without steel back plate

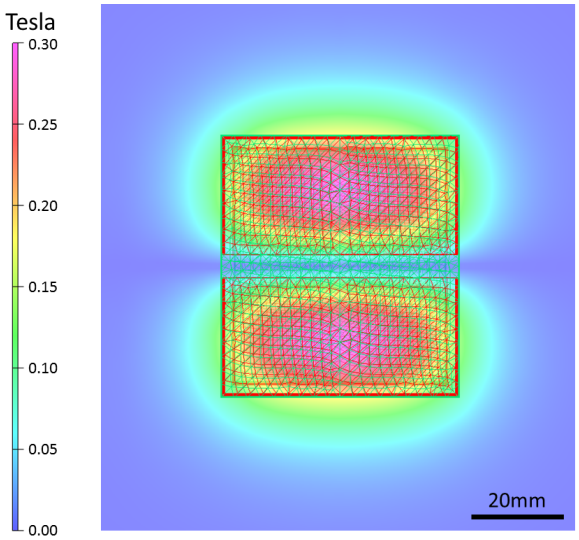


(c) Line on which field was calculated



(d) With 5mm thick steel back plate

Figure 3.23: Magnetic field around two NdFeB block magnets



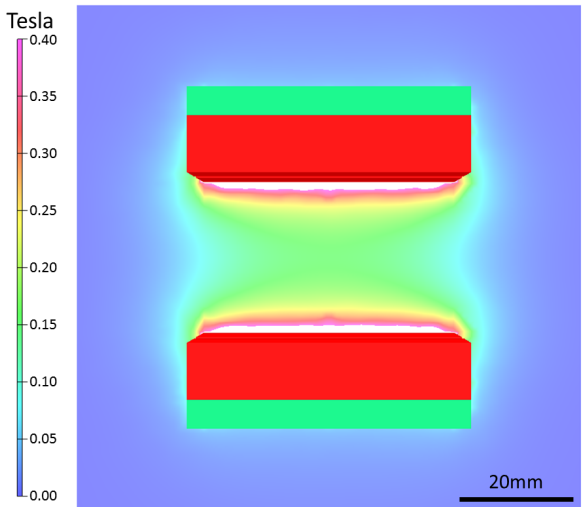


Figure 3.24: Stray magnetic fields generated by two pairs of magnets with steel back plates

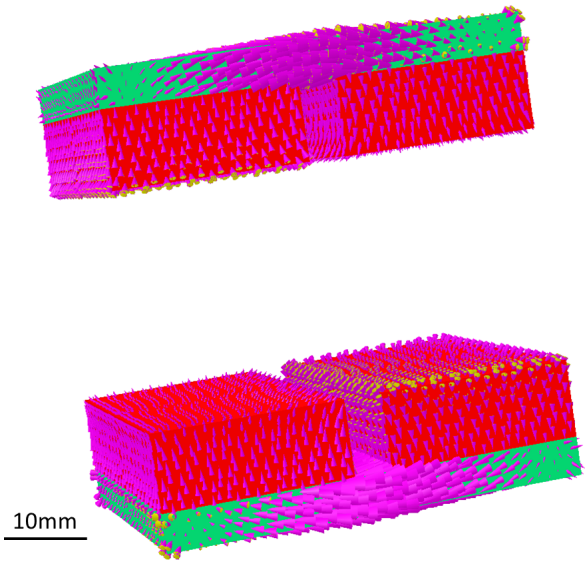


Figure 3.25: Magnetic vector orientations of two pairs of magnets with steel back plates

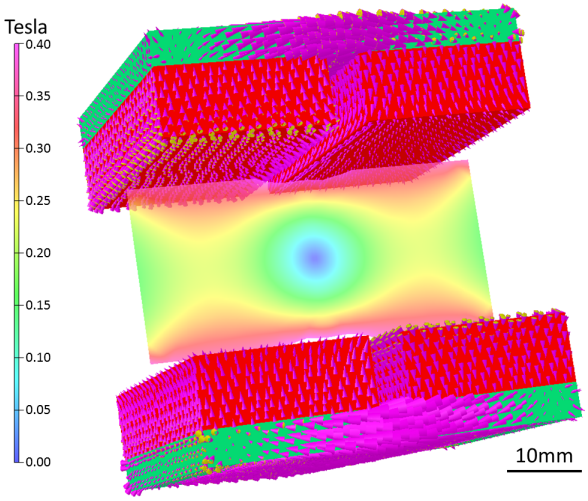


Figure 3.26: Perpendicular magnetic field generated by two pairs of magnets 30mm apart

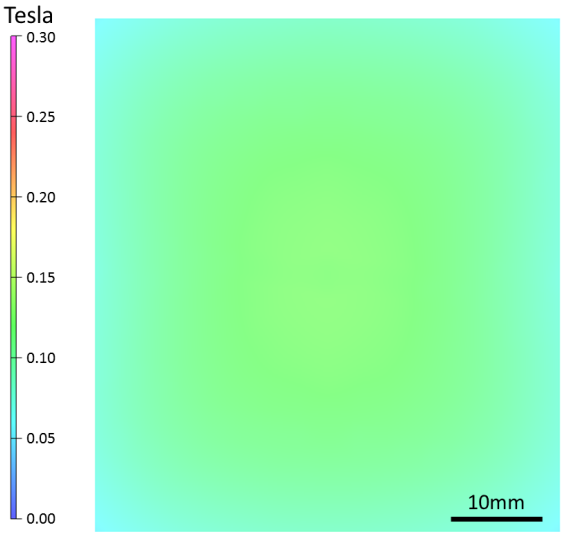


Figure 3.27: Magnetic field 15mm from and parallel to the surface of two block magnets with a steel back plate

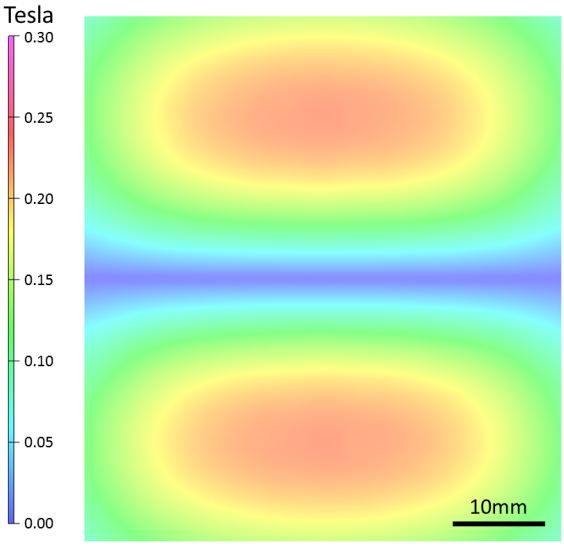


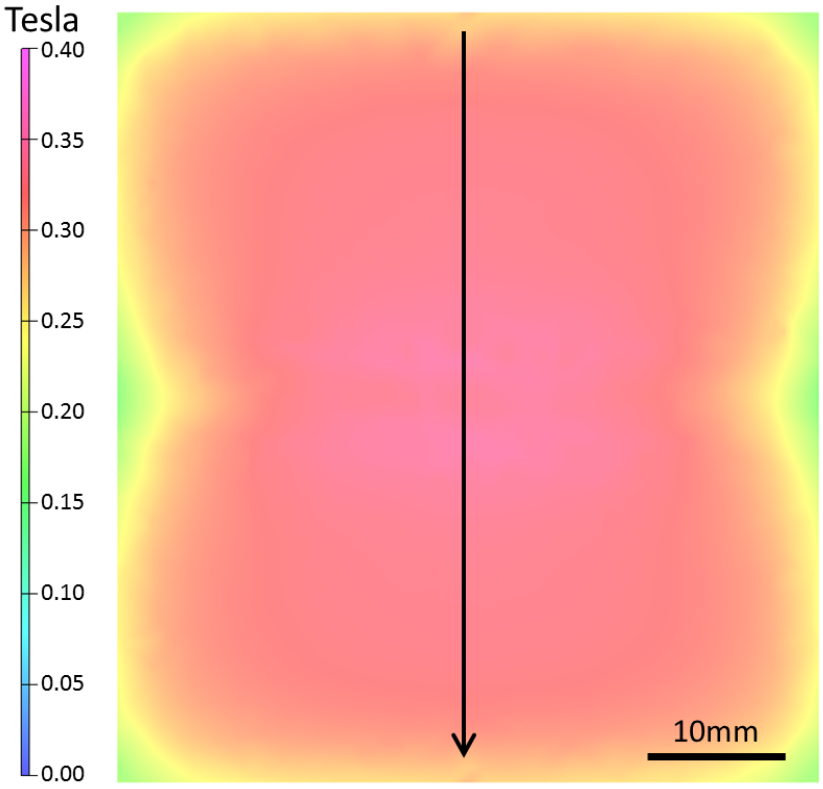
Figure 3.28: Magnetic field parallel to and halfway between two pairs of block magnets with steel plates

This ensures that, while there is an area of weak capture force in the centre of a chamber placed between the pairs, there are no paths through the chamber with consistently low magnetic field.

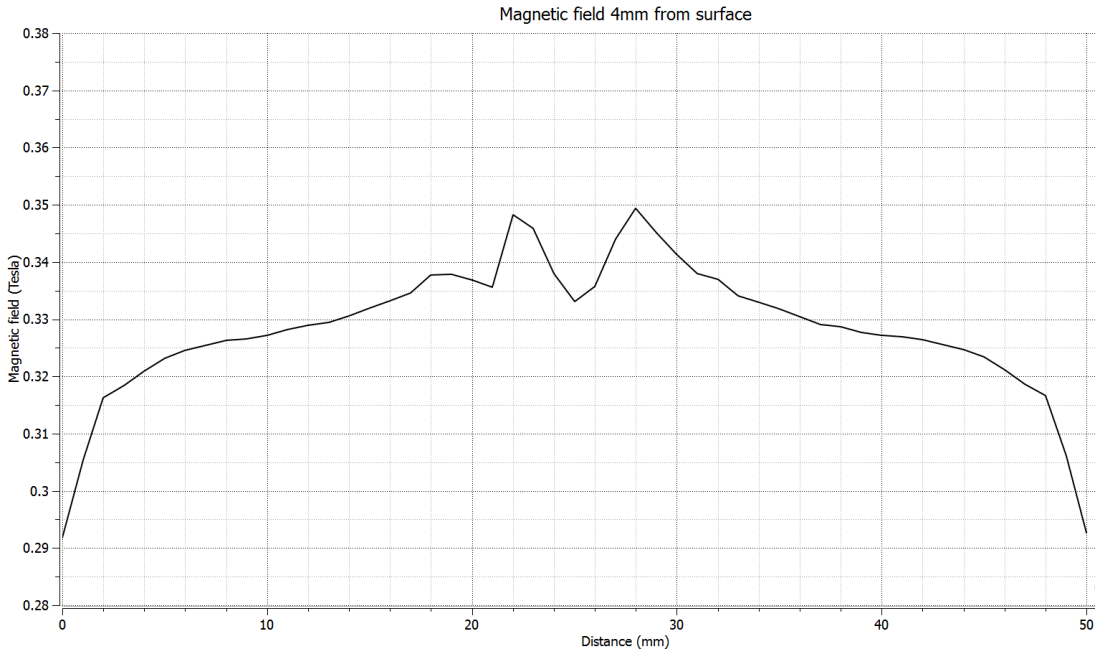
This arrangement of four magnets with steel back plates is simple, easy and cheap to assemble, generates strong magnetic capture forces, avoids escape channels, and is modifiable. Not only can the distance between the two pairs be varied to either increase the magnetic field strength or allow for separation chambers with varying heights, but it can also easily be expanded to increase the space available for the separation chambers. The assemblies of four magnets can be placed in series alongside one another, accommodating either several chambers or one larger chamber, as shown in Fig. 3.30. This magnetic assembly, where each magnetic pair is separated from its neighbour by 5mm, is the one that was built and used in the experiments described in Chapter 4. The magnetic field throughout this assembly when the two sides are separated by 30mm is shown in Fig. 3.31. Note that due to symmetry, the field at 3mm is equal to the field at 27mm, the field at 6mm is the same as at 24mm, and so on.

3.3.2 Frame and construction

Once the magnetic arrangement had been determined, the assembly was designed so that the distance between the two sides of the assembly could be varied while ensuring that they could not snap together. The frame holding the magnets was constructed out of aluminium to ensure solidity (a first plastic prototype quickly snapped) and avoid modification of the magnetic field. Aluminium has a very low magnetic permeability so any magnetisation is negligible.

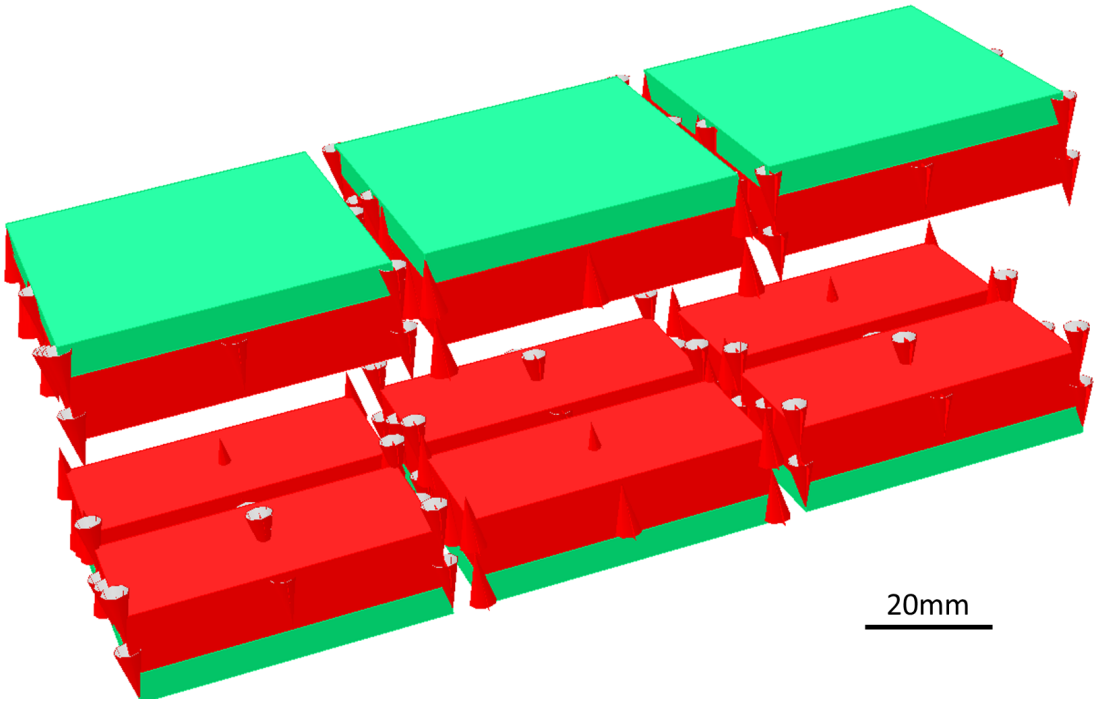


(a) Magnetic field parallel to magnets

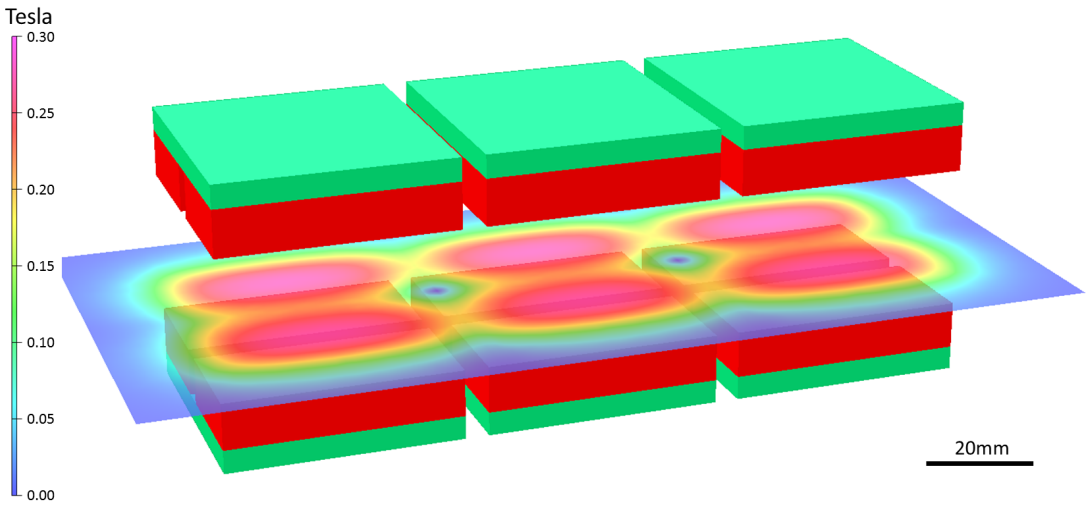


(b) Magnetic field along line shown adjacent

Figure 3.29: Magnetic field 4mm from surface of one pair of magnets



(a) Vector orientation of magnets within assembly



(b) Magnetic field 7mm from surface of magnets at bottom of assembly

Figure 3.30: Complete magnetic assembly, consisting of six pairs of 50x25x10mm NdFeB magnets with steel backs

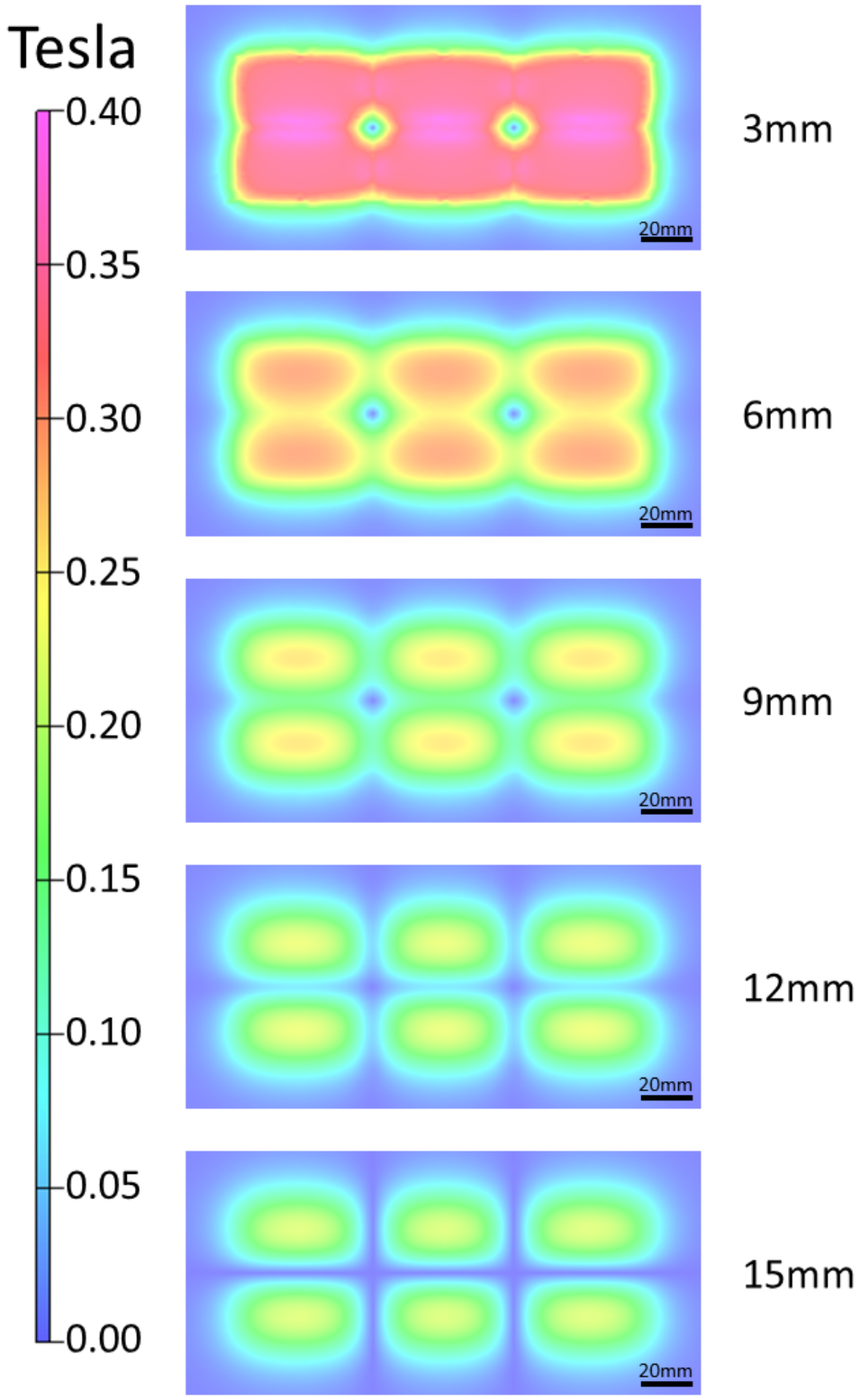


Figure 3.31: Magnetic field within magnetic assembly, at different distances from magnet surfaces

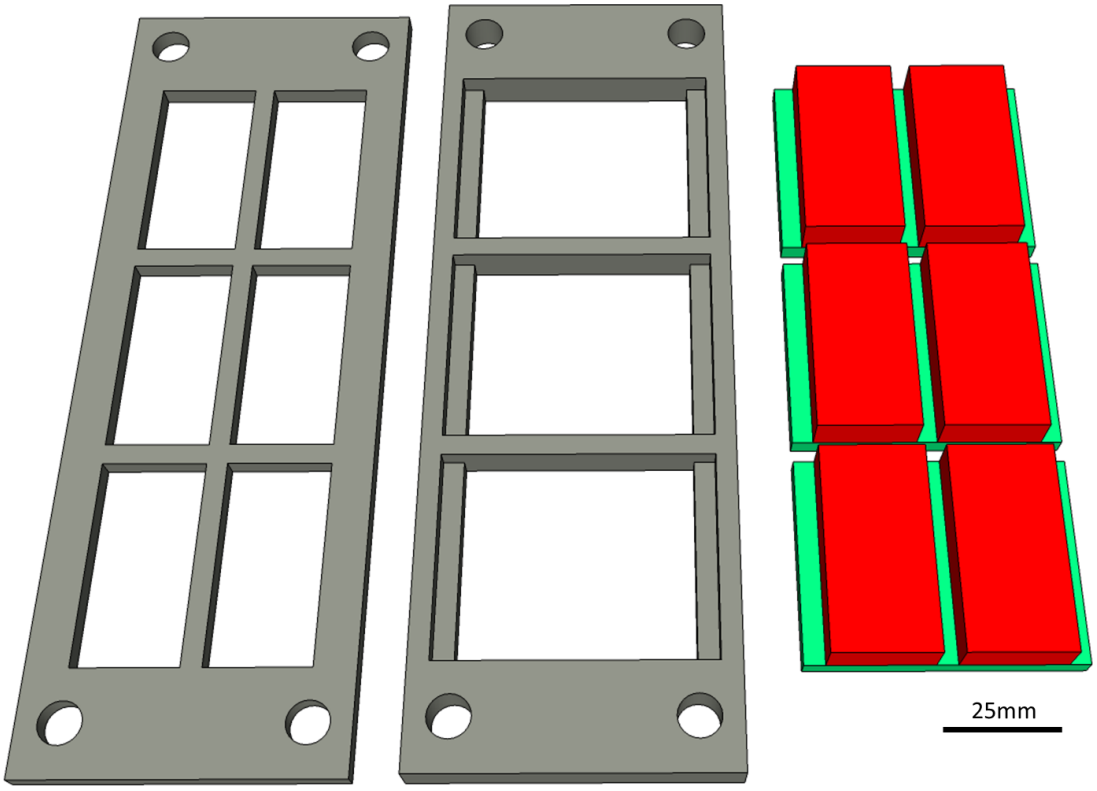


Figure 3.32: Constituent parts of each side of the magnetic assembly: aluminium frame and three magnet pairs

Each side of the frame consisted of two parts designed to hold the magnets in position and prevent any one pair with steel back plate from snapping to the opposite side of the assembly. Indeed, it is of paramount importance that the magnets are all securely held in position and there is no risk of them snapping together. Strong NdFeB magnets such as the ones used in the assembly can crush fingers caught between them. Once placed on the steel plates, the magnetic attraction to the plate prevents any magnet from jumping off said plate - using Opera Vector Fields, the force holding each magnet onto the steel plate was calculated to be 119N. When two pairs were separated by 30mm, the force on a single pair was calculated to be 45N; at 10mm however, it is 200N.

The parts which constitute each side of the assembly are shown in Fig. 3.32. To prevent the pairs snapping together, the steel back plates were cut to a size of 65x50mm,

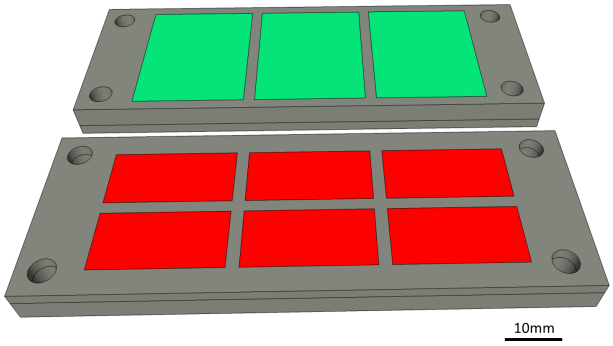


Figure 3.33: Top and bottom of one side of the magnetic assembly after being assembled

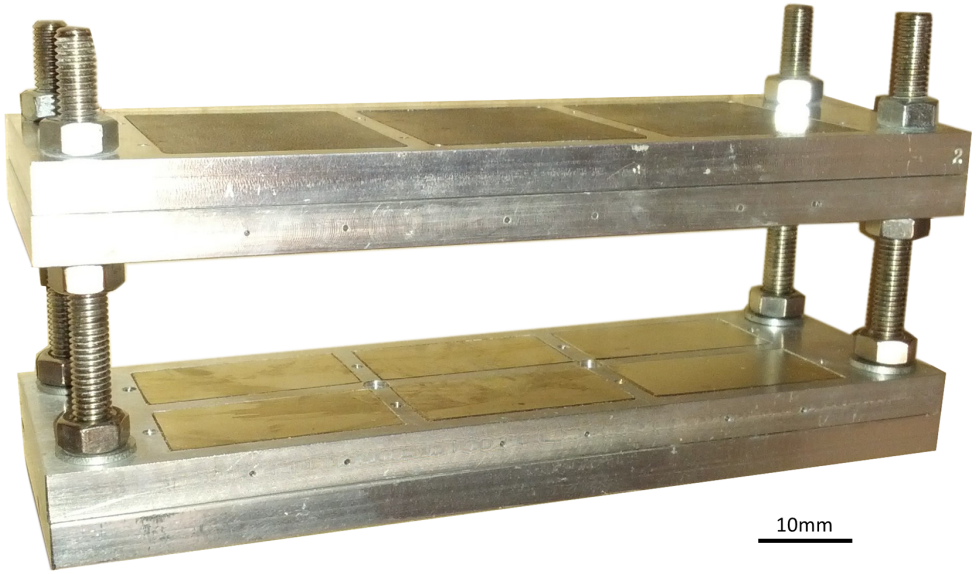


Figure 3.34: Completed magnetic assembly

providing a 5mm 'lip' on each side which serves to hold the pairs within the frame. Each pair was assembled individually, with the magnets carefully placed on them with the correct positions and orientations. These were then placed in the first part of the aluminium frame, before the second part was placed on top (Fig. 3.33). The total size of the frame was 210x75mm. This design enabled the surfaces of the magnets to be exposed, so that the separation chambers could be placed within the area of highest magnetic field.

The four holes of one side of the assembly were threaded to allow four threaded bars to be inserted, which constitute the assembly's support structure. Nuts were then used

to control the separation between the two sides of the assembly. The final result is shown in Fig. 3.34.

3.4 Forces within chamber

The magnetic modelling data can be used to calculate the magnetic force on a malaria infected erythrocyte near a wire of the mesh. As per Eq. 1.4, the required variables are:

- χ_{mol}, the molar magnetic susceptibility of haemozoin. This taken to be $11 \times 10^{-3} ml/mol = 11 \times 10^{-9} m^3$, according to Moore *et al.* [5].
- N_{mol}, the quantity of haemozoin in an infected cell. This was taken from the data shown in Fig. 1.5, also from Moore *et al.* [5].
- B and ∇B, the magnetic field and gradient respectively. These were taken from the modelling data in Fig. 3.6. $10\mu m$ from the wire, these are $B = 0.455T$ and $\nabla B = 11.4 \times 10^3 T/m$.

These data are combined to give the magnetic force on infected cells at different times after parasite infection of the cell. It should be noted that these values correspond essentially to the 'trapping force' holding a cell onto a wire. The force further away from the wires of the mesh is much lower, decreasing to almost zero due to the lack of a field gradient (see Fig. 3.7). Taken as it is at $10\mu m$ from directly above or below a wire, a cell essentially needs to collide with a wire to feel these forces, which are among the strongest within the separation chamber.

Using Eq. 1.2, the magentic forces felt by infected erythrocytes can then be compared with the drag force for different flow rates through the benchtop version. The variables

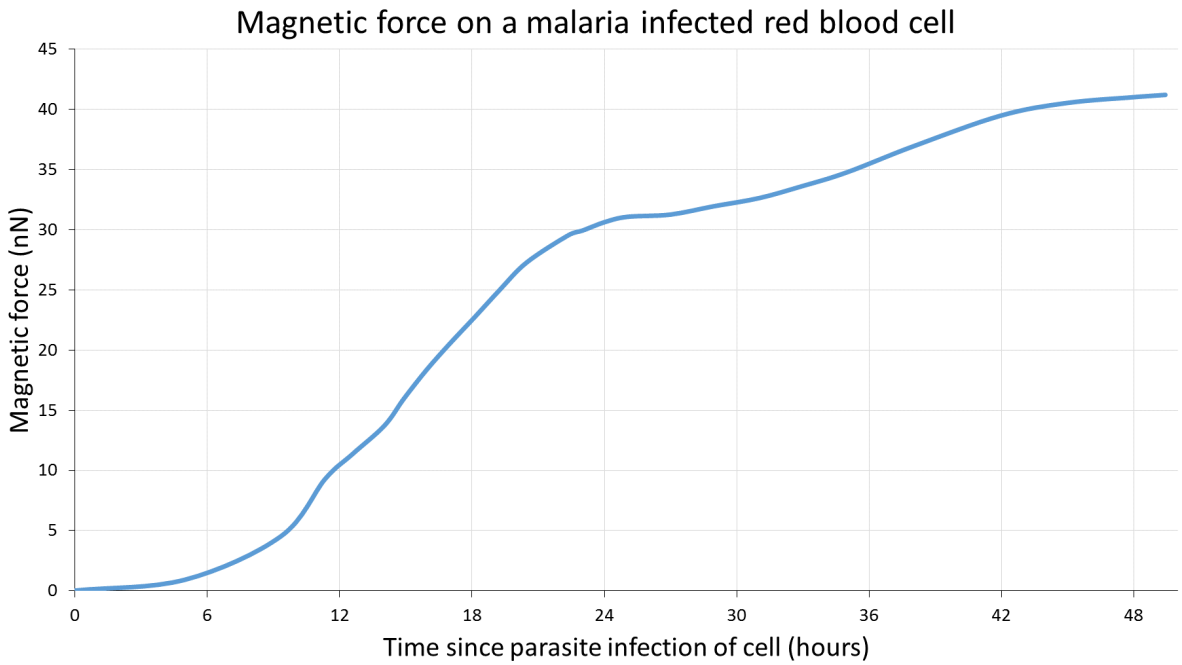
used in these calculations were:

- R, the radius of an erythrocyte. This value is an approximation - the Stokes drag force applies to spherical objects, while red blood cells are more disc-like. However, the formula is an acceptable approximation, and the radius was taken to be $4\mu m$[220].

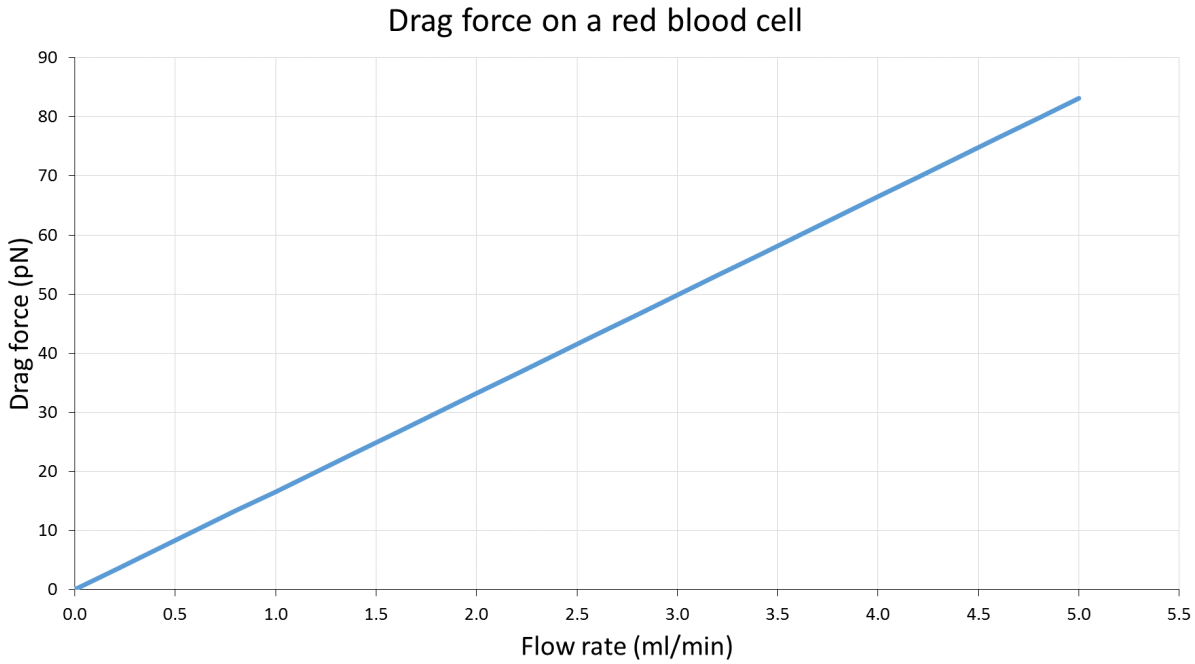
- μ, the viscosity. The viscosity of blood can vary widely and is particularly susecptible to variations in haematocrit. Here, a typical normal value of $3.5 \times 10^{-3} \frac{kg}{m \cdot s^2}$ was chosen[221, 222].

- The mesh was assumed to occupy 30% of the area of the cross-section of a chamber, decreasing the area through which the blood could flow and thus increasing the flow rate.

The results of these two calculations can be seen in Fig. 3.35, demonstrating that at this position the magnetic force exceeds the drag force by a factor of 1,000. It is worth noting that this is the strongest magnetic strength within the chamber, and it deceases rapidly with increase distance from the wires of the mesh - by moving from $10\mu m$ distance from the wire to $25\mu m$, for example, the force decreases five-fold. The drag force, however, remains constant throughout. This analysis demonstrates that most infected cells should be magnetically retained within the separation chamber if they collide with a wire, even those with very small haemozoin content.



(a) Magnetic force on an infected cell $10\mu m$ from a wire within a separation chamber at different times after infection with a malaria parasite



(b) Drag force on a red blood cell at different flow rates in the separation chamber

Figure 3.35: Comparison of magnetic and drag forces felt by malaria infected red blood cells within the scaled-down separation chamber. Note the different force scales between 3.35a and 3.35b

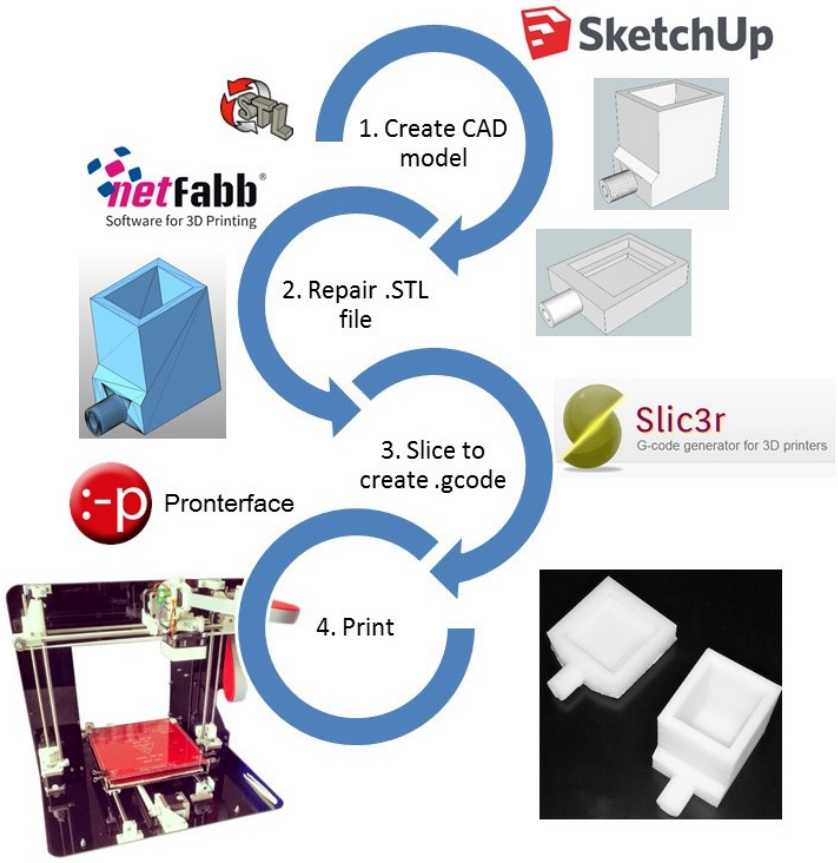


Figure 3.36: Method utilised to 3D print prototype separation chambers

3.5 Manufacture of small scale prototypes

3.5.1 3D printing

The first manufacturing step was to produce the chambers described in section 3.2.6. The two parts - the chamber and cap - were 3D printed in acrylonitrile butadiene styrene (ABS). The steps that were taken in order to manufacture an object using a 3D printer can be seen in Fig. 3.36 and are described in more detail below.

The model is first built using computer aided design (CAD) software as described in section 3.2.6. The SketchUp STL extension was used to export the design in the .stl file format, which contains information relating to the surface geometry of a 3D object

Figure 3.37: Chamber and cap at completion of printing process

(i.e. excluding colour, texture, material or other features). It renders the model into a triangulated mesh that can be read by a vast array of different software. The .stl file is checked for errors (holes in the mesh, for example) using NetFabb Studio Basic (netfabb, Germany, `http://www.netfabb.com/`) and repaired if necessary.

The repaired .stl file is then imported into a 'slicing' program. The opensource software Slic3r (chief author: Alessandro Ranellucci, Italy, `http://slic3r.org/`) was used for this project. The purpose of slicing software is to translate the 3D model into a series of instructions for the 3D printer. .stl files are sliced into the .gcode format, essentially a list of commands fed to the 3D printer in the form of x, y and z coordinates. .gcode files can also include temperature commands telling the printer what temperature to heat the material to before beginning the print. This is achieved by dividing the model into layers of a given height, each layer with a set of coordinates telling the 3D printer where to deposit material. There are a large number of different settings that can be changed,

including layer height, print speed, percentage infill and infill pattern - in order to save material, the objects can be made partially hollow. Typically, the settings need to be optimised for each print in order to get a reliable and reproducible print.

The .gcode is loaded onto the 3D printer. The method of doing this depends on the printer in use, but many use SD cards plugged directly into the printer. The printer itself is controlled either by a UI directly on the printer or using a computer connected via USB. Before launching the print, the correct temperatures need to be set. The extruder temperature will depend on the print material being used - typically it is around 180C for PLA and 250C for ABS, but optimal settings can vary with material supplier and colour. Some printers have heated beds on which the object is printed, or enclosed chambers in which the temperature can be controlled. The bed needs to be cleaned and prepared before the print is launched.

For this project, the opensource Mendel 90 (nophead, UK, `http://hydraraptor.blogspot.co.uk/2011/12/mendel90.html`) was used with a heated carbon fibre print bed. Before printing, the bed is coated with an ABS/acetone solution, made by melting ABS in acetone until the solution is saturated - visible because of the presence of solid plastic bits at the bottom of the solution. A thin coat of 'ABS juice' dried onto the surface of the heated bed helps the first layer of the print to adhere well to the print bed and helps avoid failed prints and the problem of the edges of printed objects curling upwards, a common undesirable feature of ABS prints.

In order to communicate with the printer, the opensource 3D printer controller software Pronterface (chief author: kliment, `https://github.com/kliment/Printrun`) was used to set the temperatures and, once they were reached, launch the print. The settings

used to produce the chambers are detailed in 3.1. Each chamber and cap pair took about one hour to print, and up to eight were produced at a time; Fig. 3.37 shows a chamber and cap still on the printer bed at the end of a print.

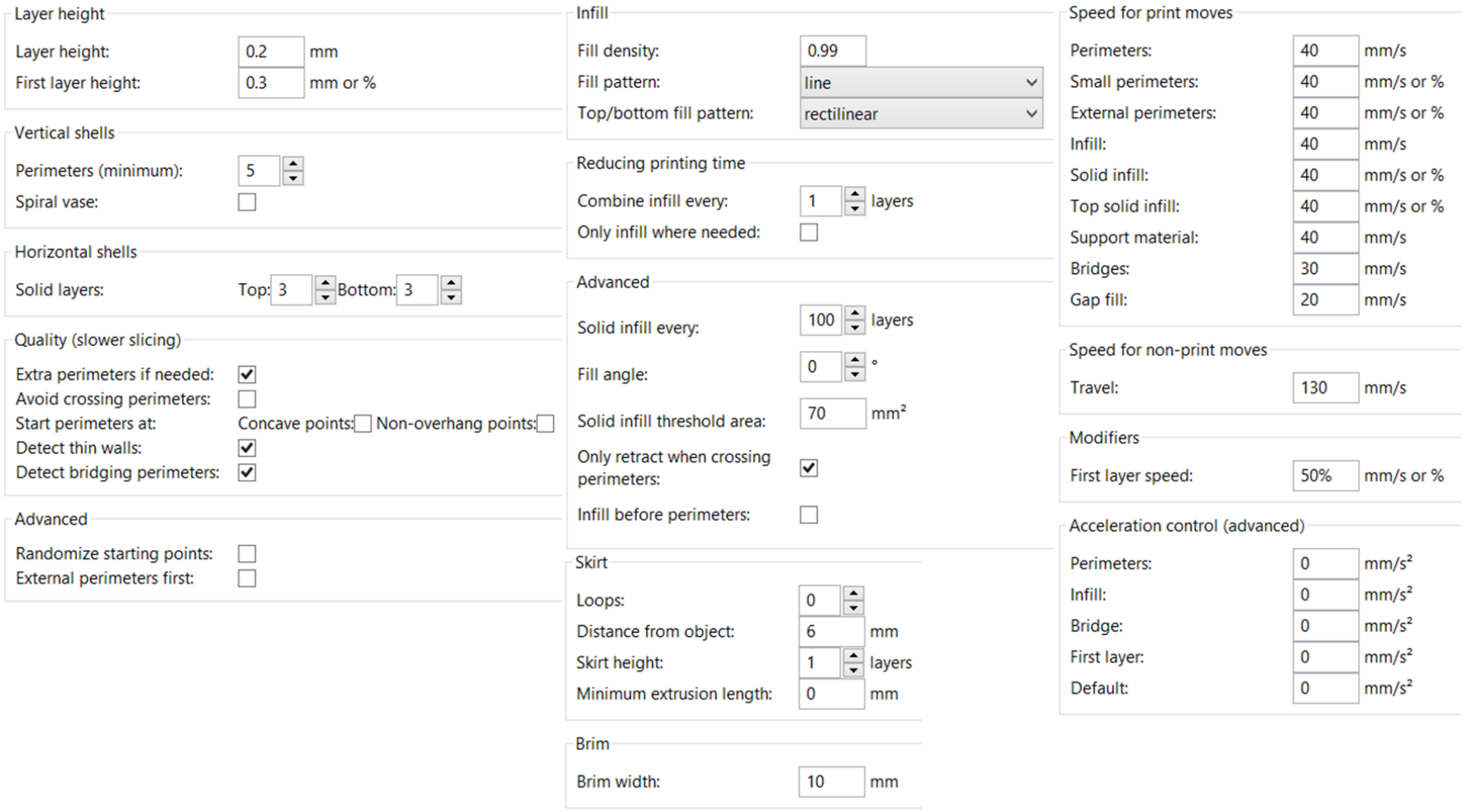


Table 3.1: 3D printing settings used in Slic3r

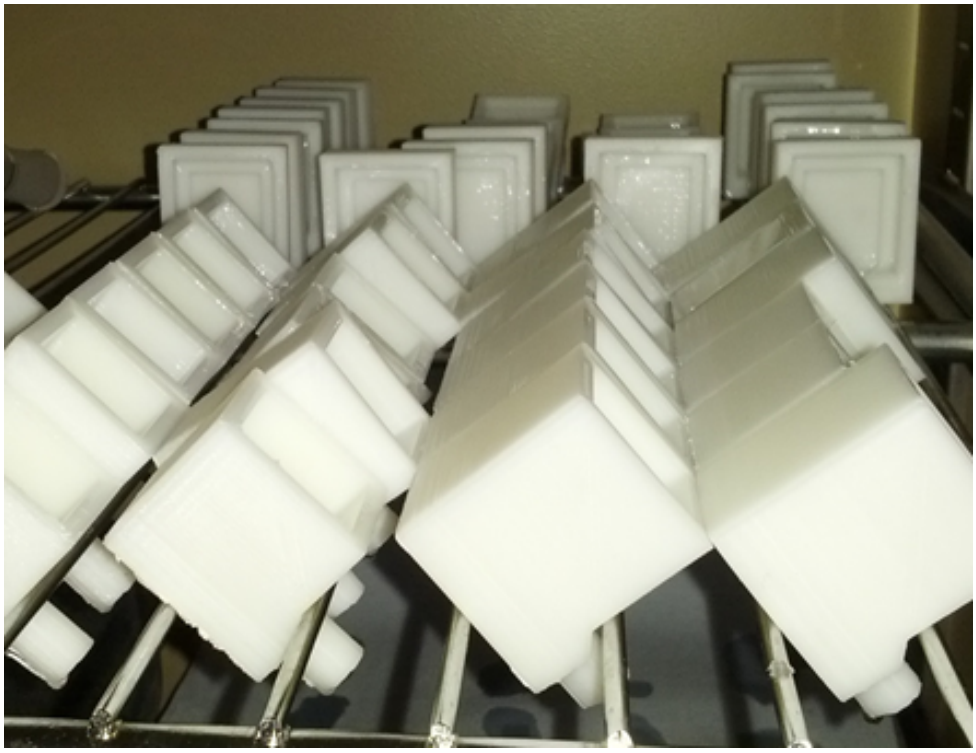


Figure 3.38: Prototype chambers curing after application of hydrophobic coating

3.5.2 Hydrophobic coating

Once printed, the internal faces of the chambers and caps were coated with the silicone elastomer Sylgard® 184 (Dow Corning, USA), a silicone based polymer known as polydimethylsiloxane (PDMS). The kit supplied consisted of a base and curing agent, which are mixed at a ratio of 10:1. The mixture was applied manually using gloves, ensuring the coating covered all areas. The coated chambers were then cured at 80^{o}C for at least 12 hours. For efficiency, this process was carried out on several chambers at the same time (Fig. 3.38).

The purpose of the coating was to render the chamber's internal surfaces hydrophobic. The edges of the chambers are the areas most likely to provide 'escape channels' for cells. Their ridged profile which results from the 3D printing process, could also cause localised dead zones and encourage clotting. Finally, the biocompatibility of the ABS utilised in

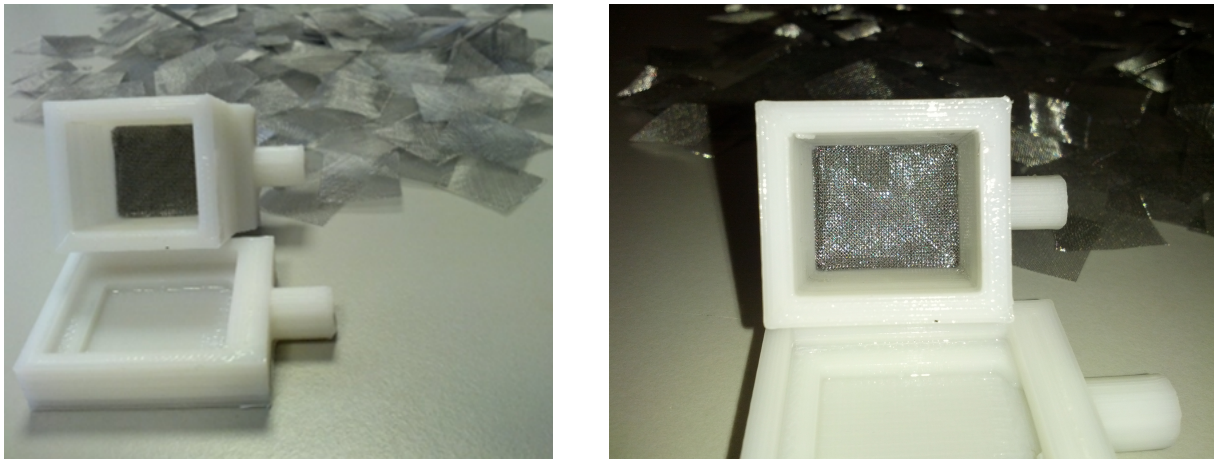


Figure 3.39: Mesh stacked horizontally in chamber

the fabrication process was uncertain, so the PDMS coating served to protect the sample and eliminate a variable that could potentially affect the results.

3.5.3 Inserting ferromagnetic mesh

Once coated, the chambers were filled with SS430 stainless steel mesh (The Mesh Company, Warrington, UK). The mesh had a wire diameter of $50\mu m$ and an aperture size of $250\mu m$ and was purchased in large sheets which needed to be cut to size. The fineness of the mesh enabled it to be cut using scissors and a guillotine paper cutter. Each piece was inserted into the chamber manually, with both horizontally and vertically stacked versions made, in order to investigate if one or the other was more effective (Fig. 3.39). The mesh was packed tightly, with as few non-filled areas as possible. Once the chamber was filled, the cap was placed and sealed in position using acetone, melting the ABS at the interface between the chamber and the cap. The final result can be seen in Fig. 3.40.

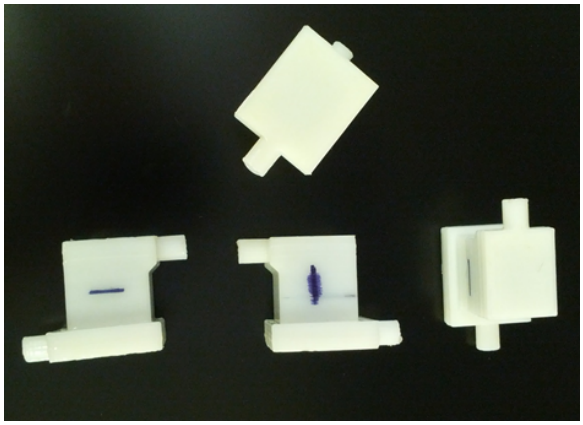


Figure 3.40: Completed and sealed prototype separation chambers (lines represent direction of mesh stacking)

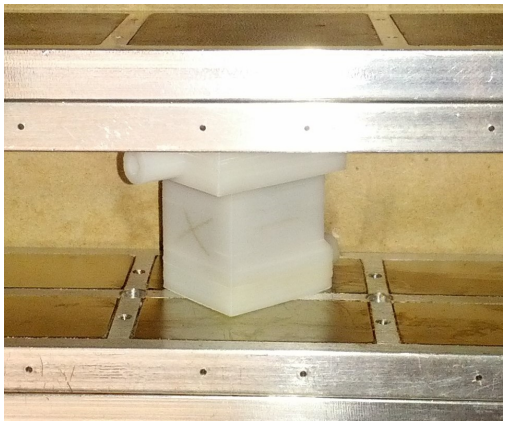


Figure 3.41: Separation chamber in magnetic assembly

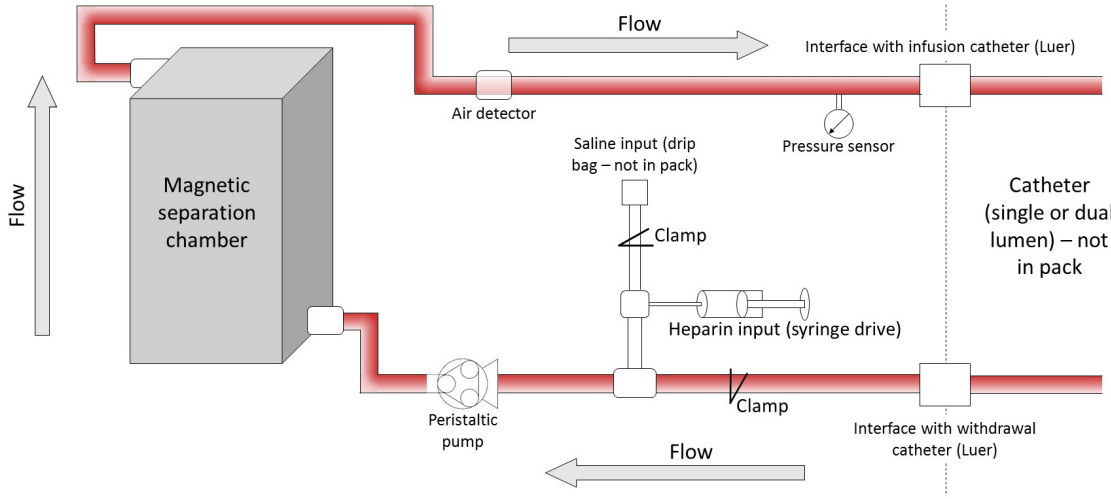


Figure 3.42: Disposable Blood circuit used for each treatment with the magnetic haemofilter. The pumps, air detector and pressure sensor are part of the instrument to which the consumables are connected, and the separation chamber is placed within the magnetic assembly

3.6 The medical device

The medical device will comprise an instrument which drives functionality. It includes the magnetic assembly presented in section 3.3, pumps, UI and safety mechanisms such as the air bubble detector and pressure monitors. The consumables, blood circuits which include the separation chambers presented in section 3.2.4, are simply connected to the instrument and the patient. An example blood circuit including its interface with parts of the instrument is shown in Fig. 3.42.

The inlet and outlet pressure sensors allow for monitoring of the patient's blood pressure as well as the pressure within the separation chamber for safety reasons. In the case of an unexpected variation in the patient's blood pressure to abnormal levels, the system would shut off automatically. An increase in pressure within the separation chamber would be indicative of saturation within the filter - the captured cells having caused a blockage due to their gradual build up on the ferromagnetic mesh. Alternatively, high pressure within the chamber could be caused by clotting.

The air detector will monitor for the presence of any significant air bubbles, an important safety feature - air bubbles circulating within the bloodstream can cause pulmonary embolisms. If any bubbles are detected, the device will automatically stop pumping. The presence of some microbubbles may be inevitable, so the circuit will also include a system for removing them. An effective method would be the use of gas permeable plastic for all or part of the tubing.

As with any extra-corporeal treatment, anti-coagulant will be required to ensure that the blood does not coagulate within the device. This is essential for both safety reasons and functional reasons. Firstly, blot clots can cause serious clinical side-effects such as

embolisms and strokes, so it is essential to ensure that no clots are returning to the patient's circulatory system. Secondly, clotting within the separation chamber could cause blockages and inhibit the device's ability to capture target cells, reducing the efficacy of the treatment. In the case of malaria, the most sensible anticoagulant to use would be heparin, since the hospital or clinic would need to provide the anticoagulant, and heparin is the most affordable and widely available anticoagulant.

In its normal mode, the instrument will automate the treatment, with only a few essential inputs required by the user. The instrument then automatically calculates flow rates, total volume to be processed etc. An 'expert' mode will also be provided, giving more control to the user. The user inputs will be:

- Required:
  - Patient weight
  - Separation chamber version
  - Central or peripheral cathetar
- Optional:
  - Initial parasitaemia
  - Flow rate
  - Total treatment time or volume to process
  - Target final parasitaemia

The device will provide a number of outputs throughout the procedure to inform the user of the current status of the treatment. These will include:

- Current status (e.g. filtering)
- Flow rate (for both blood and anti-coagulant)
- Current pressures - in loop and patient blood pressure
- Total volume filtered
- Total treatment time and estimated remaining time
- Estimated current parasitaemia

The protocol for using the device will be as simple as possible, with much of it automated. The protocol will be as follows:

1. Prepare device, consumables, patient and working area:
    - Patient comfortable and ready (not neccessarily in a bed).
    - User inputs provided when instrument asks for them.
    - Saline and heparin ready.
2. Insert central or peripheral cathetar into patient.
3. Remove consumables from packaging and connect to device:
    - Chamber locked within magnetic assembly.
    - Heparin syringe loaded into syringe pump and connected to circuit.
    - Saline bag connected to circuit.
    - Tubes placed within persitaltic pump.
    - Circuit connected to pressure monitor and air bubble monitor.

4. Priming of the circuit (automated).

5. Connect circuit to cathetar.

6. Start treatment

At the end of the treatment, the circuit is disconnected from the patient and disposed in a controlled manner. If necessary, the circuit may be cleared with saline at a controlled rate to return all the blood in the circuit to the patient, preventing any unnecessary blood loss.

3.7 Conclusion

In this section, the design of the magnetic haemofilter has been presenting, outlining the process that was employed to produce a design which meets the required specifications from section 3.1.1. The main results of that process are the separation chambers and magnetic assembly, which integrate together to create a system which generates high magnetic forces, allowing throughputs orders of magnitude higher than any of the magnetic separators from the literature (see Chapter 2).

The haemofilter has been designed to be cheap to manufacture and easy to modify, allowing for easy optimisation for different volumes or applications. Clinical-scale separation chambers appropriate for patients of any age have been designed so that the two determiners of performance - linear flow rate and magnetic force - are constant. A small-scale chamber for benchtop proof-of-concept experiments was designed and manufactured in-house using 3D printing, significantly reducing product development costs[223].

The magnetic assembly is modular - two sets of a pair of NdFeB magnets with steel

back plates can easily be added or removed. The distance between the magnets can be changed at will, increasing or decreasing the magnetic field in the separation chamber area. The simple magnetic arrangement used results in low-cost, easy manufacturing while ensuring a strong, focussed magnetic field with near zero stray fields. A prototype version was manufactured to enable benchtop testing.

In conclusion, the designs presented in this Chapter are simple, yet should be effective. The main objective was to produce a magnetic haemofilter which generates strong magnetic forces and allowed high throughput with low drag forces. The designs not only achieve that, but are also cheap to manufacture and easy to optimise. The magnetic assembly and separation chamber designs presented in this Chapter are the underlying foundations of the magnetic haemofilter as a medical device.

Chapter 4

Evaluation of Haemofilter

4.1 Aims and methods

4.1.1 Aims

This Chapter presents the experiments that were conducted in order to evaluate whether the designed haemofilter from Chapter 3 could be effective at capturing malaria infected cells from a flowing sample. In order to test the device, samples containing malaria infected erythrocytes were passed at controlled flow rates through the scaled-down version of the device detailed in section 3.2.6. The parasitaemia of the initial sample was compared with the final sample's parasitaemia in order to investigate the degree to which the infected cells were captured. The aims of these experiments were to:

1. Establish the maximum separation efficiency (percentage reduction in parasitaemia) that can be achieved

2. Investigate how the flow rate affects the separation efficiency

3. Investigate other variables that affect the separation efficiency

4. Demonstrate that human samples show the same results as mouse samples, thus validating the bench-top animal model, as well as demonstrating that the device works on both *P. vivax* and *P. falciparum*

5. Quantify the infection stages (i.e. time since parasite has infected RBC) that are successfully removed

6. Check for any adverse effects on other components of the blood that might be caused by the filtration process

7. Investigate degree of false positive capture

4.1.2 Giemsa staining

The parasitaemia was quantified using Giemsa staining and optical microscopy, the current gold standard for malaria diagnosis[224]. Giemsa stains DNA - as erythrocytes do not naturally contain any DNA, Giemsa clearly highlights any parasites within the cells so that they can be identified using optical microscopy. The different stages of infection (ring, trophozoite, schizont) are also visually distinguishable, enabling the parasitaemia to be broken down into the individual contributions of each infected stage. This enables an investigation into the stage at which infected cells are successfully captured (i.e. contain sufficient haemozoin), as well as the quantification of the separation efficiency for each stage.

The quantification of the parasitaemia was carried out with the generous assistance of malaria microscopist experts from the London School of Hygiene and Tropical Medicine

Figure 4.1: Thin film blood smears: unstained (top) and stained (bottom)

(LSHTM) and the Universidad de Antioquia in Medellín, Colombia. Their independence and expertise in differentiating between both different infection stages and false positives serve to increase the credibility of the results.

4.1.2.1 Protocol

To stain the samples, a thin film blood smear is created by placing a drop of sample of about $100\mu m$ onto a glass microscope slide, and smearing it using the edge of a second slide (Fig. 4.1). Once the sample has dried, it is fixed by dropping methanol onto the slide. The slides are then covered with a solution of Giemsa diluted 4:1 in water (Fig. 4.2). After 25 minutes, the slides are delicately rinsed with water and are ready to be counted using an optical microscope using oil immersion 100x magnification.

The microscopist first locates an area with a good distribution of cells, packed close

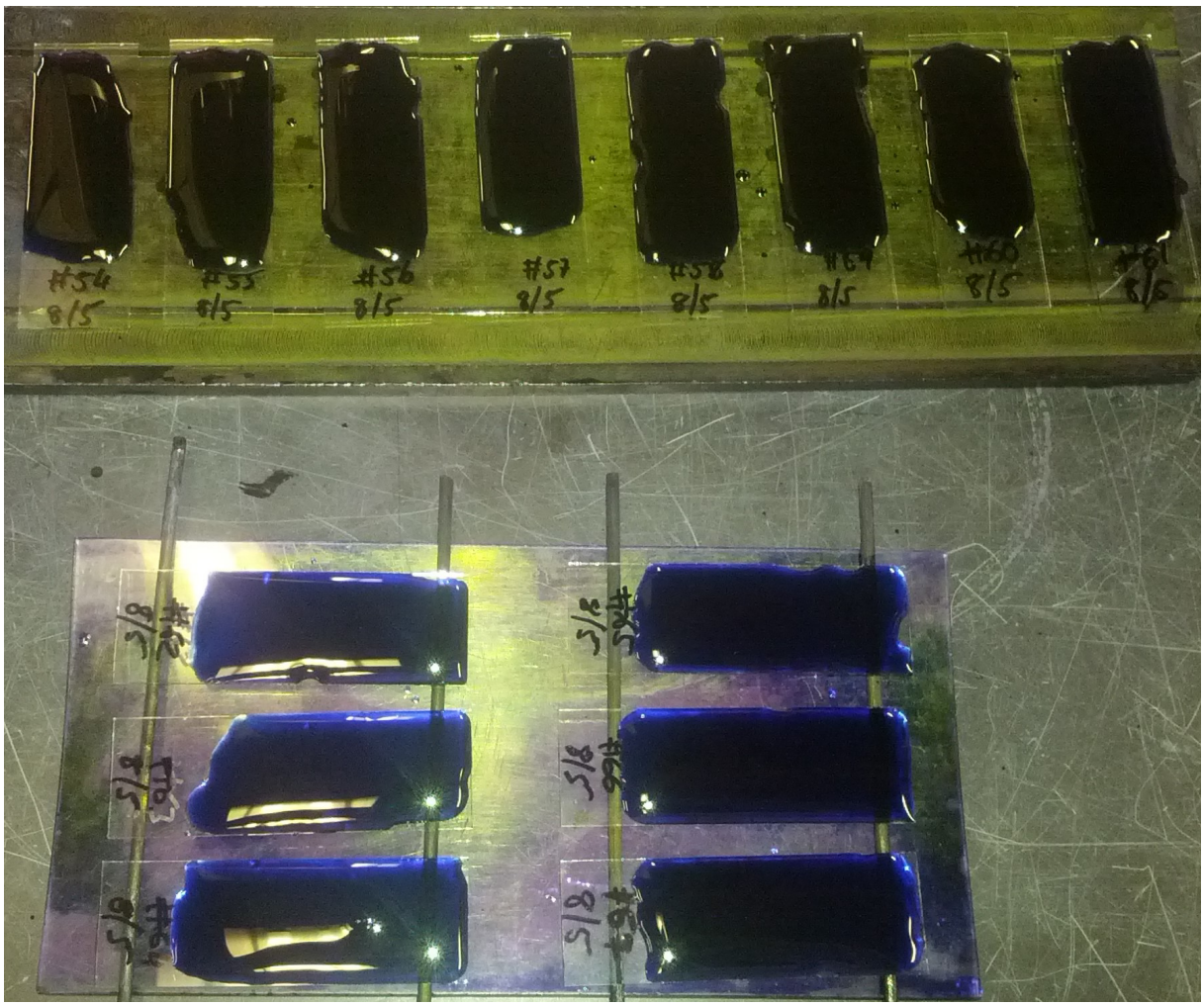


Figure 4.2: Giemsa staining of microscope slides

together but not overlapping each other - the purpose of the thin film blood smear is to ensure that such an area can be found on the slide. They then count the number of rings, trophozoites and schizonts visible in the current field of view. This is repeated for 11 total fields per slide. The parasitaemia of each stage (and of the total) is calculated using:

$$P_{\%} = \frac{3 \cdot \sum c}{100} \tag{4.1}$$

where P is the parasitaemia as a percentage, and c is the number of infected cells. This formula assumes that there are on average 303 cells in every field of view:

$$P_{\%} = \frac{\sum c}{RBC_{total}} \times 100 = \frac{3 \cdot \sum c}{100}$$

$$RBC_{total} = RBC_{field} \times 11$$

$$\therefore RBC_{field} \times 11 = \frac{10^4}{3}$$

$$\therefore RBC_{field} = \frac{10^4}{33} = 303 \tag{4.2}$$

4.1.3 Apparatus

All of the experiments looking at the device's ability to remove malaria infected erythrocytes used the same apparatus (Fig. 4.4). Samples containing malarial erythrocytes were flowed through the scaled-down separation chamber detailed in section 3.2.6, placed

into the magnetic assembly detailed in section 3.3. Magnetic forces pull the chamber into the position of highest magnetic field within the assembly, ensuring that the chambers are always placed in the same, optimal position. The separation chambers were mass produced using 3D printing (see section 3.5.1 for 3D printing details and method) so that a new separation chamber could be used in each experiment (Fig. 4.5). Their internal surfaces were rendered hydrophobic by coating them with PDMS, before they were filled with ferromagnetic mesh as described in section 3.5.2. The lines on the chambers represent the direction in which the mesh was stacked - a horizontal line represents horizontal stacking (Fig. 4.5e), and a vertical line vertical stacking.

The initial blood samples were loaded into a 20ml Chromacol® syringe (Essex Scientific Laboratory Supplies, UK), which was connected to the separation chamber via Versilic® silicone tubing (Scientific Laboratory Supplies, UK). The samples entered at the bottom of the chamber, in order to ensure that the experiment was not affected by gravitational forces, particularly as the chambers are initially empty and were not primed before the start of the experiments. The samples exited at the top and opposite side of the chamber, flowing via silicone tubing into a BD Biosciences Falcon™ tube (Farnell, UK). The flow rates were controlled by an Aladdin-220 programmable syringe pump (World Precision Instruments, Hitchin, UK).

4.1.4 Cultured *P. falciparum*

The experiments detailed in this section were carried out in the research labs of the Grupo Malaria, in the SIU of the Universidad de Antioquia, Medellín, Colombia. We are very grateful to the research group for providing this opportunity.

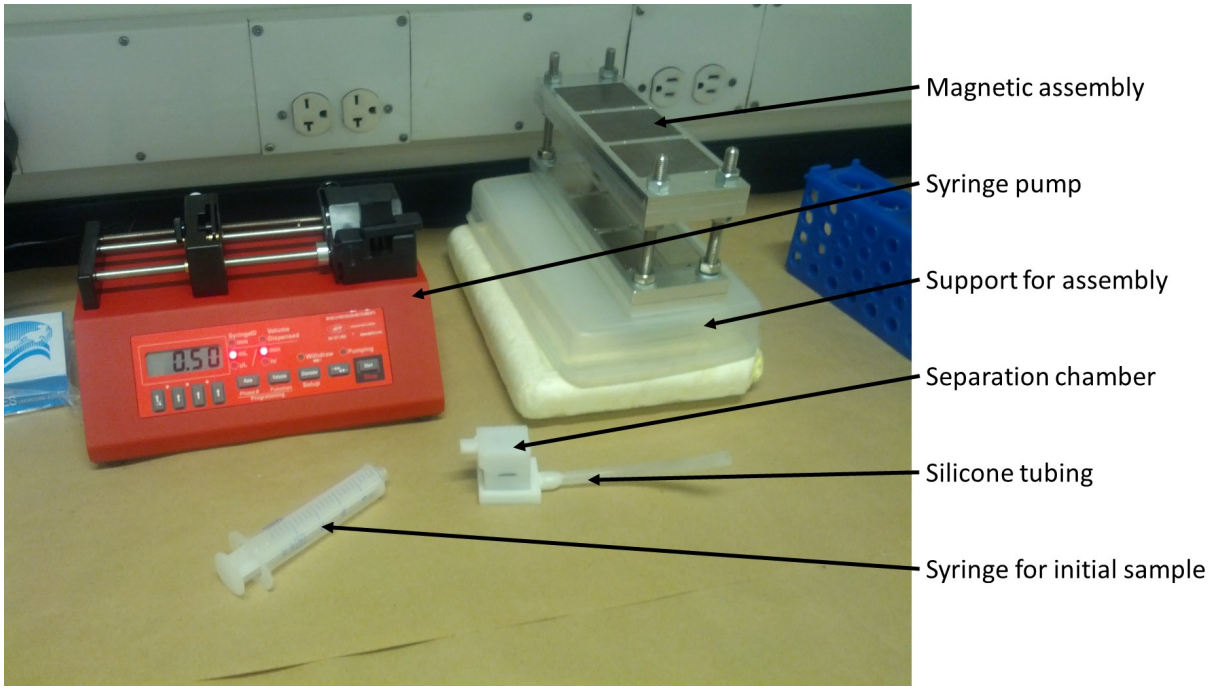


Figure 4.3: Constituent parts of apparatus

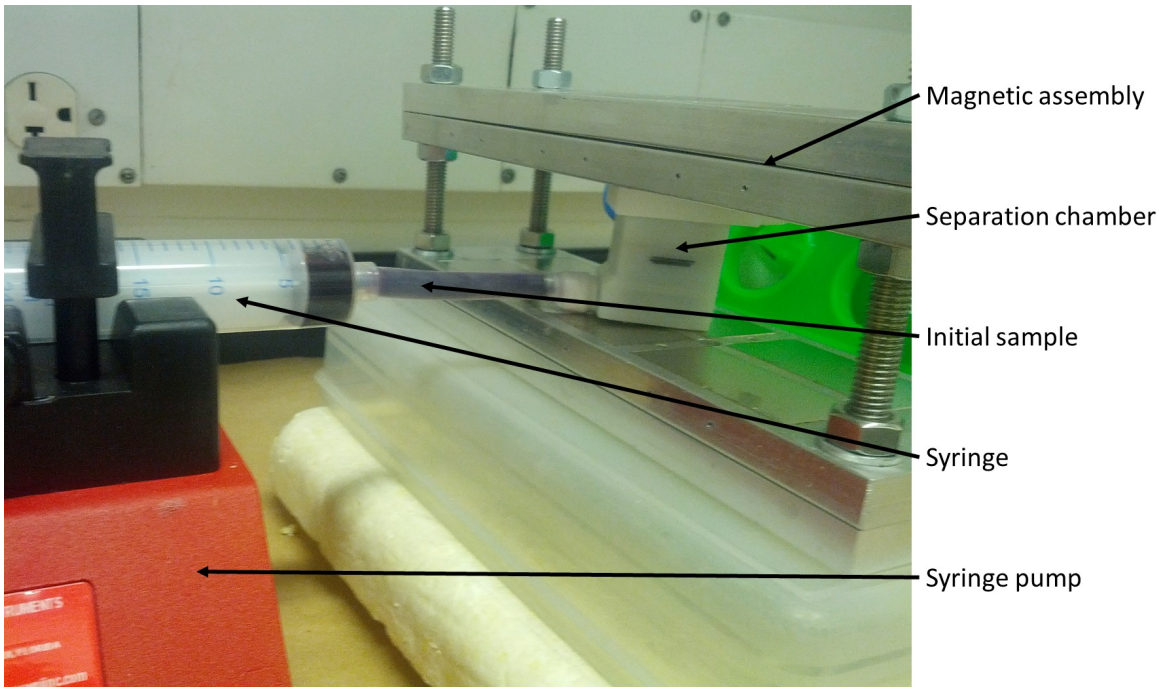
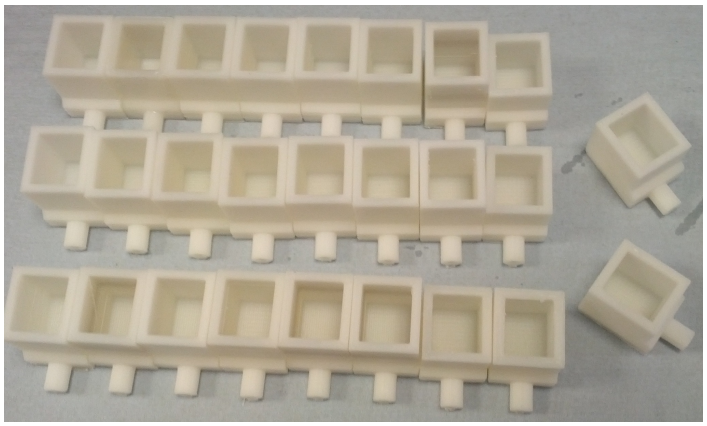
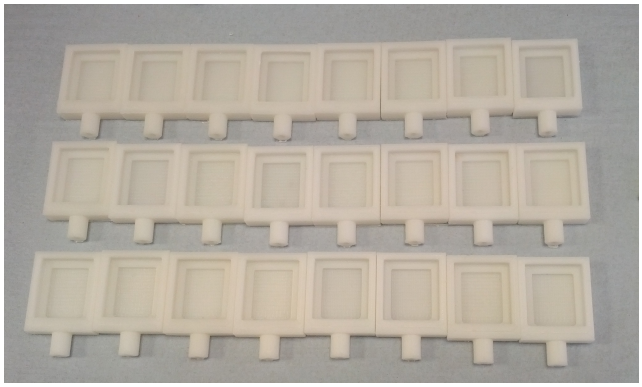


Figure 4.4: Assembled apparatus with experiment running

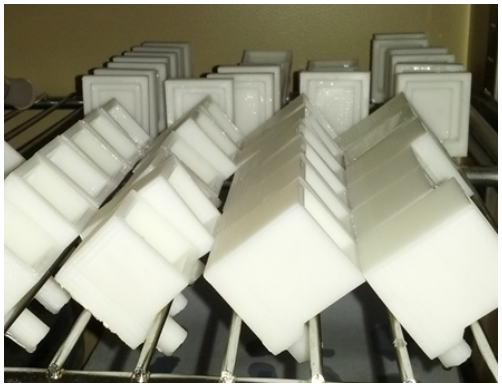
(a) Chamber and cap 3D printed



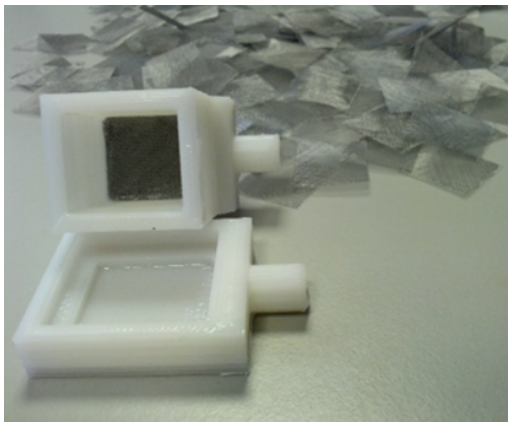
(b) 3D printed chambers



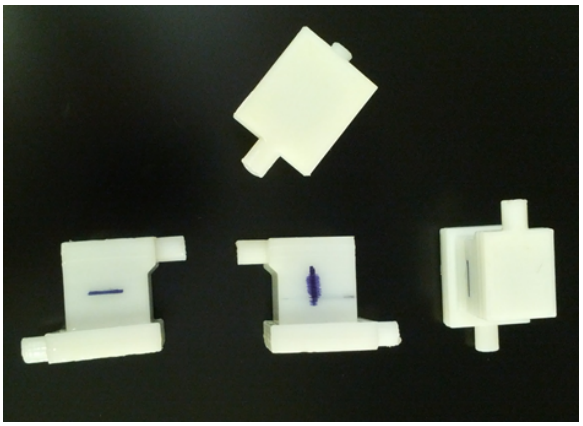
(c) 3D printed lids for chambers



(d) Chambers curing at 70^{o}C



(e) Ferromagnetic mesh stacked horizontally in chamber



(f) Chambers sealed and ready for use

Figure 4.5: Production of separation chambers

4.1.4.1 Cultures

The first experiments were conducted on samples of cultured *P. falciparum*. These were human red blood cells infected with parasites which had previously been isolated from a patient and cryopreserved. Small samples can be taken from this isolate and cultured at 37^{o}C in medium, in this case Roswell Park Memorial Institute (RPMI). The sample progresses through the different erythrocytic stages of the malaria life-cycle (see Fig. 1.2 and section 1.2.2), and the parasitaemia increases approximately every 48 hours when the schizonts rupture, releasing merozoites which infect new erythrocytes in the culture.

In order to control the parasitaemia of the samples used in the experiments, the cultures were allowed to progress to high parasitaemia and then diluted with healthy erythrocytes. The cultures were then centrifuged and the cell pellet re-suspended in RPMI. The haematocrit was kept constant for all experiments, by using 1ml RPMI for every $7\mu l$ of cell pellet. A typical sample consisted of a $700\mu l$ pellet suspended in 40ml RPMI. This was then divided into two 20ml samples in order to conduct two experiments.

The cell cultures and sample preparations for this project were carried out by Alexandra Marcela, from the Grupo Malaria.

4.1.4.2 Filtration

Before each experiment, the area was cleaned and cleared of clutter. Appropriate protective equipment (gloves, lab coat, goggles) were worn. The apparatus was set up as shown in Fig. 4.4, with 20ml of sample loaded into the syringe which was connected to a clean separation chamber. The syringe pump was programmed to the desired flow rate, and the experiment started. Once the syringe was empty, the experiment was paused while it was

carefully removed from the syringe pump and loaded with air. The experiment was then resumed with air pumped at the same flow rate in order to push as much of the sample as possible through the chamber. All the experiments were conducted at room temperature.

4.1.4.3 Sampling

Samples of 1ml were taken at various points throughout the experiment in order to measure the parasitaemia. One sample was taken of the 'initial' sample, before filtration. Two or three samples were then taken from the 'filtered' sample: one of the first ml to exit the separation chamber, one when the volume within the syringe chamber had been reduced by half, and one once the syringe was empty. In some experiments, a sample of the 'filtered' sample was also taken after air had been used to push as much of the sample as possible through the chamber.

Once the sample had been filtered, the chamber was removed from the magnetic assembly and reconnected to the empty syringe. The chamber was then vigorously flushed with air in order to remove any sample remaining in the chamber, typically about 1ml. This recovered sample constituted the final sample of an experiment, and was used to investigate the cells that had been captured within the chamber. Thus every experiment had four or five 1ml samples:

1. One from the initial unfiltered sample, as provided at the beginning of the experiment. This was generally used as the 'initial sample' for two experiments, since two experiments were conducted with the 40ml of cultured cells.

2. The first ml to emerge from the separation chamber after the start of the experiment.

3. After the syringe was empty - 20ml had been pumped, but only 5-8ml had typically

been filtered with the rest of the sample in the chamber or tubing.

4. One after as much of the sample as possible had been passed through the separation chamber, which had been flushed with air at the same flow rate as the experiment.

5. One from the sample remaining in the separation chamber at the end of the experiment.

For each of these, one microscope slide was prepared as described in section 4.1.2, and the parasitaemia established by a trained microscopist. The microscopy was carried out by Alexandra Marcela and Briegel de Las Salas, from the Grupo Malaria.

4.1.5 Donated *P. vivax*

In order to test whether the haemofilter functioned on malaria patient blood, samples voluntarily donated by *P. vivax* patients were passed through the device and their initial parasitaemia compared to their parasitaemia after filtration. Furthermore, in order to see whether the device had any undesirable effect on blood components, complete blood counts were performed on the samples before and after being passed through the haemofilter. The samples were taken from donors infected with *P. vivax* in the El Bagre municipality in the north-east of Antioquia, Colombia, an area with relatively high incidence. Patients presenting with symptoms at the malaria diagnostic post were first diagnosed using thick blood smears. If positive, they were invited to volunteer to take part in the study.

In four of the experiments, the samples were chilled on ice and shipped to the Universidad de Antioquia in Medellin, where the experiments were performed. As such, the time between taking of the samples and performing the experiment varied considerably

between each sample, from eight hours up to 24. While the experiments were performed at room temperature, the blood samples were still chilled. Temperature affects the rheology of the blood, importantly affecting the viscosity of blood, a key contributor to the drag force felt by cells (see Eq. 1.2)[225]. The temperature can also affect the function of platelets and other cells[226].

Ethics approval for this research was provided by the Human Ethics Committee of the Universidad de Antioquia and can be seen in Appendix C (internal document number BE-IIM). The patients were fully informed of the purpose of the study, and each signed a patient consent form, the template for which is in Appendix D, while the patient registration form is in Appendix E. The inclusion criteria for the patients were:

- Exclusive infection with *P. vivax*
- At least 18 years of age
- Diagnosed with non-complicated malaria, using clinical evaluation methods recommended by the WHO

The exclusion criteria were:

- Pregnant women
- Recognised concomitant pathologies
- Anaemia

The blood was collected by experienced researchers from the Grupo Malaria of the Universidad de Antioquia, in 2.5ml vacutainer blood tubes containing heparin; five tubes (12.5ml) were taken per patient. The samples were chilled in ice and immediately shipped

by air to the Universidad de Antioquia in Medellín, where they were filtered through the device at various flow rates (1-4ml/min). Samples pre and post filtration were immediately sent to the university's haematology laboratory for full blood count analysis.

4.2 Flow rate analysis

The chambers used in the experiments had a cross-sectional surface area of $21 \times 18mm = 378mm^2$. The surface areas of the clinical versions described in section 3.2.4 are:

- Infant: $40 \times 25mm = 1,000mm^2$. 2.7 times small-scale version.
- Small: $75 \times 40mm = 3,000mm^2$. 7.9 times small-scale version.
- Medium: $100 \times 50mm = 5,000mm^2$. 13.2 times small-scale version.
- Adult: $160 \times 50mm = 8,000mm^2$. 21.2 times small-scale version.

These provide the multipliers that can be used to calculate the flow rates that could be used within the clinical scale device. If an experiment is conducted at 2ml/min in the scaled-down chamber, for example, a flow rate of 42.4ml/min in the adult version would generate the same linear flow rate, and thus the same drag force. Since the magnetic forces are the same, this indicates the throughput that would theoretically produce the same result *in vivo*.

As discussed in section 3.1.2, significantly more than TBV needs to be filtered in order to achieve a high total parasitaemia reduction. For example, taking a TBV of 5l for an adult and assuming a separation efficiency of 90%, 12.8l of blood need to be processed in order to remove 90% of infected cells from a patient, a total treatment time of just over

two hours if filtering at 100ml/min. Using this same model, the total treatment times for different flow rates were calculated for an infant, child, youth and adult. The assumptions were:

- Initial parasitaemia: 2%
- Separation efficiency of device: 90%
- Infant TBV: 500ml (weight 6.5kg)
- Child TBV: 1,500ml (weight 20kg)
- Youth TBV: 3,200ml (weight 40kg)
- Adult TBV: 5,000ml (weight 65kg)

The calculated total volumes to be processed to achieve a 90% reduction in total parasitaemia were:

- Infant: 1,275ml
- Child: 3,830ml
- Youth: 8,180ml
- Adult: 12,785ml

It is interesting to note that the total volume that needs to be processed is equal to approximately 2.5x TBV for all patients. This number should be taken as the 'filtration ratio' and used to calculated the total volume that will need to be processed to treat any given patient.

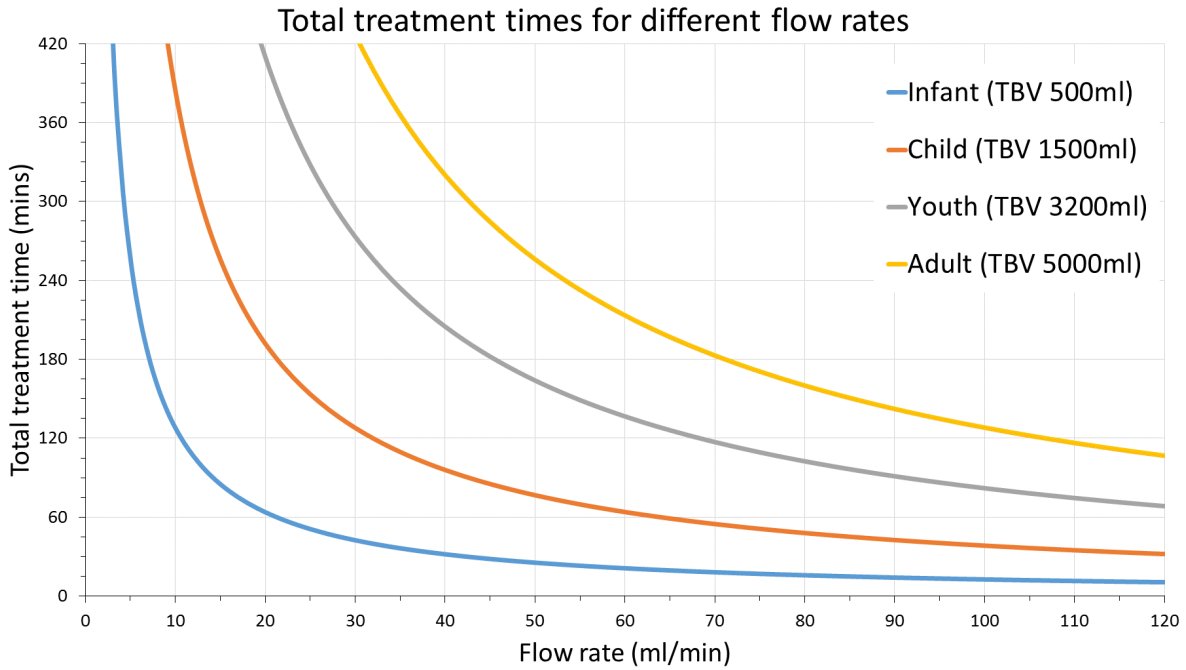


Figure 4.6: Time required to achieve a 90% reduction in parasitaemia for different patient TBV, assuming 2% initial parasitaemia and 90% device separation efficiency

The total time needed to reduce the total parasitaemia by 90% for different flow rates is shown in Fig. 4.6. Because of the dimensions of the separation chambers, the flow rates used in the small, medium and adult versions can be respectively three, five and eight times the flow rate used in the infant version. This means that if the experiments indicate that a 90% separation is possible using a flow rate of, for example, 3ml/min, the flow rate used in the clinical versions could be 8ml/min, 24ml/min, 40ml/min and 64ml/min for the infant, small, medium and adult versions respectively. Using our model patients, these correspond to total treatment times of:

- Infant: 2 hours 40 mins
- Child: 2 hours 40 mins
- Youth: 3 hours 15 mins
- Adult: 3 hours 10 mins

All of these are respectable treatment times, although the ultimate target should be to reduce them as much as possible.

These data were used to help choose the flow rates to be used in the scaled-down experiments. While the first priority was to demonstrate that the separation chamber was successful in capturing malaria infected erythrocytes, the second goal was to check whether its performance was maintained at higher flow rates. The first experiments were therefore carried out at 1-2ml/min, followed by further experiments at up to 4ml/min.

4.3 Results - cultured *P. falciparum*

The 24 experiments using cultured *P. falciparum* were conducted with flow rates ranging from 0.2ml/min to 4 ml/min. The initial samples had varying parasitaemias and different combinations of ring-stage and mature infected erythrocytes. The results are summarised in Table 4.1 and Fig. 4.7; the two bars for each experiment represent the initial and filtered samples after a single pass through the magnetic haemofilter. Across all experiments, there was an average 44.4% reduction in the parasitaemia with a standard deviation of 32.0%, with separation efficiencies ranging from -34.7% (i.e. an observed increase in parasitaemia) to 81.8%. Fig. 4.8 shows the separation of only ring stage infected cells, while Fig. 4.9 shows the separation of only mature stage infected cells.

It is difficult to draw a conclusion from the data in Fig. 4.7. While 87.5% (21 out of 24) showed an overall reduction in the parasitaemia, the overall separation efficiency varied significantly and showed no evident link to the flow rate. One immediate conclusion, however, is that the three experiments in which no reduction in parasitaemia was observed all saw slight (35%, 7% and 8%) increases in the number of ring stage cells, but decreases

Flow rate	Initial parasitaemia			Filtered parasitaemia			Separation efficiency		
(ml/min)	Total	Rings	Mature	Total	Rings	Mature	Total	Rings	Mature
0.2	2.76%	0.36%	2.40%	0.63%	0.00%	0.63%	77.2%	100.0%	73.8%
0.5	1.29%	0.42%	0.87%	0.65%	0.33%	0.32%	50.0%	21.4%	63.8%
0.75	7.14%	2.19%	4.95%	1.58%	0.55%	1.03%	77.9%	75.1%	79.2%
1	11.13%	4.83%	6.30%	6.45%	4.20%	2.25%	42.0%	13.0%	64.3%
1	11.13%	4.83%	6.30%	3.68%	2.16%	1.52%	67.0%	55.3%	76.0%
1	1.56%	0.63%	0.93%	0.29%	0.15%	0.14%	81.7%	76.2%	85.5%
1	1.08%	1.08%	0.00%	1.46%	1.43%	0.03%	-34.7%	-31.9%	N/A
1	1.41%	1.35%	0.06%	1.51%	1.47%	0.04%	-7.1%	-8.9%	33.3%
1.5	2.73%	1.05%	1.68%	1.07%	0.42%	0.65%	60.8%	60.0%	61.3%
1.5	1.60%	0.16%	1.44%	0.41%	0.14%	0.27%	74.4%	12.5%	81.3%
1.5	6.66%	0.90%	5.76%	1.22%	0.50%	0.72%	81.8%	45.0%	87.5%
2	2.64%	2.31%	0.33%	2.86%	2.68%	0.18%	-8.3%	-16.0%	45.5%
2	3.12%	2.85%	0.27%	1.97%	1.88%	0.09%	37.0%	34.2%	66.7%
2	1.62%	0.36%	1.26%	1.07%	0.45%	0.62%	34.3%	-25.0%	51.2%
2	2.01%	2.01%	0.00%	1.52%	1.51%	0.01%	24.2%	24.7%	N/A
2	2.01%	2.01%	0.00%	1.69%	1.62%	0.07%	15.9%	19.4%	N/A
2.4	1.60%	0.16%	1.44%	0.42%	0.15%	0.27%	73.8%	6.3%	81.3%
2.5	1.80%	0.03%	1.77%	1.46%	0.02%	1.44%	19.2%	50.0%	18.6%
2.5	3.09%	0.00%	3.09%	1.43%	0.00%	1.43%	53.9%	N/A	53.9%
3	1.68%	0.60%	1.08%	1.35%	0.61%	0.74%	19.6%	-1.7%	31.5%
3	1.44%	0.00%	1.44%	1.07%	0.00%	1.07%	25.7%	N/A	25.7%
3.5	1.71%	0.93%	0.78%	0.38%	0.23%	0.15%	77.8%	75.3%	80.8%
4	1.71%	0.93%	0.78%	0.39%	0.24%	0.15%	77.2%	74.2%	80.8%
4	2.76%	0.36%	2.40%	1.52%	0.27%	1.25%	44.9%	25.0%	47.9%
						Average	44.4%	31.1%	61.4%
						Standard deviation	32.0%	35.8%	20.6%

Table 4.1: Summary of results from 24 experiments with cultured *P. falciparum*

in the parasitaemia of trophozoites and schizonts. This supports the hypothesis that the device is only effective at removing mature infected cells which contain sufficient haemozoin. To investigate this further, the separation of rings and of mature cells were analysed separately.

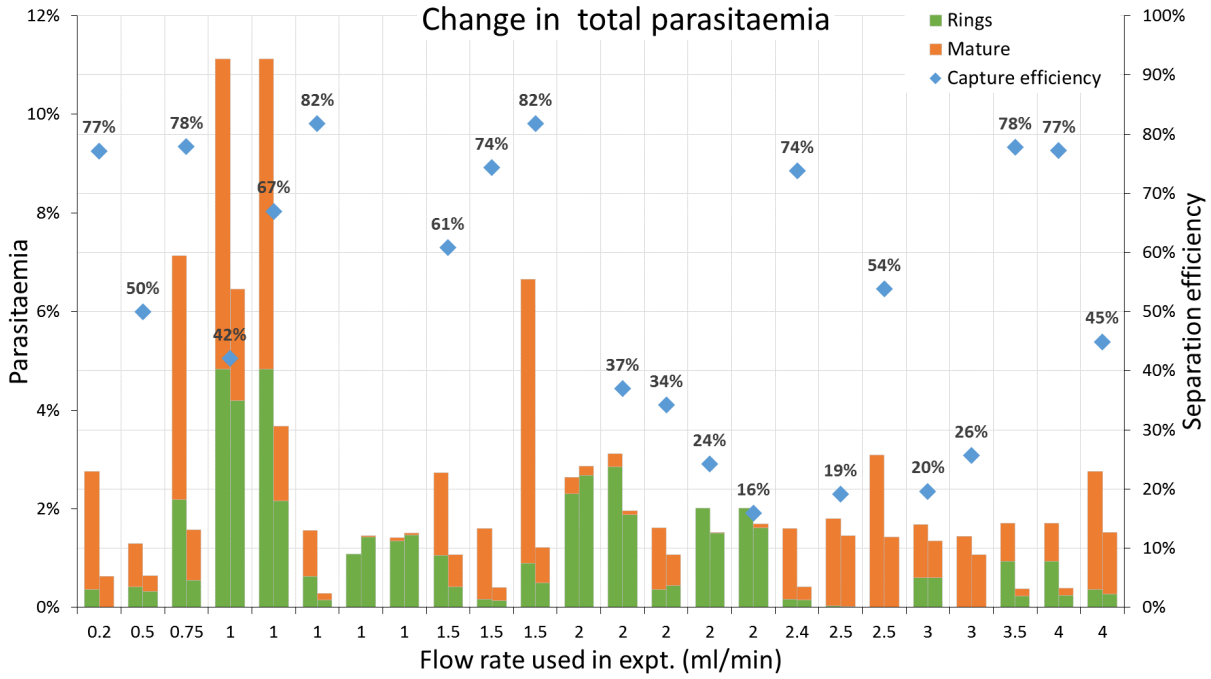


Figure 4.7: Parasitaemia of initial and final samples and the percentage reduction (capture efficiency) in parasitaemia for a single pass through the magnetic haemofilter for all 24 experiments conducted with cultured *P. falciparum* (n.b. capture efficiency not included if below 0)

4.3.1 Separation of ring-stage parasites

Fig. 4.8 presents the variation in parasitaemia of only the ring-stage infected cells, with experiments in which the initial ring-stage parasitaemia was at or close to 0% excluded. It shows the unpredictability of the separation of ring-stage cells - some show significant reductions, while others remain flat or even show small increases. The average separation efficiency was 31.1%, but with a high standard deviation of 35.8%.

Ring-stage cells contain only small quantities of haemozoin, so should theoretically not be captured by the magnetic haemofilter. However, about 24 hours after infection, some parasites still appear as rings but can contain 75% of the haemozoin that a schizont contains at cell rupture[5]. It is therefore possible that some of the cells in the 'ring-stage' populations contain sufficient haemozoin to be captured by the device. Indeed, out of 19

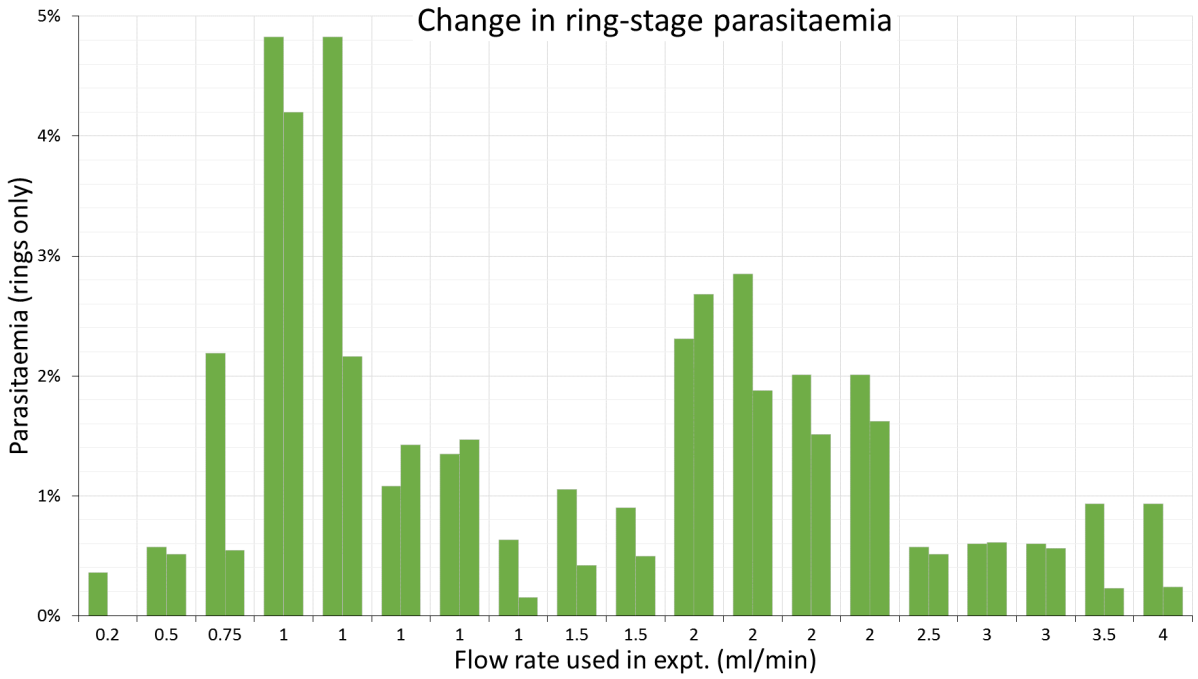


Figure 4.8: Parasitaemia of ring-stage cells, before and after for a single pass through the magnetic haemofilter

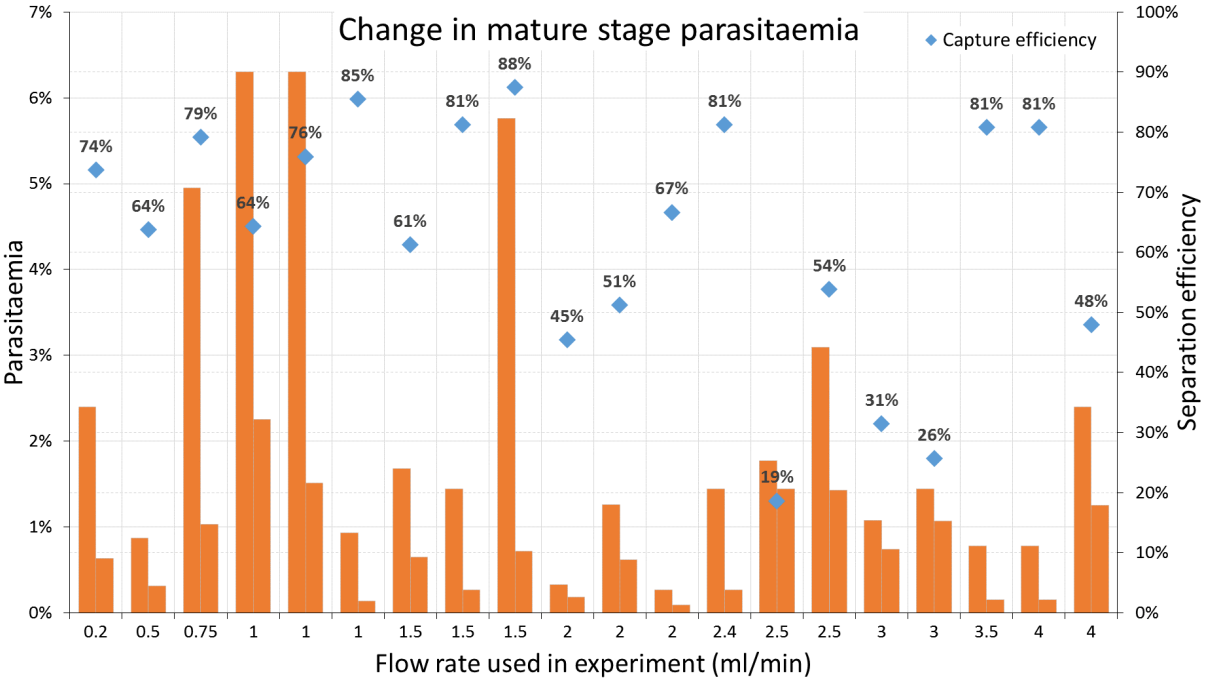


Figure 4.9: Mature stage parasitaemia of samples before and after for a single pass through the magnetic haemofilter, and separation efficiency of mature infected cells

experiments presented in Fig. 4.8, 11 (58%) saw a decrease in ring-stage parasitaemia of over 50%, including six (32%) over 75%, clearly indicating that some ring-stage cells were captured by the haemofilter.

However, there is no clear link between ring-stage separation efficiency and flow rate, and no discernible relationship with the initial ring-stage parasitaemia. This supports the conclusion that the device is capturing 'late-stage' rings, or early stage trophozoites - the separation efficiency in this case would be determined by the proportion of ring-stage cells that contain sufficient haemozoin, which could vary significantly from one experiment to another.

This conclusion is further supported by photographs of the samples. Fig. 4.10b shows a typical sample after filtration, and contains almost exclusively early-stage rings. The ring-stage parasitaemia of this particular slide was found to be 2.53%, and was one of the slides in an experiment run at 1ml/min, with a calculated ring-stage separation efficiency of 55%. The immediate conclusion when comparing it to Fig. 4.10a is that the mature cells have been very successfully captured, but a closer look shows the presence of larger rings in the process of becoming mature trophozoites. There is only one of these in Fig. 4.10b, suggesting that these cells are being captured, accounting for the decrease in ring-stage parasitaemia seen in some of the experiments.

Only five experiments (26%) showed a negligible (between -25% and 25%) change in ring-stage parasitaemia. While it was predicted that most of the experiments would fall into this category, the conclusion that the device also captured 'late-stage' ring-stage parasites accounts for this small number - only about a quarter of the initial samples didn't contain any late-stage rings. Indeed, it is worth noting that three out of these five

are from initial samples that contained no trophozoites or schizonts, and were likely to contain almost exclusively early-stage rings.

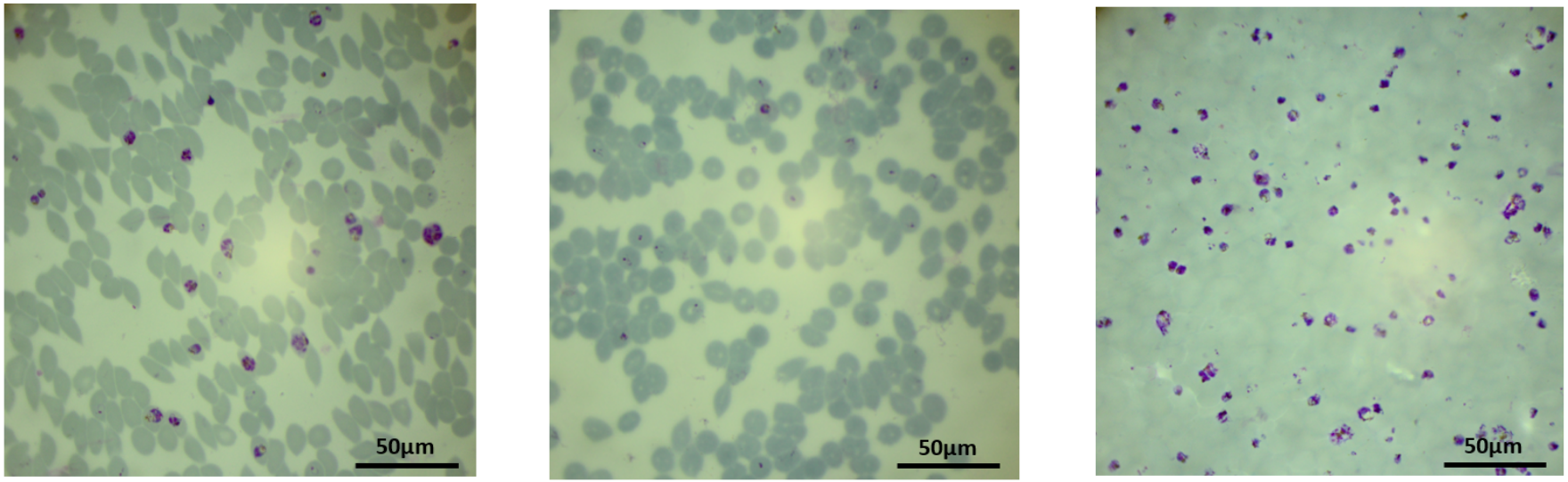
Four experiments (21%) showed an overall increase in the ring-stage parasitaemia between the initial and filtered samples. Since all the separation chambers used were clean, it was highly unlikely that new infected cells be added to the sample during the procedure. This increase, which peaked at a 32% increase (from 1.08% to 1.43%) in an experiment with a flow rate of 1ml/min, could therefore be due to:

- *False positive capture of uninfected erythrocytes.* If the separation chambers were retaining more uninfected than ring-stage infected erythrocytes, then an increase in the ring-stage parasitaemia would be expected. However, this seems unlikely. Even if uninfected cells were susceptible to magnetic capture, which is not impossible, there is no reason why a ring-stage infected cell would be less likely to be captured than an uninfected cell. Furthermore, if this was the case, one would expect regular increases in the parasitaemia throughout the experiments, rather than in only 21%.

- *Experimental errors.* This could include, for example, coincidental problems in the sampling procedure, or during preparation of the slides, favouring either higher infection rates for the filtered samples, or lower infection rates for the initial samples.

- *Uncertainties.* As discussed in section 4.5.1, there are presumptions in the Giemsa method for establishing parasitaemia which could account for the increase in ring-stage parasitaemia - in fact, no cells have been captured, and the difference between the initial and filtered samples is within the margin of error. This is likely to be the case for those experiments where the increase is small (7% and 8%), but perhaps could not account for the experiment with an increase of 32%.

In conclusion, the results indicate that early stage rings are not captured by the device. However, the large number of experiments with reductions in the ring-stage parasitaemia indicate that at least some of these infected cells are being captured, most likely those that are beginning or about to begin their transformation into mature trophozoites.



(a) Initial sample **(b)** Filtered sample **(c)** Recovered from chamber

Figure 4.10: Giemsa stained blood smears at 100x magnification

4.3.2 Separation of mature parasites

In contrast to the ring-stages, all of the experiments in which the initial sample contained mature stage infected erythrocytes showed a reduction in the mature stage parasitaemia, as demonstrated in Fig. 4.9. The separation efficiency ranged from 19% to 88%, with an average of 61.4% and standard deviation of 20.6%. 65% (13 out of 20) of the experiments had separation efficiencies of over 60%, while 30% (six) had efficiencies above 80%. Five experiments (25%) had separation efficiencies of less than 50% and only one (5%) less than 25%.

The data clearly indicate that the separation chamber successfully captured mature stage infected cells. This conclusion is further supported by comparing images of a filtered sample, which contains almost exclusively rings, and a sample recovered from a separation chamber after an experiment (see Fig. 4.10). The initial and recovered samples clearly contain a significant number of infected cells, while the filtered sample contains almost exclusively ring-stage cells.

4.3.2.1 Relationship between separation efficiency and flow rate

Initially, there does not seem to be a significant link between the separation efficiency and the flow rate. This is particularly emphasised by Fig. 4.11. In this graph, the data points are connected by lines as a visual aid only, as successive experiments are not related. However, connecting the data points for the initial parasitaemia, filtered parasitaemia and separation efficiency makes it easier to see that there is a more apparent link between the initial and final paraistaemia, while the separation efficiency appears unrelated to the other two data sets. There is not the anticipated decrease in separation

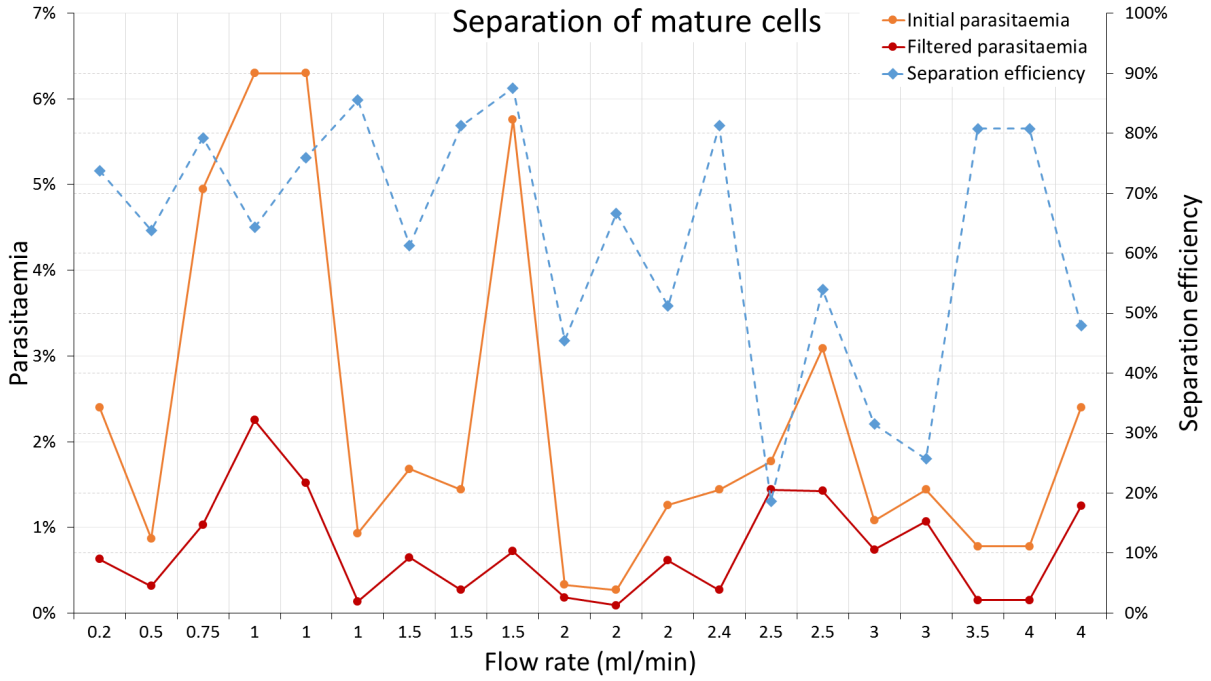


Figure 4.11: Initial and filtered parasitaemia and separation efficiency of mature stage infected erythrocytes

efficiency as the flow rate is increased. Two experiments in particular (at flow rates of 3.5ml/min and 4ml/min) show oddly high separation efficiencies (81%) at high flow rates. Experiments conducted at the same flow rates also do not show much consistency. The three experiments performed at 1ml/min showed efficiencies of 61%, 81% and 88%, while the three at 2ml/min showed efficiencies of 45%, 51% and 67%.

This suggests that either the role of the flow rate is less important in this regime than was anticipated, or there is another variable which affects the separation more than the flow. One important factor could be the variation between two different chambers. Since these were manufactured by hand, a degree of heterogeneity was inevitable. In particular, the mesh in some chambers could have accidentally been stacked in a way which allowed escape channels up the side of the chamber, leading to poor separation performance.

However, further analysis suggests that there is a link between the separation effi-

		Efficiency		
		High *(>60%)*	*Low* *(<60%)*	**Total**
Flow	*High* *(>2ml/min)*	20%	35%	55%
	Low *(<=2ml/min)*	45%	0%	45%
	Total	65%	35%	**100%**

Table 4.2: Contingency table showing relationship between flow rate and separation efficiency of mature stage cells

Flow rate (ml/min)	Average efficiency	Standard deviation
<1.5	73.7%	7.8%
1.5-2.4	67.8%	14.0%
>2.4	48.5%	23.3%

Table 4.3: Average separation efficiency of mature stage cells for different flow rate ranges

ciencies and the flow rate. Grouping the experiments into high and low flow rates and separation efficiencies shows that 80% of the experiments fall into the anticipated categories of high efficiency with low flow, and low efficiency with high flow (see Table 4.2). Furthermore, all of the remaining 20% fall into the high flow and high efficiency category, with none in the low flow and low efficiency. In other words, the experiments that had unexpected separation efficiencies had unexpectedly high ones - an encouraging result that suggests the flow rate could be pushed up further.

In fact, grouping the results into flow rate ranges - low, medium and high - and calculating the average separation efficiency of each group demonstrates that there does seem to be some correlation between flow rate and separation efficiency (see Table 4.3). The six experiments with flow rates below 1.5ml/min had average separation efficiencies of 74%, and were reasonably consistent with a standard deviation of only 8%. As the flow rate increases, both the average separation efficiency and the consistency of the results decrease, with the efficiency dropping to below 50% for experiments with flow rates 2.5ml/min and above, with a standard deviation of 23%.

4.3.2.2 Filtered parasitaemia

The parasitaemia of the filtered sample is arguably more important than the separation efficiency. After all, the goal of the device is to reduce a patient's parasitaemia as much as possible in as short a time as possible. Fig. 4.12 shows the percentage of experiments whose filtered parasitaemias were below given values. It shows that the parasitaemia of the filtered sample was reduced to less than 1% in 65% of cases, and less than 0.5% in 40% of experiments. It is worth noting that the 13 experiments with filtered parasitaemia less than 1% are not the same as the 13 with separation efficiencies over 60%.

It would be more valuable for the device to reduce the mature parasitaemia to below a critical value (e.g. 0.25%) regardless of the initial parasitaemia. For example, an initial sample with parasitaemia of 1% reduced to 0.25% has a separation efficiency of 75%, while a sample with initial parasitaemia of 5% that is reduced to 1% has an efficiency of 80%. Looking at the separation efficiency, the second experiment appears more successful, whereas in reality the final parasitaemia may have a more important impact in terms of patient benefit, so the first experiment could be considered more successful. This effect can be seen in the results. One experiment with one of the highest separation efficiencies at 76% had a final mature stage parasitaemia of 1.5%, with the high efficiency largely due to the high 6.3% initial parasitaemia. Meanwhile, one of the experiments with low separation efficiency at 45% had one of the lowest filtered parasitaemias at 0.2% - the low efficiency was due to the small initial parasitaemia of 0.3%.

Looking at Fig. 4.11, the final parasitaemia seems to bear no correlation with the flow rate, but does seem related to the initial parasitaemia. Indeed, the net difference between initial and final parasitaemia shows a strong correlation with the initial parasitaemia, as

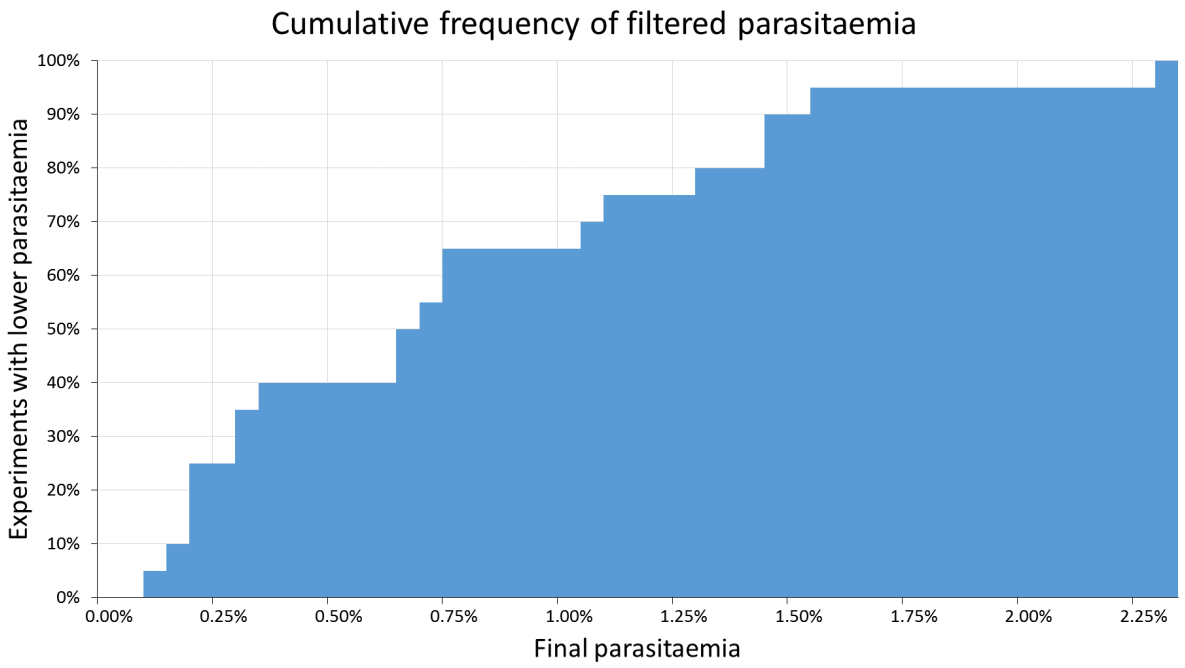


Figure 4.12: Cumulative frequency of experiments with filtered parasitaemia below given values

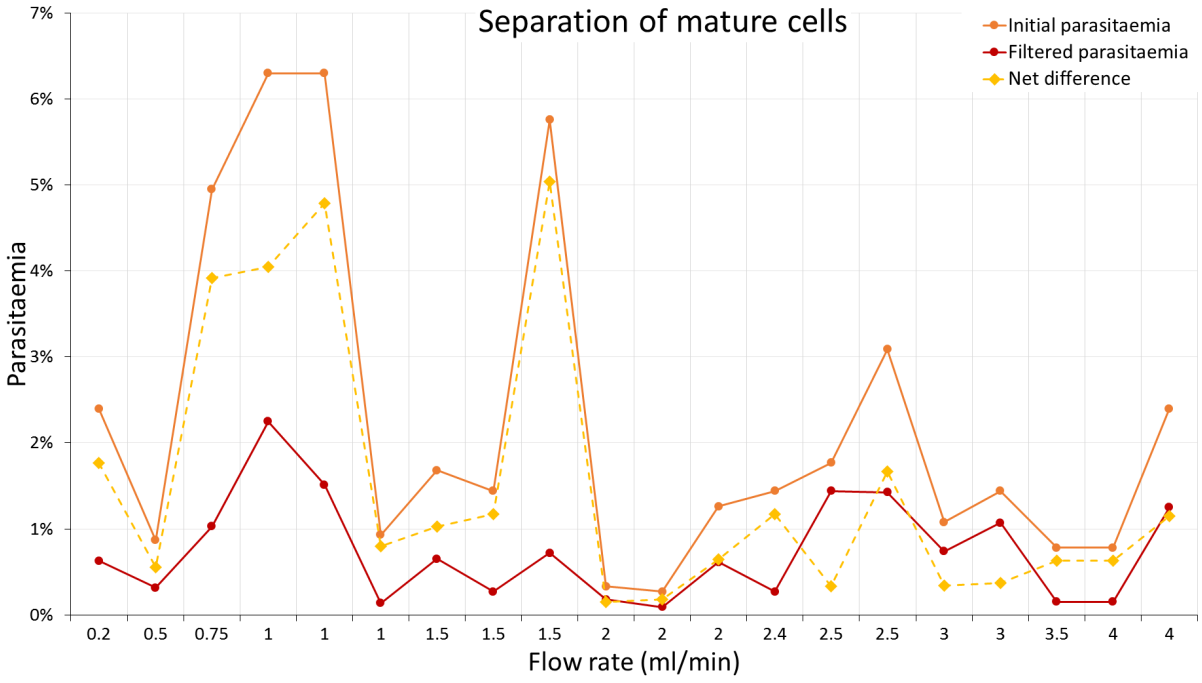


Figure 4.13: Net difference between initial and filtered mature stage parasitaemia at different flow rates

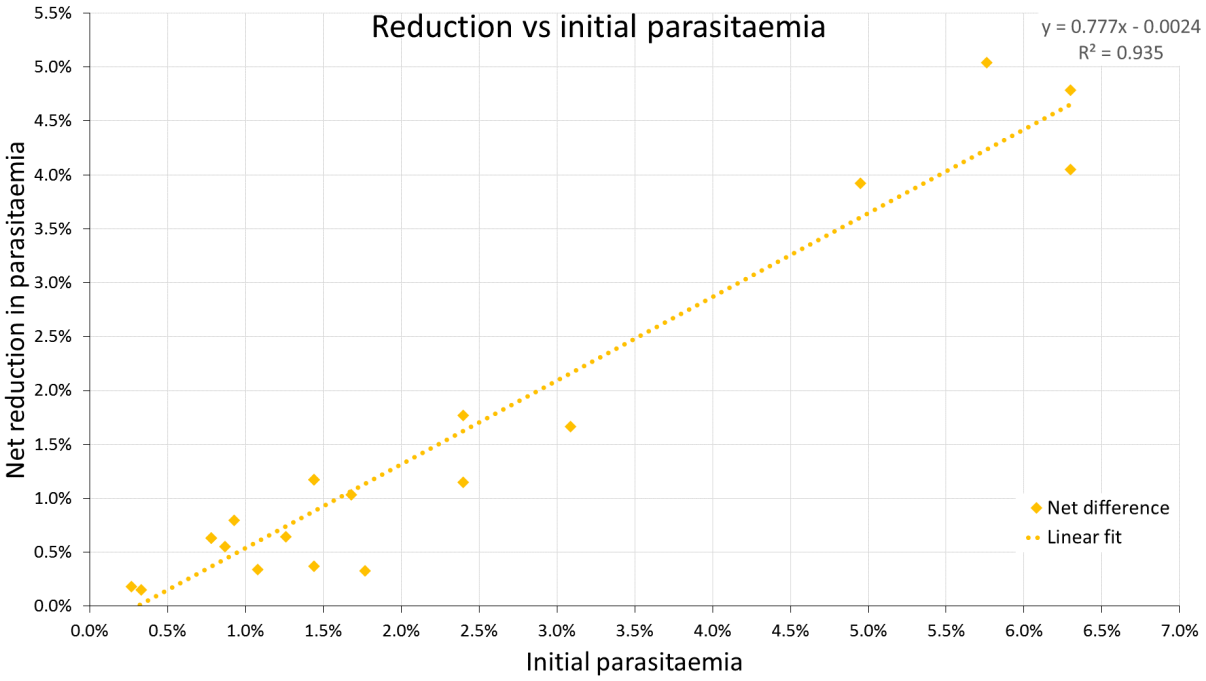


Figure 4.14: Net reduction in mature parasitaemia vs. initial parasitaemia

can be seen in Fig. 4.13. With the exception of one experiment at 2.5ml/min, both the net difference and the final parasitaemia appear to track the initial parasitaemia. Fig. 4.14 shows the relationship between the initial parasitaemia and the net reduction in parasitaemia for mature cells. The linear trend-line suggests a good correlation with a coefficient of determination of 0.935; with a gradient of 0.78, it suggests that the device removes 78% of the infected cells.

4.3.2.3 Conclusion

While this data certainly suggests a significant correlation between the initial parasitaemia and its reduction, it is only one factor affecting the performance of the device and the linear trend-line should not be taken as a rule. Given the two conclusions that the flow rate affects the separation efficiency, and the initial parasitaemia affects the filtered parasitaemia, it could be assumed that the relationship between the initial and filtered

parasitaemias would be linear for a given flow rate. Alternatively, for a given initial parasitaemia, one could expect a strong correlation between the separation efficiency and the flow rate.

The device is certainly able to capture a significant proportion of the mature trophozoites and schizonts that pass through it, but the data clearly indicates that both the initial parasitaemia and the flow rate are important factors in determining the success of the filtration. In a clinical environment, the device's ability to reduce a patient's parasite burden - and the time it takes to do so - will depend primarily on these two factors. Initial parasitaemia will vary considerably and cannot be controlled. Testing of the clinical version described in section 3.2.4 should therefore focus on flow rate optimisation, bearing in mind that the optimal value may depend on the patient's current parasitaemia, and the best scenario may therefore be to vary the flow rate as the treatment progresses and the patient's parasitaemia is reduced.

4.4 Results - donated *P. vivax*

4.4.1 Reduction in parasitaemia

Through the collaboration with the Universidad de Antioquia in Colombia, it was possible to obtain samples of blood from six patients infected with *P. vivax*, as described in section 4.1.5. The intention was to check whether the device successfully extracted infected cells from the blood of patients, confirming that the results reported in section 4.3 were repeatable when (1) the infected cells were in whole blood, rather than just medium, and (2) with *P. vivax* as well as *P. falciparum*.

Patient number	Flow rate (ml/min)	Initial parasitaemia				Filtered parasitaemia				Separation efficiency			
		Para/ µL	*Rings*	*Mature*	*Total*	*Para/ µL*	*Rings*	*Mature*	*Total*	*Para/ µL*	*Rings*	*Mature*	*Total*
1	1.5	7040	0.12%	0.21%	**0.33%**	Unknown	0.09%	0.21%	**0.30%**	N/A	25.00%	0.00%	**9.09%**
2*	1.5	1225	Unknown	Unknown	**0.03%**	Unknown	0.03%	0.04%	**0.07%**	N/A	N/A	N/A	**-128.57%**
3	2.0	Unknown	0.00%	0.30%	**0.30%**	Unknown	0.00%	0.00%	**0.00%**	N/A	N/A	100.00%	**100.00%**
4	1.0	Unknown	0.00%	0.06%	**0.06%**	Unknown	0.00%	0.03%	**0.03%**	N/A	N/A	50.00%	**50.00%**
5	1.0	9615	0.02%	0.16%	**0.18%**	6040	Unknown	Unknown	**0.09%**	37.18%	N/A	N/A	**50.00%**
6	1.0	2000	0.01%	0.05%	**0.06%**	520	Unknown	Unknown	**0.01%**	74.00%	N/A	N/A	**83.33%**

*Patient 2: Giemsa staining failed. % parasitaemia estimated from number of parasites per µL

Table 4.4: Initial parasitaemia, filtered parasitaemia and resultant separation efficiency of donated *P. vivax* samples

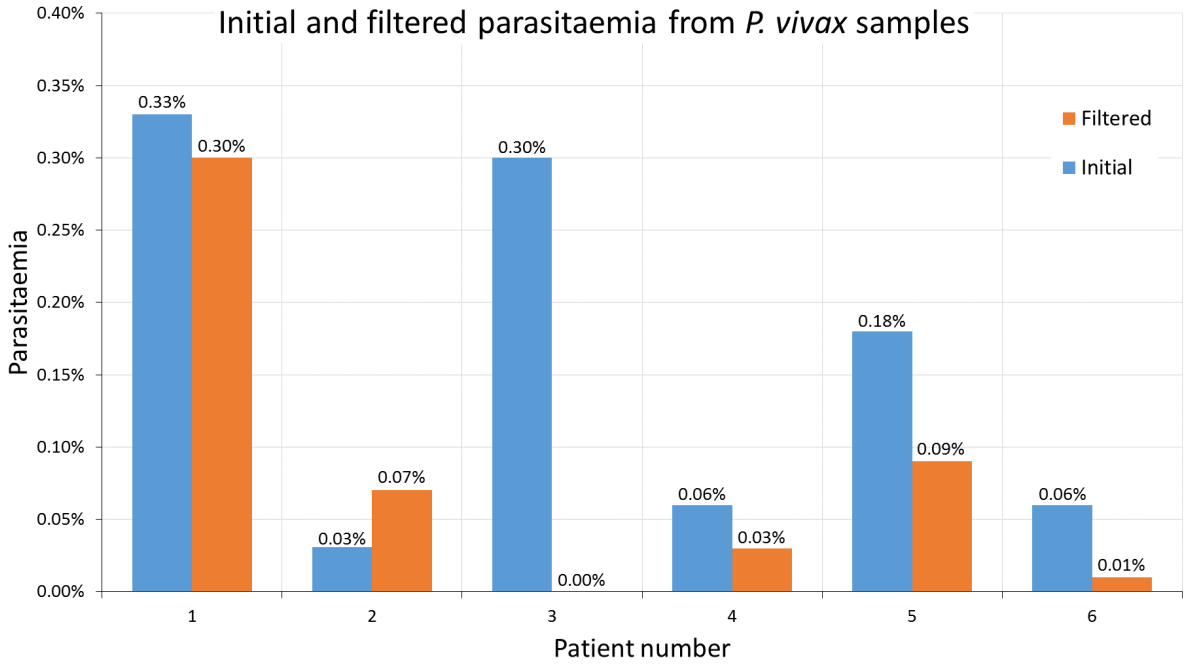


Figure 4.15: Initial and filtered parasitaemia for samples donated by *P. vivax* patients

The blood samples were flowed through the haemofilter using the method described in section 4.1 and the same apparatus as the *P. falciparum* experiments from section 4.3. The change in parasitaemia resulting from a single pass of the samples through the device are preseted in Table 4.4 and Fig. 4.15. All of the experiments were conducted at room temperature, although the temperature of the samples was not monitored. For patients 1-4, the samples were collected in El Bagre and shipped on ice to Medellin, where the experiments were performed in the laboratories of the Grupo Malaria at the Universidad de Antioquia. For patients 5 and 6, however, the experiments were performed on site at the hospital in El Bagre by Briegel de las Salas from the Grupo Malaria. The temperatures of the samples for the first four patients may therefore have been different than those of patients 5 and 6.

Unfortunately, the patient's parasitaemias were too low to draw a significant conclusion from the results - initial parasitaemia ranged from 0.03% to 0.30%, most of which

was constituted of ring-stage cells. Low parasitaemias are common in *P. vivax* patients, particularly in uncomplicated cases, so it was unsurprising that it was not possible to obtain donated samples with high levels of infected cells, particularly considering the fact that "uncomplicated malaria" was one of the inclusion criteria for volunteers.

The measured values were all close to or lower than the margin of error of the Giemsa staining method of establishing the parasitaemia, preventing a meaningful conclusion from being drawn. Ideally, the parasitaemia of these samples would have been checked using a highly sensitive technique such as RT-PCR, but no such equipment was available. However, the results do indicate that infected cells are captured, with a decrease in parasitaemia observed in all experiments except patient 2.

In this case, an increase in parasitaemia was observed, but this result is particularly unreliable, firstly because of the very low initial parasitaemia, and secondly because the Giemsa staining of the microscope slide containing the initial sample was unsuccessful. The sample underwent almost complete haemolysis and it was not possible to read any data from the slide. As a result, it was not possible to establish the initial parasitaemia for patient 2 in the same manner as the other samples. While the number of parasites per microlitre was established when the sample was taken from the patient, this was not established for the filtered sample.

4.4.2 Preliminary safety tests

	Patient 1 (1.5ml/min)			Patient 2 (2.5ml/min)			Patient 3 (2ml/min)			Patient 4 (1ml/min)			Patient 5 (1ml/min)			Patient 6 (1ml/min)		
	Before	*After*	*Diff.*	*Before*	*After*	*Diff.*	*Before*	*After*	*Diff.*	*Before*	*After*	*Diff.*	*Before*	*After*	*Diff.*	*Before*	*After*	*Diff.*
WBC (10^3/µl)	7.2	6.8	-0.4	5	5.2	0.2	8.1	8.1	0	5.6	4.4	-1.2	5.9	4.91	-0.99	6.04	4.75	-1.29
Neutrophils	64.5%	61.6%	-2.9%	84.0%	77.8%	-6.2%	45.7%	45.2%	-0.5%	78.5%	78.8%	0.3%	-	-	-	-	-	-
Lymphocytes	19.7%	25.5%	5.8%	9.8%	11.1%	1.3%	29.0%	30.5%	1.5%	19.3%	18.4%	-0.9%	38.6%	40.9%	2.3%	11.1%	24.0%	12.9%
Monocytes	11.3%	10.9%	-0.4%	5.8%	10.0%	4.2%	20.9%	19.8%	-1.1%	0.7%	1.5%	0.8%	-	-	-	-	-	-
RBC (10^6/µl)	5.67	5.27	-0.4	4.22	3.86	-0.36	4.34	4.36	0.02	4.38	4.53	0.15	4.96	4.19	-0.77	4.38	4.58	0.2
Haemoglobin (g/dl)	16.4	15.4	-1	12.1	11.2	-0.9	10.8	10.9	0.1	12.1	12.4	0.3	14.4	12.5	-1.9	13	13.6	0.6
Haematocrit	49.4%	45.3%	-4.1%	36.1%	33.1%	-3.0%	32.6%	33.1%	0.5%	37.2%	38.8%	1.6%	41.7%	35.4%	-6.4%	37.1%	38.9%	1.8%
PLT (10^3/µl)	81	92	11	36	43	7	135	138	3	72	64	-8	73	50	-23	98	57	-41

Table 4.5: Summarised complete blood count results for *P. vivax* patient's blood flowed through the magnetic haemofilter

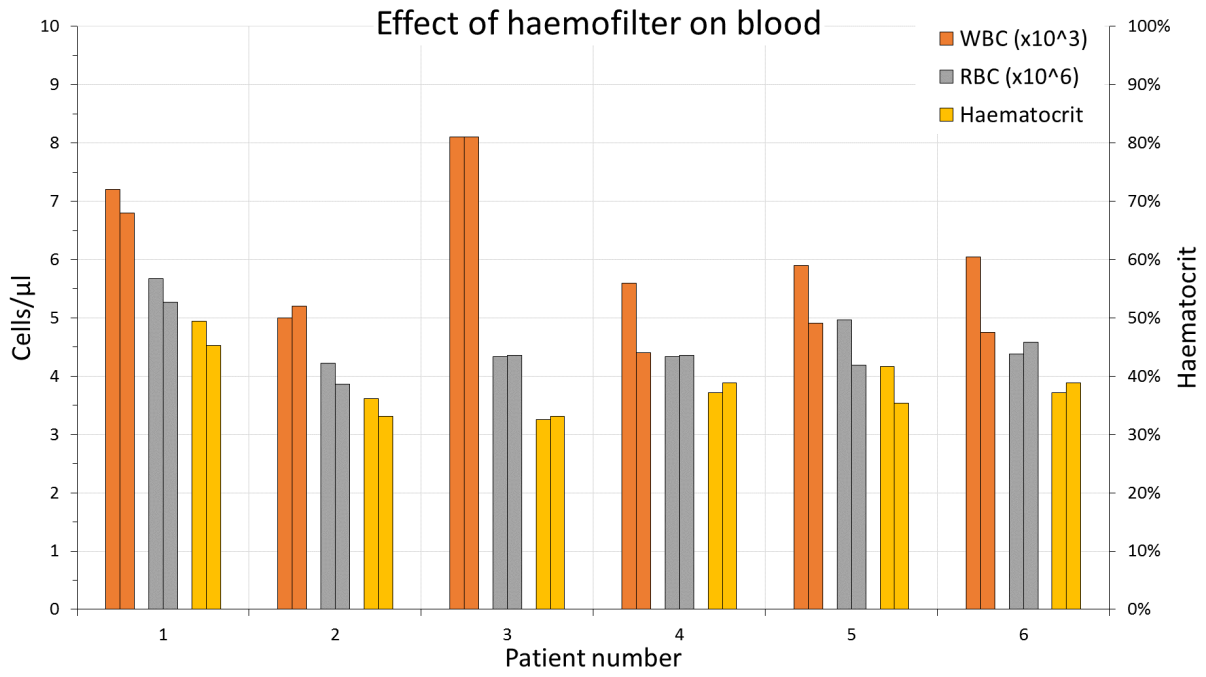


Figure 4.16: Complete blood count results (white blood cells, red blood cells and haematocrit) for *P. vivax* patients' blood before and after being passed through the haemofilter

The samples did, however, allow for testing of whether the haemofilter causes any harm to other blood components. Complete blood counts of the blood samples before and after filtration through the device were performed; the results can be seen in Table 4.5 and Fig. 4.16; the complete, raw results can be seen in Appendix A. In addition to the complete blood counts, all of the samples were observed using light microscopy to check for any cell lysis or other observable damage.

The most significant variations were observed in the RBC counts. These may be expected to decrease slightly, due to the capture of infected erythrocytes, but this effect should be very small owing to the very low parasitaemias present in the sample. Some patients saw significant RBC decreases much higher than would be expected; there was no apparent link between the samples which saw a decrease and either the flow rate or the temperature (i.e. patients 1-4 vs. patients 5 and 6). It is also surprising that

some patients saw increases in their RBC counts, and these remain unexplained. One possible explanation is error or inaccuracy of the full blood count tests, although this seems unlikely to account for changes in the values of up to 15% (in patient 5). It is possible that there is some false positive capture of RBC for some patients, which could explain the large decreases, and this will need to be further investigated.

At the slowest flow rate of 1ml/min, there is a slight but repeated reduction in white blood cells. This is likely due to slight sedimentation of these heavier cells - indeed, in the experiments with cultured *P. falciparum*, significant sedimentation of the cells in medium was observed at the lowest flow rates, so it is natural to assume some effect with the white blood cells. This could be reversed by having the separation chamber inlet at the top and outlet at the bottom.

While little difference between before and after filtration was observed, many of the samples exhibited the signs of infection that would be expected, including in particular low platelet and high neutrophil counts; the former was observed in all patients (range: 36-$138\times10^3/\mu l$). Importantly, the haemoglobin did not decrease significantly or consistently, indicating that there is, as expected, no capture of haemoglobin. None of the samples showed any visual signs of cell lysis when observed under a microscope.

Significant variations between the initial and filtered samples were observed in the white blood cell population proportions. For example, Patients 1 and 6 saw a significant increase in their proportion of lymphocytes, from 20% to 25% and from 11% to 24% respectively. Patient 1, the only patient with a significant initial basophil proportion, also saw a huge decrease in basophils, from 2.3% to 0.2%. Patient 2 meanwhile saw a significant increase in monocytes, from 6% to 10%. As isolated cases, these are not of

	Patient 2 (2.5ml/min)		
	Initial	*Filtered*	*Recovered*
WBC (10^3/ul)	5.0	5.2	3.4
Neutrophils	84.0%	77.8%	80.7%
Lymphocytes	9.8%	11.1%	13.1%
Monocytes	5.8%	10.0%	5.2%
RBC (10^6/ul)	4.22	3.86	6.83
Haemoglobin (g/dl)	12.1	11.2	19
Haematocrit	36.1%	33.1%	58.1%
PLT (10^3/ul)	36	43	14

Table 4.6: Complete blood count results for Patient 2, showing the difference between before filtration, after filtration, and the blood captured in the haemofilter

significant concern, but should be monitored during future development of the device.

In addition to the initial and filtered samples, the blood that was captured within the device was analysed for Patient 2 - these results can be seen in Table 4.6. While the filtered sample is not significantly different from the initial sample (bar the increase in monocyte proportion, mentioned above), the sample captured in the chamber has a significantly higher red blood cell count, haemoglobin and haematocrit, with significantly lower WBC and platelet counts. This indicates a purification of red blood cells, which may be indicative of false positive capture of RBC. The higher haemoglobin content may be due to the capture of deoxygenated haemoglobin, which is slightly paramagnetic (see section 1.2.8).

4.5 Error analysis

4.5.1 Giemsa staining uncertainties

The Giemsa staining method is a rapid and established method of diagnosing malaria and establishing its severity, but its reliability has been questioned[227]. However, it has lim-

itations in terms of accurately quantifying parasitaemia for the purposes of this project, in which we are investigating potentially small changes in the parasitaemia. It is crucial, for example, for the microscopist to choose a field of view with a good distribution, corresponding to 303 cells per view, and this is typically only estimated rapidly. Furthermore, small parasitaemias (i.e. $<1\%$) correspond to a very small number of counted infected cells - if one infected cell per viewing field is counted, that corresponds to a parasitaemia of 0.33% - presenting increased uncertainty, particularly for the filtered samples in which the parasitaemia is predicted to be reasonably low.

We can demonstrate these inaccuracies with two hypothetical samples. The first microscopist chooses an area of the slide with an average of 321 cells per field of view. They count a total of 30 cells, of which 7 are schizonts. The calculated total parasitaemia is $30 \times 3/100 = 0.90\%$ and the schizont parasitaemia is $7 \times 3/100 = 0.21\%$. Due to the number of healthy cells being 6% higher than is assumed when using eq. 4.1 ($321/303 = 1.06$), the 'real' parasitaemias should be total infected cells divided by total cells, times 100. This gives a total parasitaemia of $(30 \times 100)/(321 \times 11) = 0.85\%$ and a 'real' schizont parasitaemia of $(7 \times 100)/(321 \times 11) = 0.20\%$, 5.5% and 4.8% lower than the calculated values respectively.

The second microscopist chooses an area with an average 285 cells per field of view (6% lower than the assumed 303). They also count a total of 30 cells with 7 schizonts. The results are identical with a total parasitaemia of 0.90% and schizont parasitaemia of 0.21%. However, the 'real' parasitaemia here should be $(30 \times 100)/(285 \times 11) = 0.97\%$ and 'real' schizont parasitaemia $(7 \times 100)/(285 \times 11) = 0.22\%$.

In this scenario, we have two samples which appear identical in terms of the calculated

parasitaemias. However, the 6% error each side in the estimation of the cell distribution, which is imperceptible to the microscopist, indicates that there is actually a 14% difference in the total parasitaemia - 0.85% compared to 0.97% - and a 10% difference in the schizont parasitaemia - 20% compared to 22%.

We can use these numbers to indicate the effect that the uncertainties have on the separation efficiency (i.e. percentage of infected removed by the device). Assuming the calculated initial schizont parasitaemia was 0.90%, and the final parasitaemia was 0.21%, we calculate that 76.7% of the schizonts were removed. However, using the 'real' parasitaemias from above, we could actually have an initial parasitaemia of 0.85% and a final parasitaemia of 0.22% a separation efficiency of 74%. We could also have an initial parasitaemia of 1.06% and final parasitaemia of 0.20%, a separation efficiency of 81%.

This hypothetical scenario is conservative in terms of the potential error as it considers only the uncertainty in the average number of cells per field of view, ignoring other contributors to error, particularly the observed number of infected cells - the same microscopist can look at the same sample twice and count only 5 schizonts the first time, and 12 the second. Clearly, this can have a significant effect on the results, particularly when looking at separation efficiency or dealing with low parasitaemias. Based on these considerations, it is reasonable to assume that the error in establishing parasitaemia using the Giemsa staining method could be as high as 10%.

4.5.2 Confidence of result

In order to confirm the validity of the results from section 4.3, a paired student t-test was performed on the mature cell results. The null-hypothesis for this analysis was "the

Mean difference	1.64
Degrees of freedom	19
T-value	4.62
P-value	0.0002
Correlation	0.76
Correlation Probability	<0.0001

Table 4.7: Results from paired t-test

magnetic haemofilter has no effect on the mature cell parasitaemia", or in other words there is no difference between the parasitaemia before and after filtration across all 20 experiments. The data sets compared were the 20 'initial' and 'filtered' parasitaemia of mature infected erythrocytes, shown above in Table 4.1. The analysis was performed using KaleidaGraph 4.5 (Synergy Software, USA). The results from the analysis can be seen in Table 4.7.

The results demonstrate that the haemofilter was successful in reducing parasitaemia with a very high confidence (p=0.0002). The correlation between the initial and filtered samples was calcuated to be 0.76, also with very high confidence. This is very similar to the average separation efficiency of 78%, found by comparing the initial parasitaemia to the final parasitaemia, plotting a linear trendline and calculating the gradient (see Fig. 4.14) here the confidence was high too, with an R^2 value of 0.935.

The high confidence of the conclusion is not surprising. Even allowing for a pessimistic error of 10% in the parasitaemias, all of the experiments still showed a reduction in parasitaemia, with only two experiments showing a 'minimum' reduction in parasitaemia of less than 10%, and only three less than 30% (see Table 4.8). Meanwhile, the 'maximum' parasitaemia reductions were as high as 90%.

Expt. #	Flow rate	Initial parasitaemia			Filtered parasitaemia			Separation efficiency		
		Min	*Observed*	*Max*	*Min*	*Observed*	*Max*	*Min*	*Observed*	*Max*
1	0.2	2.2%	2.4%	2.6%	0.6%	0.6%	0.7%	68%	74%	79%
2	0.5	0.8%	0.9%	1.0%	0.3%	0.3%	0.3%	56%	64%	70%
3	0.75	4.5%	5.0%	5.4%	0.9%	1.0%	1.1%	75%	79%	83%
4	1	5.7%	6.3%	6.9%	2.0%	2.3%	2.5%	56%	64%	71%
5	1	5.7%	6.3%	6.9%	1.4%	1.5%	1.7%	71%	76%	80%
6	1	0.8%	0.9%	1.0%	0.1%	0.1%	0.1%	82%	85%	88%
7	1.5	1.5%	1.7%	1.8%	0.6%	0.7%	0.7%	53%	61%	68%
8	1.5	1.3%	1.4%	1.6%	0.2%	0.3%	0.3%	77%	81%	85%
9	1.5	5.2%	5.8%	6.3%	0.6%	0.7%	0.8%	85%	88%	90%
10	2	0.3%	0.3%	0.4%	0.2%	0.2%	0.2%	33%	45%	55%
11	2	0.2%	0.3%	0.3%	0.1%	0.1%	0.1%	59%	67%	73%
12	2	1.1%	1.3%	1.4%	0.6%	0.6%	0.7%	40%	51%	60%
13	2.4	1.3%	1.4%	1.6%	0.2%	0.3%	0.3%	77%	81%	85%
14	2.5	1.6%	1.8%	1.9%	1.3%	1.4%	1.6%	1%	19%	33%
15	2.5	2.8%	3.1%	3.4%	1.3%	1.4%	1.6%	44%	54%	62%
16	3	1.0%	1.1%	1.2%	0.7%	0.7%	0.8%	16%	31%	44%
17	3	1.3%	1.4%	1.6%	1.0%	1.1%	1.2%	9%	26%	39%
18	3.5	0.7%	0.8%	0.9%	0.1%	0.2%	0.2%	76%	81%	84%
19	4	0.7%	0.8%	0.9%	0.1%	0.2%	0.2%	76%	81%	84%
20	4	2.2%	2.4%	2.6%	1.1%	1.3%	1.4%	36%	48%	57%

Table 4.8: Minimum and maximum mature cell parasitaemia of initial and filtered samples, allowing for a 10% error, and resulting minimum and maximum separation efficiencies

4.6 Conclusions

The experiments evaluating the haemofilter indicate firstly that the magnetic haemofilter is able to separate malaria-infected erythrocytes from uninfected cells. The results detailed in section 4.3 are very promising in that that was a reduction in the parasitaemia of trophozoites and schizonts in every experiment and at all flow rates. Considering the uncertainties in establishing the parasitaemia using the Giemsa staining method, the maximum expected change in the calculated separation efficiencies would be less than 10%, while the experiment with the smallest reduction in parasitaemia was 19%. In other words, the worst performing experiment still showed a reduction in parasitaemia. Looking at Fig. 4.9, this experiment could even be considered an outlier, most likely due to poorly stacked mesh within the separation chamber.

Some experiments did show a reduction in ring-stage parasitaemia, including one particularly significant reduction from 4.8% to 2.2%. This was unexpected - theoretically, ring-stage cells do not contain the paramagnetic haemozoin required to give the cells a magnetic susceptibility and enable their capture. However, it can be difficult to differentiate between early and late ring-stages, and the latter can contain significant quantities of haemozoin (see Fig. 1.5 and section 1.2.8). Some experiments may have contained a large quantity of late ring-stage parasites containing sufficient haemozoin to be captured, particularly at low flow rates. Other experiments, meanwhile, may have contained mostly early ring-stage cells, explaining the lack of capture. The experiments in which small increases were observed are most likely due to uncertainties in parasitaemia quantification; the only other possible explanation would be a false capture of healthy RBC, but there was no data to indicate this was occuring.

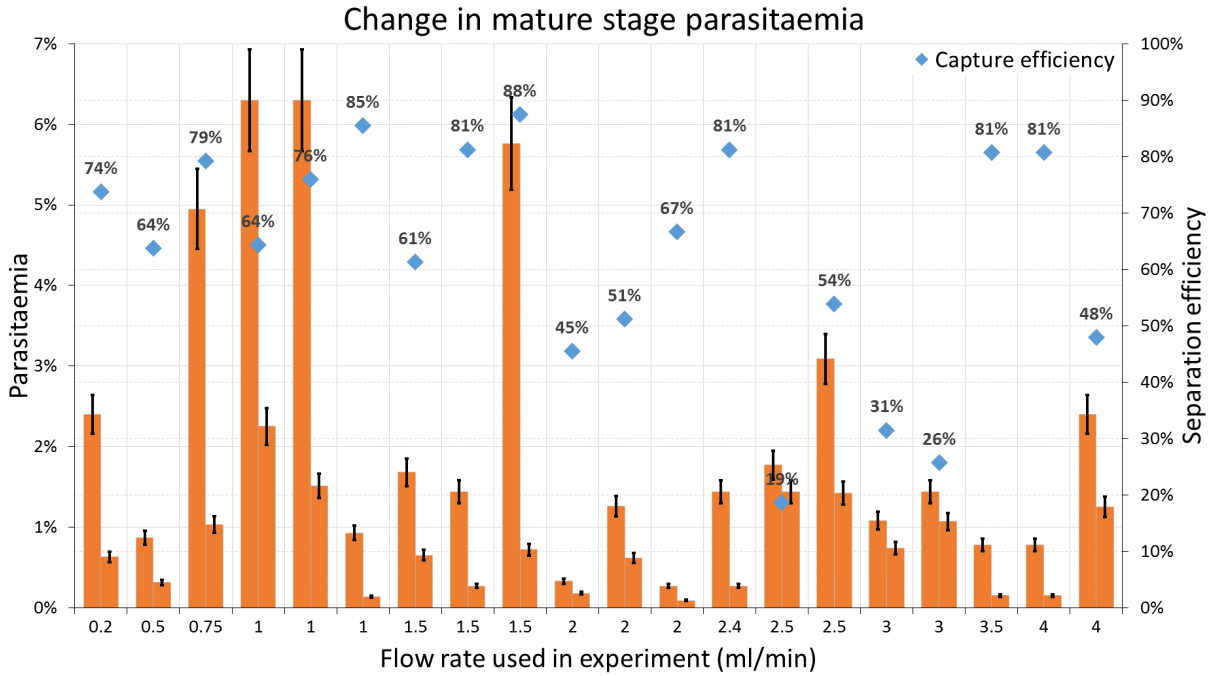


Figure 4.17: Mature stage parasitaemia of samples before and after filtration, including error bars assuming an error of 10% in estimating the parasitaemia using the Giemsa staining method, and separation efficiency of mature infected cells

The separation efficiencies achieved were reasonably high, although did not meet the hoped for target of 90%. However, allowing for a 10% error in the parasitaemia, some of the separation efficiencies could approach 100% (see Fig. 4.17). Initially, there was also no apparent link between the separation efficiency and the flow rate within the values used, contrary to what was expected. However, simplifying the data by dividing it into low and high flow rate and separation efficiencies indicates that there is a link between the two, with 80% of the experiments falling into the expected categories (low flow and high efficiency, or high flow and low efficiency). The 20% that didn't had high flow and high efficiency; in other words, when the experiments did not perform as expected, it was in a positive way.

Because new separation chambers were used for each experiment, to prevent cross-contamination, it is possible that the variation in separation effieciency is due to some

chambers being manufactured to a higher standard than others. The insertion of the mesh was done by hand, and small gaps could easily allow for the formation of escape channels - paths through which cells can pass without being captured. If the experiments which showed significantly lower flow rates than others performed at the same flow rate are removed from Table 4.8, namely experiments 10, 14, 16 and 17, the average efficiency rises to 71%±6%.

It was not possible to reliably conclude that the device successfully reduced the parasitaemia of donated *P. vivax* samples after a single pass, due to the very low initial parasitaemias of the samples. Furthermore, while there was an apparent reduction in parasitaemia in five out of the six experiments, there are insufficient data points for a conclusion to be made with any confidence. That being said, the reduction seen in most experiments (and the fact that the only case in which a reduction was not observed was when the data for the initial sample was particularly low and unreliable due to unsuccessful Giemsa staining) is indicative that the device might work on whole blood samples as well as on cultured samples.

The experiments using blood samples donated by *P. vivax* patients, combined with light microscopy observations of the samples, indicated that the device was not causing any harm to blood cells - there were no signs of significant haemolysis. However, there were some significant changes in the cell counts which may be indicative of false positive capture of uninfected red blood cells. The very slight reduction in WBC seen at lower flow rates was almost certainly down to sedimentation as the direction of flow was from the bottom of the chamber to the top. The full blood counts should be taken as preliminary safety data which are positive in the sense that there does not seem to be any drastic

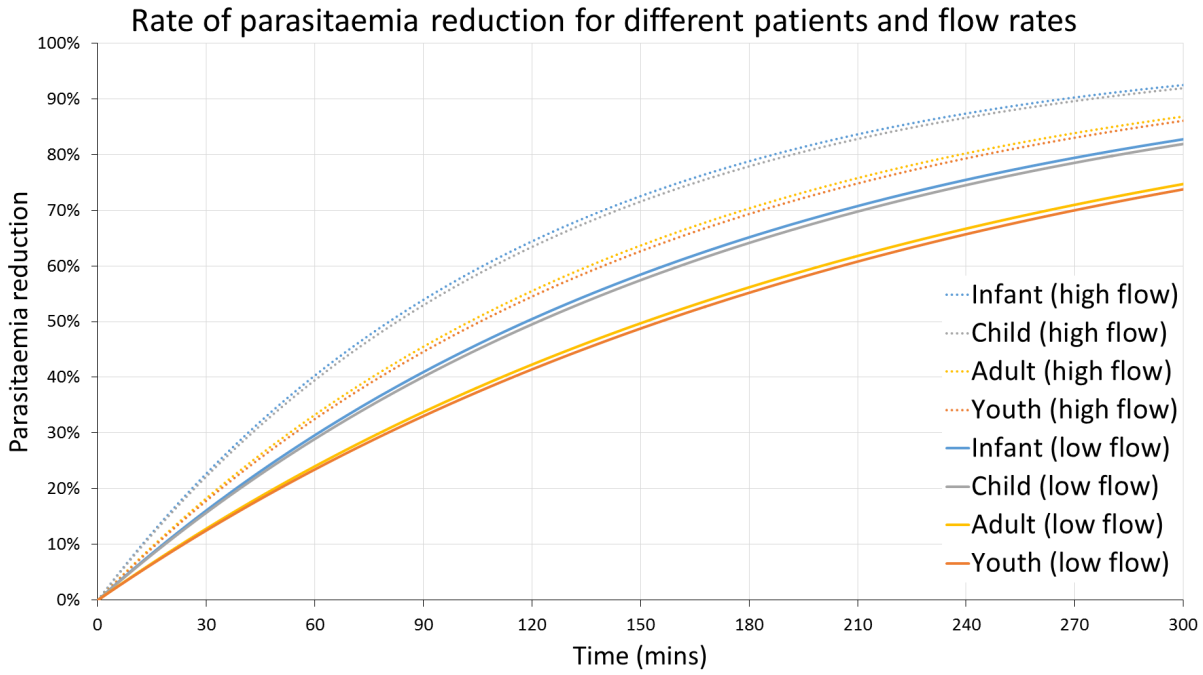


Figure 4.18: Theoretical parasitaemia reduction for four model patients with high flow rate (3ml/min; separation efficiency 53%; dotted lines) and low flow rate (1.5ml/min; separation efficiency 72%; solid lines)

effect caused by the device, but insufficient to conclude that the device as it is currenty designed is safe for use in humans.

A further limitation to the *P. vivax* experiments is that the temperature of the samples was not monitored during the experiments. Ideally experiments looking at the performance and safety of the device should be performed at body temperature, since the rheology of the blood and function of certain cells varies with temperature (see section 4.1.5). This fact contributes further to the conclusion that the safety data collected is only very preliminary and more experiments are required before any meaninful conclusion as to the safety of the haemofilter can be made.

4.6.1 Flow rates

The data did not provide a conclusive link between the flow rate and the separation efficiency, although experiments performed at lower flow rates were more successful on average. This could be due to outliers, or simply because the flow rates used were not within a range in which the variations had an observable effect. However, in order to calculate estimated treatment times based on this data, average separation efficiencies at 'low' and 'high' flow rates can be taken. Eliminating the lowest and highest separation efficiencies for each group, the averages were:

- Low flow rate (1.5ml/min): 72%
- High flow rate (3ml/min): 53%

Fig. 4.18 shows the theoretical parasitaemia reduction rates that could be achieved in four different model patients using these flow rates and separation efficiencies in the clinical versions of the device described in section 3.2.4. It shows that, despite the lower efficiency, the higher flow rate yields better parasite reduction with faster treatment times. With the results achieved in these experiments, a child's or infant's parasitaemia level could be reduced by 50% in only 80 minutes. A 90% reduction would be achievable in 4.5 hours. For youths and adults, the treatment times are longer because of higher TBV - 1 hour 45 mins for a 50% reduction, and over five hours for 90%.

These results demonstrate that the device's performance is reasonably close to what would be clinically acceptable. While a reduction in treatment time (i.e. faster reduction of parasitaemia) would be desirable, the ability to halve a child's parasitaemia in less than an hour and a half could already provide significant clinical benefits.

4.6.2 Summary

The results were very positive and certainly indicate that the device as designed could be a positive medical device, achieving material parasite reduction in acceptable times. Indeed, as initial results, they are particularly promising - there is still scope for optimisation in terms of design which could improve separation efficiencies and allow for higher flow rates. The ferromagnetic mesh within the chamber, for example, has not been investigated, and different gauges or materials could provide improved performance.

The full blood count results were also good, indicating that the haemofiltration process does not cause any harm to cells through shear or stress. However, the possibility of accidental capture of both RBC and WBC by the device needs to be studied further. Further toxicology studies and blood analyses could be performed to investigate any long term toxicity, particularly of the clinical versions of the device. The potential false capture of other blood components such as trace metals, though unlikely, should also be investigated.

Further investigation into the device's performance at higher flow rates and, importantly, on patient samples are required. The effect of the flow rate on the separation efficiency has not be quantified, and is crucial to the optimisation of the haemofilter's performance. Tests on patient blood are important to see how the results are affected by filtering whole blood rather than cultured samples. Results are likely to be less good, due to the higher viscosity of whole blood when compared to RPMI medium.

As discussed in section 3.6, anticoagulant will need to be added to the system during each procedure with heparin the most likely candidate. In these experiments, both Ethylene Diamine Tetra Acetic Acid (EDTA) and heparin were used as anticoagulants, and the

dosages used were determined by the fact that the samples needed to be shipped from El Bagre to Medellin, rather than to be representative of the dosage that would be used when treating a human patient. As such, the effective use of anti-coagulant and risk of clotting, particularly within the separation chamber, will need to be carefully investigated when developing the medical device.

Finally, in order to justify the potential applications detailed in Chapter 2, the ability of the haeomfilter to separate MNP and magnetically labelled cells and other agents from whole blood would need to be demonstrated with similar experiments to those presented in this Chapter. This would justify the argument that the device is a platform, easily adaptable to any new application.

Chapter 5

Commercial Evaluation of Device

5.1 Introduction

In this Chapter, the commercial evaluation that has been undertaken is outlined. The aim was to establish whether commercialisation of the haemofilter as a medical device to treat malaria is commercially viable, addressing the following questions:

1. Does the haemofilter meet an unmet need?
2. Can it be profitable?
3. What is the size of the market?
4. What is the go-to-market strategy?
5. How much investment is required to get to market?

Each of these questions has a number of sub-questions, and to answer these both primary and secondary research was conducted. The malaria treatment market and industry were analysed, mainly through a literary review, and experts were consulted, including malaria

clinicians and researchers, medical device entrepreneurs, nephrologists, business and start-up experts, medical device manufacturers and industry regulators, amongst others.

The method devised to ascertain whether the project is commercially viable was:

1. Establish where the device fits into the current treatment pathway. Discuss with clinicians how and when they would use the treatment, and which patients stand to benefit most. From their answers, identify the best niche in which to start using the device, considering the best target market to drive rapid adoption.

2. Identify the first country in which the device would be sold. Analyse data of the number of patients in that country who fit into the identified niche, where they go to get treatment, and how much it currently costs the customer (i.e. patient or health service).

3. Look at whether the device could provide an economic benefit as well as a clinical benefit, by reducing inpatient time for example, and estimate the cost per death averted using the device. This provides an estimate for how much the customer would be willing to pay for the treatment.

4. Estimate the cost of a single treatment to the patient or health service, and compare it to what they currently pay. The main task here is to estimate how much a single set of consumables would cost, once the manufacturing and distributing processes are established.

5. Combine the results from 2 and 3 to establish whether or not the company could make a margin on each treatment, and if so how large, and as a result how big the total addressable market is.

6. Create a go-to-market strategy to reach the customer niche, detailing what is required to make the first commercial sale (e.g. regulatory approval), and outlining any specific device capabilities or specifications (e.g. able to treat both adults and children).

7. Construct a financial plan, estimating the total costs of getting to market, the time it will take, and the total investment required to get there. Identify potential funding sources and put together a timeline including both development and investment strategy.

Finally, successful commercialisation of medical devices often depends largely on the recommendations of respected members of the healthcare community. During this process, profiles were made of the individuals who will be essential to the project's success. These include members of the board or team of the potential commercialising vehicle, as well as key opinions leaders in the principal target markets, who will need to be champions of the technology and enhance its credibility.

5.2 The clinical need

In order to establish how the haemofilter would fit into the current treatment pathway, malaria experts, both clinicians and researchers, were asked what effects they thought the haemofilter might have on a patient, whether used in isolation or in combination with existing therapies. Based on these discussions, patients who stood to benefit from the treatment were broken down into three categories - untreatable, hospitalised severe and non-severe - with different value propositions for each group.

5.2.1 Untreatable patients

Patients in this group are defined as those for whom there is currently no effective pharmaceutical treatment. For these patients, the haemofilter could be used independently of drugs, with the goal of reducing and maintaining the parasitaemia at a low level so as to prevent the manifestation of symptoms. As the device does not remove early ring-stage infected erythrocytes (see section 4.3.1), two treatments would be required initially, performed 18-36 hours apart, at which point the infected cells that escape capture during the initial treatment have matured into trophozoites and schizonts, but have not yet ruptured to release merozoites. The patient could then be discharged from hospital, but should be regularly monitored, and possibly still given a course of anti-malarial tablets.

Ideally, a second round of treatments would take place when the parasitaemia rises above a pre-defined critical value and before symptoms reoccur. Realistically however, it is likely that patients would only return to be treated once symptoms manifest again, and constant monitoring of the parasitaemia would be economically and logistically challenging. Trials would need to be conducted to ascertain the average time between first treatment and patient relapse, which could vary significantly for different patients and malaria species. The ideal scenario would be to keep a patient alive and symptom free indefinitely with regular treatments. Eventually, particularly for young children, it is possible that the controlled presence of a small number of parasites could act like a vaccine, helping the patient's immune system to build up an immunity and successfully fight the parasite without further intervention.

Patients in this category include those with drug resistant strains, such as those currently emerging in the Mekong Delta (see section 1.2.7). These currently have very limited

treatment options and, in the absence of a better option, are usually prescribed an anti-malarial drug course regardless of its effectiveness. These patients could benefit substantially from treatment with the haemofilter, regardless of the severity of their condition. Furthermore, drug resistance arguably gets more coverage in the press than any other malaria related news, and the ability to offer an alternative treatment to these patients could create significant buzz around the device, helping to drive adoption.

5.2.2 Hospitalised patients

The majority of malaria patients who are admitted to hospital in endemic countries, whether in public or private units, are complicated or severe cases, or at risk of becoming so. Other patients are simply given a course of anti-malarial tablets and sent home. The definition of complicated and/or severe varies from country to country, but the WHO has a definition of severe, which is typically used to categorise patients. However, using this method, most patients admitted would not be considered severe - one study in found that only 17% of malaria patients admitted to a hospital were 'severe' according the WHO definition, while all would be considered 'complicated'[228].

When a patient presents at hospital with malaria-like symptoms (e.g. fever), malaria is typically confirmed within about 30 minutes using Giemsa stained blood smears, whereby the patient is admitted and immediately given anti-malarial medication, usually either quinine or artesunates intravenously. Their chances of survival, which varies considerably between countries, depends largely on their parasitaemia upon admission[229, 230]. In this scenario, the haemofilter would be used as soon as possible, in order to reduce the patient's initial parasitaemia and increase the chances of recovery.

The immune response that causes malarial symptoms is caused by rupturing infected cells, which result in circulating debris within the bloodstream (see section 1.2.4). In the case where a patient with high parasitaemia is treated with intravenous drugs, these symptoms can worsen immediately after drug administration, as the immune system responds to the debris created by the large number of cells killed. In some cases, this response can be so severe as to cause death. Using the haemofilter to remove the infected cells, particularly mature trophozoites and schizonts, would reduce or even eliminate these side-effects.

Using the haemofilter alongside existing drugs to treat hospitalised patients could not only decrease mortality in severe cases, but also reduce average inpatient time. The time between administration of treatment and patient discharge from hospital, and indeed symptom elimination, is linked to the initial parasitaemia. Reducing this using the haemofilter could therefore help patients to recover faster, enabling hospitals to discharge them in a shorter amount of time.

5.2.3 Non-severe patients

The haemofilter could have a benefit for all malaria patients when combined with the anti-malarial tablet courses, such as Coartem, typically given to acute non-complicated patients. Currently, complete recovery using typical drug courses frequently takes over a week (see Appendix B). Symptom elimination time is linked to the length of time between infection and beginning the drug course, or in other words is linked to the parasitaemia when the course is started. Furthermore, as with severe patients, the courses can cause side effects similar to malaria symptoms, such as head-aches and vomiting[231]. Treating

a patient with the haemofilter on the day they start the drug course could therefore reduce the time to symptom elimination by increasing the drug's efficacy and reducing its side-effects.

Anti-malarial tablet courses are often heavily subsidised by the state, so the cost to the patient is generally low or negligible. Furthermore, many patients in endemic countries acquire the drugs from pharmacies where there can be a wide choice, including very cheap (although less effective) options. Haemofiltration cannot compete on cost with these treatments, and it is unlikely that the health systems of endemic countries, or indeed any other sponsor such as a charity, would subsidise a treatment that does not save lives, but simply quickens recovery. Therefore, patients in this group would be those in private healthcare, with health insurance or paying for their own treatment.

5.3 Beachhead market

5.3.1 Patient segment

Each of the three patient segments detailed in section 5.2 stands to benefit from the treatment. From a commercial perspective, each has its advantages and difficulties, but one needs to be selected as the niche segment on which to focus initially. The best group in which to start is not necessarily the one with the largest total addressable market, but rather the one which would best facilitate getting to market and best drive adoption to the other groups. Furthermore, the development of the haemofilter into a product is likely to heavily depend on grants, be they business, engineering, research or medical (see section 5.6). The patient segment focus should therefore be the one that provides the

best chances of winning a wide range of supporting grants; most grants that would be targeted focus either on human impact or commercial potential.

The group that best fulfils these criteria is the hospitalised severe group, which strikes the right balance of human impact - it is the group in which most lives stand to be saved - and commercial potential - there is a large and growing hospitalised malaria patient population, driven by urbanisation and rising middle classes in endemic countries. Since clinical trials would have to be performed in hospitals in any case, it is easiest to perform clinical trials on this group, as they are all concentrated in one place. It is also the best group from which to drive adoption, as the clinical trials will demonstrate not only if there is reduced mortality but also if there is reduced recovery time and, importantly, the size of these reductions. The treatment could be available in both public and private hospitals.

Non-severe patients in private healthcare will only pay for a treatment which is proven to provide the benefits they are paying for with no risks of complications, and their pain is much smaller than severe patients. The value proposition to the former is to return to work faster, while to the latter it is to increase their chances of surviving the disease. Non-severe patients seeking haemofiltration through private healthcare would also be harder to reach, as they often attend small clinics rather than large hospitals. It is also a bad segment to focus on initially because haemofiltration would be perceived as a treatment for the rich, attracting negative publicity and making it more difficult to raise development money from grants. However, once the benefits of haemofiltration have been demonstrated, and it has generated some noise and excitement, this segment could be the most profitable in the long term.

Billing the haemofilter as a treatment for patients who are currently untreatable, particularly drug resistant patients or pregnant women, could be a very useful marketing tool. Drug resistance in particular gets a lot of media attention. Proposing a drug-free treatment in an era of emerging drug resistance (as discussed in section 1.2.7) could also increase the liklihood of being awarded grants to develop the device. However, it is a challenging segment from a commercial perspective, firstly because there is a relatively small number of untreatable patients, and secondly because they are widely dispersed. Particularly in cases of drug resistance, the patients can in fact be very difficult to locate and identify. The ability to 'treat the untreatable' should therefore be a core part of the marketing of the device, but not the main initial focus of product development and market penetration.

In conclusion, hospitalised severe cases are the best segment to focus on initially, enabling the haemofilter to get a foothold in the market, demonstrating its benefits and driving wider adoption. The segment of non-severe patients in private healthcare is likely to be the most profitable in the long term due to the large numbers and high potential margins. Growth and expansion should therefore be focussed on this segment. Meanwhile, 'treating the untreable' should remain a mantra of the commercialisation vehicle in order to increase awareness of the device, but should not, at least initially, be a primary focus of commercialisation, as the segment is likely to prove the most challenging and least profitable.

5.3.2 Starting country

Having selected a niche patient segment, 7 countries with the high malaria burdens - Brazil, The Democratic Republic of Congo, India, Indonesia, Kenya, Nigeria and Thailand - were analysed in order to select the one in which to focus initially (see table 5.1). The ideal country would have:

1. A large number of patients and deaths, in order to have a large addressable market and be able to achieve the maximum human impact.

2. A large number of hospitals, since the initial focus will be on hospitalised patients, and the treatment may well be limited to tertiary healthcare facilities for some time after launch.

3. A well funded public healthcare system. Patients in endemic countries are often from poor backgrounds and rely on the public healthcare system to provide their treatment. That system will therefore be a major driver of adoption and, in many cases, a very important customer.

4. A large private healthcare market, helping to drive adoption and growth. Many patients in the initial target segment will be in private hospitals which are less price-sensitive and often more efficient than public units, facilitating sales.

5. A mixture of *P. vivax* and *P. falciparum*. As discussed in section 1.2.4.3, the treatment may be more effective on *P. vivax* than on *P. falciparum*, due to cytoadherence. However, the impact on survivability could be greater on *P. falciparum* patients, as the mortality rates are much higher. As such, the country in which the device is

first used should ideally have a mixture of both strains, so that the different effects on each can easily be tested.

The conclusion from the analysis was that the best country in which to start is India. It has a large number of malaria cases, which are widely agreed to be largely underestimated by the WHO - some estimate that malaria may account for up to 3.6% of deaths in India[17, 18]. Furthermore, despite healthcare funding varying in different states, malaria patients in India are more likely than in most endemic countries to be able to reach a hospital with relative ease. While the national healthcare system remains underfunded and faces challenges in its goal of delivering good quality healthcare to all Indians, there are increasing numbers of high quality units throughout the country, reflecting India's status as a large emerging economy with commendable healthcare ambitions. The private healthcare market is also large and growing. It accounted for 63% of hospital beds in India in 2010, up from 49% in 2002, creating over 70% of India's new hospital beds in that period[232, 233]. Finally, there is approximately a fifty-fifty split between *P. falciparum* and *P. vivax* in India[4].

5.3.3 Size of beachhead market

Comparing India to the other countries in Table 5.1 indicates that the best beachhead market is hospitalised patients in India, the majority of whom will be severe patients. However, the number of patients in this segment is unclear, with no official statistics for the number of hospitalised malaria patients available. While India does have a system for reporting on the burden of malaria in the country, it, along with the WHO, is widely criticised as being inaccurate[16, 17, 18, 235, 236]. Indeed, even Dr Neena Valetcha,

	Brazil	DR Congo	India	Indonesia	Kenya	Nigeria	Thailand
Number of cases	310,000	17 million	19 million	5.6 million	3.5 million	48 million	140,000
Number of deaths	140	69,000	28,000	9,400	12,000	180,000	170
Number of hospitals (beds/1,000)	7,700 (2.3)	- (0.8)	15,400 (0.7)	1,833 (0.9)	4,700 (1.4)	4,000 (0.5)	1,300 (2.1)
Well funded healthcare system?	Yes	No	State variable	Yes (new)	No	No	Yes
Large private healthcare market?	Yes	No	Yes	Med	Med	Yes	No
Mixture of *vivax* and *falciparum*?	85% *vivax*	100% *falciparum*	50/50	50/50	100% *falciparum*	100% *falciparum*	60% *vivax*

Table 5.1: Analysis of countries with high malaria burdens[4, 234, 233]

director of the National Institute for Malaria Research in India, who was contacted during the market research process, said that she was unable to provide an estimate, and that "no one knows".

In order to estimate the number of malaria patients admitted to hospital in India each year, six different methods were used, combining statistics and estimates from different sources. The results, shown in Table 5.2, demonstrate the difficulty in achieving a reliable estimate. The results for the total number of hospitalised severe patients range from 16,300 to 205,000, with an average of 96,000 and standard deviation of 69,000. The results for the total number of hospitalised patients, range from *ca.* 100,000 to *ca.* 1.2 million, with an average of 566,000 and a standard deviation of 406,000. These were calculated assuming that 17% of patients in hospital are severe, a number taken from Singh et al.[228].

Method 1		Method 2		Method 3		Method 4		Method 5		Method 6	
Total deaths [3]	205,000	Total deaths [1]	28,000	Total cases [1]	1.90E+07	Total cases [1]	1.90E+07	Total deaths [1]	28,000	Tot. population [2]	1.22E+09
% in hospital [3]	15%	% in hospital [3]	15%	% severe [6]	5.40%	Mortality overall [6]	0.60%	% in hospital [3]	14%	Death rate [2]	8
Deaths in hosp.	30,750	Deaths in hospital	4,200	Total severe	1.03E+06	Total deaths	114,000	Deaths in hospital	3,920	Total deaths	9.76E+06
Severe mortality [4]	24%	Severe mortality [4]	24%	Severe mortality [4]	24%	% in hospital [3]	14%	Severe mortality [4]	24%	% malaria [3]	3.6%
Severe in hosp.	**128,125**	**Severe in hosp.**	**17,500**	Total deaths	246,240	Deaths in hospital	15,960	**Severe in hosp.**	**16,333**	Malaria deaths	351,360
% in hosp. severe [5]	17%	% in hosp. severe [5]	17%	% in hospital [3]	14%	Severe mortality [4]	24%	% in hosp. severe [5]	17%	% in hospital [3]	14%
				Deaths in hospital	34,474	**Severe in hosp.**	**66,500**			Deaths in hosp.	49,190
				Severe in hosp.	**143,640**	% in hosp. severe [5]	17%			Severe mortality [4]	24%
				% in hosp. severe [5]	17%					**Severe in hosp.**	**204,960**
										% in hosp. severe [5]	17%
Hospitalised patients:	**753,676**		**102,941**		**844,941**		**391,176**		**96,078**		**1,205,647**

Sources:

1. World Health Organization
2. World Bank
3. Lancet (2010); Vol. 376(9754): 1768–1774
4. Trop. Med. Int. Health (2009); Vol. 14(3):332–337
5. Malaria Research and Treatment (2013); Vol. 2013. Article ID: 341862
6. J. Vector Borne Dis. (2012); Vol. 49:157-163

AVERAGE: 565,743

Table 5.2: Estimating number of hospitalised patients in India[4, 17, 229, 228, 237, 234]

5.3.4 Expansion

Once the haemofilter is established in the beachhead market, growth should be targeted at non-severe patients in private healthcare units in India, and hospitalised patients in other endemic countries, with Nigeria the best country to focus on second. Indonesia could also be a lucrative market, while Thailand and Cambodia (not included in the analysis in Table 5.1 due to its low comparitive burden) should not be neglected as the countries in which drug resistance is emerging (see section 1.2.7). Moving forward, growth is likely to come from large cities in endemic countries, particularly in Africa.

There may also be a significant market in non-endemic countries to treat imported cases, where hospitals are under more pressure to offer the best available treatments and margins can be significantly higher than those achievable in emerging markets. There were 1,500 imported cases in the UK in 2013, and the WHO estimated that there were 6,250 cases in Europe in 2010, although some claim the real figure could be as high as 36,000[238, 239, 235]. In the USA, the Center for Disease Control reported 2,000 cases in 2011, an increase of 14%[240].

5.4 Pricing and margin

5.4.1 Value propositions

The value propositions to patients are simple, ranging from the haemofilter being the only treatment option, to increasing the chances and speed of recovery. However, there also needs to be a strong value proposition for the hospitals or healthcare systems who will be purchasing the instrument and disposables from the company or its distributor. The

value propositions vary between private and public units, but include:

1. The chance to save lives
2. Reduced inpatient time, freeing up beds
3. Able to treat patients who would otherwise be untreatable
4. Demonstrate that the hospital is innovative and offers the latest treatments
5. Higher average profit per malaria patient
6. Reduced average cost of treating a patient
7. Reduced cost per death averted

Which of these value propositions are most important will vary from hospital to hospital, and depend largely on whether the hospital is in the public or private sector. For example, a hospital in the private sector probably cares more about increasing profits, so would be most interested in increasing the number of patients and the average profit per patient rather than freeing up beds or reducing cost per death averted. A public hospital, on the other hand, has no interest in profits, but would likely be more attracted by the possibility of reducing the average inpatient time and cost of treating a patient.

5.4.2 Cost benefits of haemofilter

If the haemofilter delivers on the promised patient benefits - decreased mortality and recovery time - value propositions one through five are likely. However, numbers six and seven require further investigation. In order to see if the haemofilter could deliver

Item	Average cost
Drug treatment	$21.60
Drug administration	$1.40
Inpatient care	$47.30
Total cost	$70 **(£43.50)**
Cost per life saved	**£55.50**

Table 5.3: Average cost of treating a severe patient in India[229]

cost reductions to hospitals, the current cost of treating a severe patient in India was investigated.

According to Lubell et al., in 2009 the average cost of treatment with artesunates is $70.30, or £43.60 ($1 = £0.62). With a mortality rate of 21.4%, the cost per death averted was £55.50. The average inpatient time of patients in Lubell's study was 4.6 days. The average inpatient care cost in India is $10.25 per day, yielding a total average inpatient care cost of $47.30 (£29.34). The artesunates, which are administered intravenously several times a day, cost on average $4.68 per day, resulting in a total drug cost of $21.60 (£13.40)[229]. These costs are summarised in Table 5.3.

These figures clearly demonstrate that reducing the average time in hospital could have a significant effect on the average cost of treating a patient and the cost per death averted. Reducing the average inpatient time to two days, for example, could provide a cost saving of £25 (Fig. 5.1). The impact that reducing both inpatient time and the average mortality rate has on the cost per death averted is shown in Fig 5.2. Reducing the inpatient time to two days and the mortality to 10% would reduce the cost per death averted by £34.50 to £21. This excludes the cost of haemofiltration, but provides an indication of how the treatment could be priced if it provides the anticipated benefits.

Item	# per set	Supplier	Part #	# per pack	Cost of pack	# packs for 1,000 sets	Cost for 1,000 sets
Male Luer connector	2	The West Group Waterlooville (UK)	MTLL004-6005	100	£30.29	20	£605.85
Female Luer connector	2	The West Group Waterlooville (UK)	FTL10-6005	100	£28.14	20	£562.80
Dual pinch clamp	2	Cole-Parmer London (UK)	WZ-95785-00	25	£13.47	80	£1,077.60
Tee junction	2	The West Group Waterlooville (UK)	T210-6005	100	£58.28	20	£1,165.50
3ml syringe, Luer connector	1	Sigma-Aldrich Gillingham (UK)	Z192112-200EA	200	£46.70	5	£233.50
1/16 ID 1/8 OD tubing (50ft roll)	4ft	The West Group Waterlooville (UK)	FLXC1-2	50ft	£26.28	80	£126.14
Chamber casing	1	N/A	N/A	1	£0.20	1,000	£200.00
Mesh (uncut)	0.25m^2	Hebei Meihua Hardware Mesh Co. Anping (China)	N/A	30m^2	£52.00	8.33	£433.10

Total cost for 1,000 sets: £4,404.50 Total cost per set: £4.40

Table 5.4: Bill of materials for consumables

With an inpatient time of two days, a mortality rate of 10%, and a haemofiltration cost of £25, the average total cost of treating a patient would not change, but the cost per death averted would decrease by £9.50. If the price of the haemofitler was such that its use would provide an overall cost saving, it would be much easier to sell to hospitals and healthcare systems, helping to drive adoption.

5.4.3 Manufacturing cost of consumables

In order to investigate the price at which the haemofilter would be sold, the manufacturing cost of the consumables required for each treatment was estimated. The parts required for each set of consumables are (Fig. 5.3):

- 2x male Luer connectors: used to connect the device to the catheter
- 2x female Luer connectors: used to connect the saline drip bag and heparin syringe

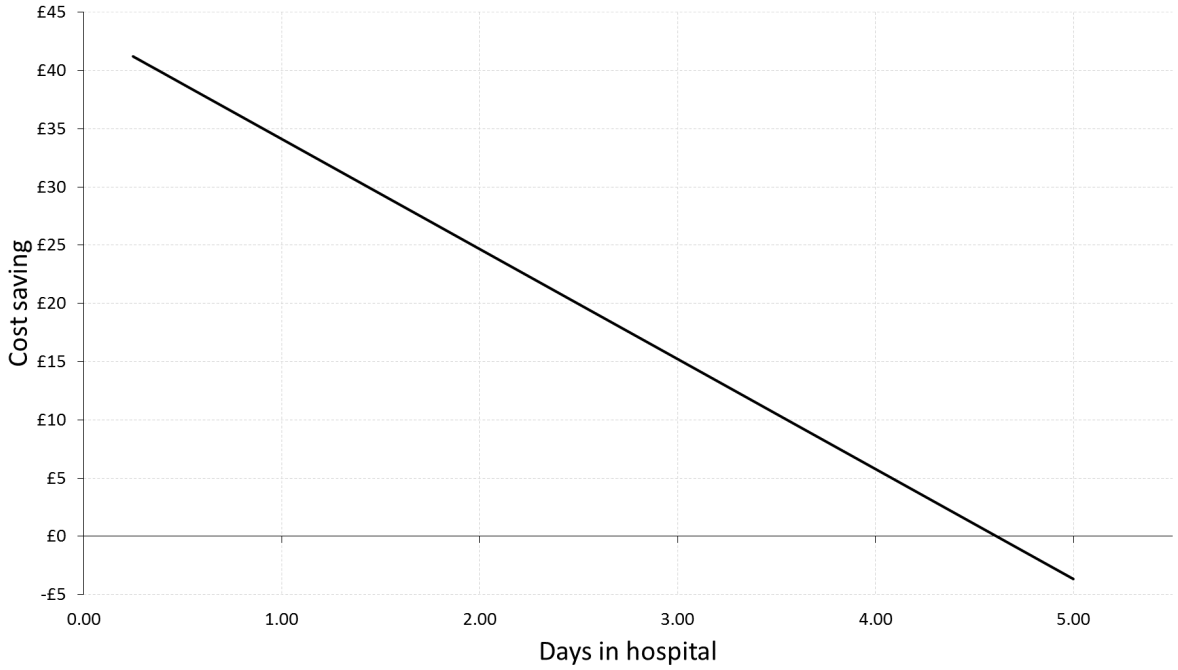


Figure 5.1: Cost savings achieved by reducing average inpatient time

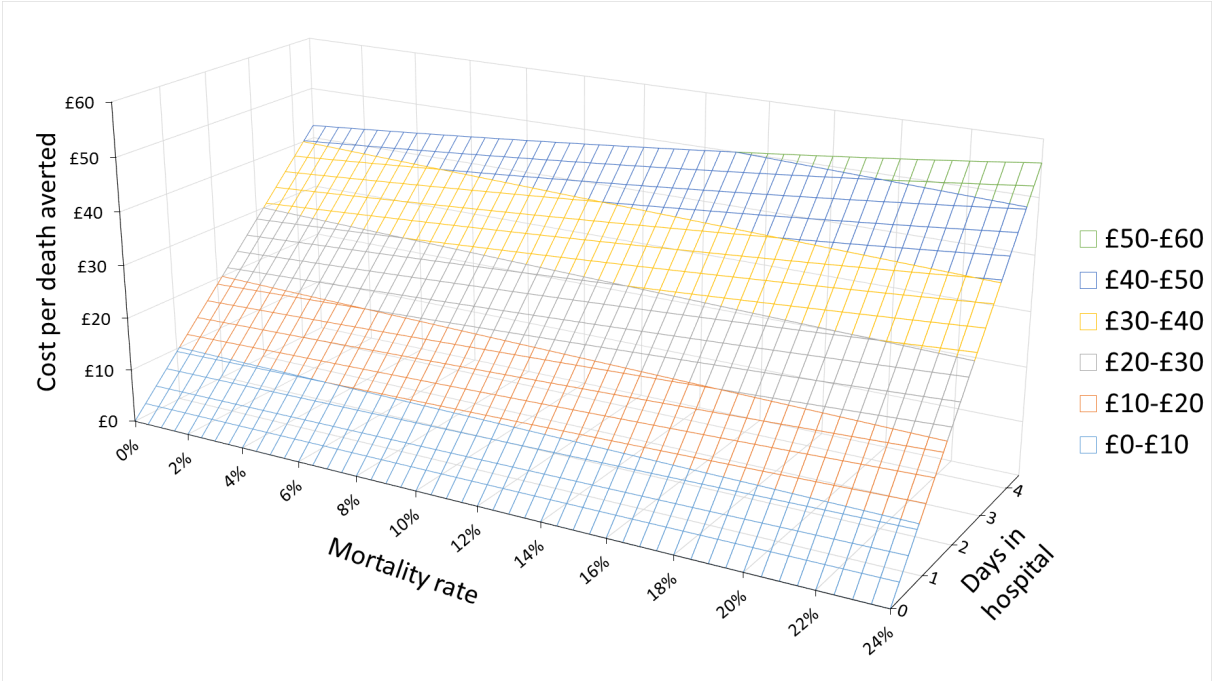


Figure 5.2: Cost per life saved for different mortalities and inpatient times

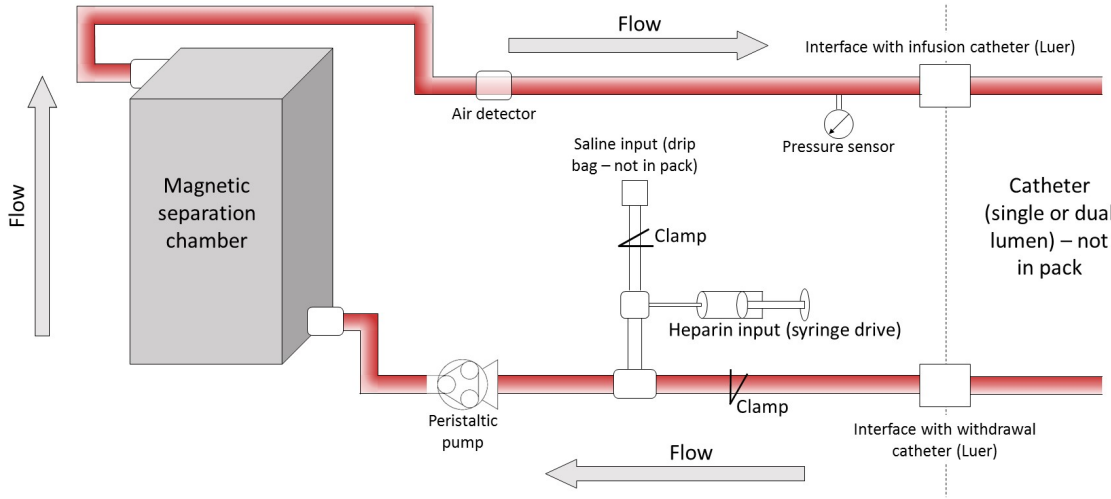


Figure 5.3: Sketch of blood circuit showing parts required for the consumables

- 2x pinch clamps
- 2x tee junctions: used to interface the saline bag and heparin syringe with the circuit
- 1x 3ml syringe: included to ensure that the syringe with which the heparin is injected is always the correct type (in terms of dimensions and interface)
- 4ft of 1.6mm inner diameter, 3.2mm outer diameter silicone or polyethylene tubing
- Separation chamber casing
- Ferromagnetic (SS430) stainless steel mesh

Note that no specific part is required for air detection within the blood circuit - the transparent tubing can be simply passed through a detector on the instrument.

Suppliers for each of these parts were approached for quotes to estimate the total cost of the materials required to manufacture 1,000 sets of consumables. These were combined

Item	UK	India
Bill of Materials	£4.40	£4.40
Labour cost per hour	£45[1]	£1.10[2]
Labour time required	20 mins	20 mins
Labour cost	£15	£0.37
Sterilisation cost[3]	£1	£1
Import tax (38%)	£6	£0
Postage and packaging	£1	£0.75
Total cost	**£27.40**	**£6.52**

[1] Quote from EG Technologies

[2] Assuming 7% yearly growth since 2010

[3] Assuming £500/cycle

Table 5.5: Comparison of manufacturing cost of consumables in UK and India[241]

in order to construct a complete bill of materials (BOM) (Table 5.4). The BOM for each set was found to be £4.40. It is important to bear in mind that this is not volume optimised, and that the price would drop significantly when manufacturing hundreds of thousands or millions of sets.

The BOM does not include the cutting of the mesh, assembly, or sterilisation, which are all factored into the estimated labour time even though they could be outsourced. To estimate the total manufacturing cost, this total labour time was estimated and combined with average hourly wages as well as import tax and shipping costs to get an estimated cost per consumable. The cost of manufacturing in the UK and in India were compared, highlighting the size of cost saving that can be achieved by manufacturing in India (Table 5.5) - at £6.52, the cost of manufacturing in India is less than 25% of the cost of manufacturing in the UK, which was estimated at £27.40. This is primarily due to the difference in labour costs, but also due to India's 38% import tax.

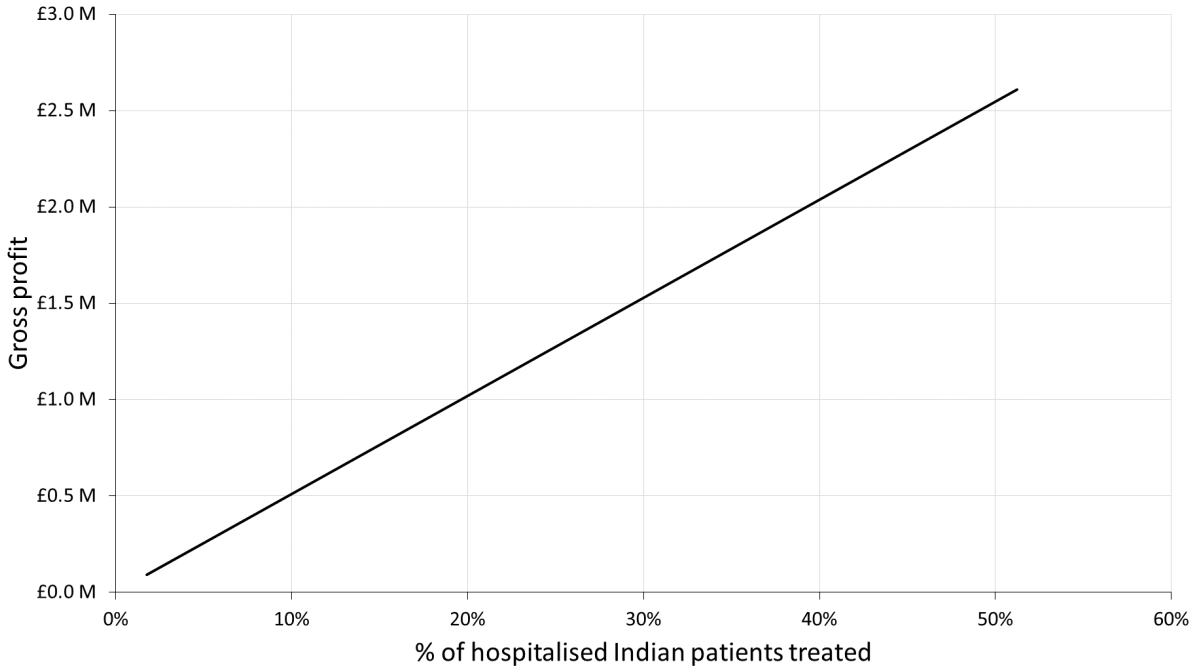


Figure 5.4: Gross profit earned vs. percentage of beachhead market reached

5.4.4 Margin and profit

Section 5.4.3 estimates a manufacturing cost of about £6.50 per consumable, while section 5.4.2 demonstrates that it could be sold to a hospital at a total cost of about £25. This implies a profit of £18.50 that the company would need to share with the distributor. The distributor would likely want a margin at least equivalent to the company's, giving a profit of £9 per treatment. This represents a margin of 58%.

Combined with the estimate from section 5.3.3, Fig. 5.4 shows the gross profit earned versus the percentage of the beachhead market that is reached. If the company had estimated fixed costs of £500k, it would need to sell to 10% of the hospitalised patients in India in order to break even.

5.4.5 Revenue model

Section 5.4.4 demonstrates that it is possible to earn a margin on the disposables that are required for each treatment. This is crucial, as the haemofilter will be commercialised using a 'razors-and-blades' model, where most of the profits come from the consumables and not the instrument[242]. This model is widely used in several industries, for example desktop printers, where companies such as HP make little or no profit from the printer itself, but earn high margins on the ink cartridges which are required for, and only compatible with, their printers. A highly relevant comparison to the haemofilter are dialysis machines, which reguarly use a similar revenue model in which they lease the instrument to hospitals who then purchase the tube sets and cartridges required for each treatment.

5.5 Route to market

5.5.1 Milestones

As the haemofilter is a medical device, a number of hoops need to be jumped through before a product is ready to be sold to the market:

1. *Safety trials*: before approval is given to conduct clinical trials (in the UK, approval is given by the Medicines and Healthcare products Regulatory Agency (MHRA)), safety trials need to be conducted on healthy subjects in order to demonstrate that the device does not cause any harm. Typically, an animal safety trial is required first, followed by a human safety trial. The latter would be conducted on volunteers (who would be compensated) in collaboration with a hospital or vetinary college with haemofiltration or extra-corporeal dialysis experience.

2. *Clinical trials*: in order to obtain regulatory approval and have a product that can be sold, its clinical benefits need to be demonstrated in one or several clinical trials. For the haemofilter, these will be conducted on patients that fit into the beachhead market segment (i.e. hospitalised in India). The indication criteria for inclusion in the trial will have to be very well defined, including details such as parasitaemia cut-off points, age, severity, etc.

3. *Regulatory approval*: in the EU, CE marking is required; in the USA, Food and Drug Administration (FDA) approval is required. Most countries have their own regulatory body which needs to certify the device before it can be sold. While they are not essential, having CE marking and/or FDA approval greatly facilitates the regulatory pathway in new markets. Getting these can be an expensive process, not least because of the required testing to prove biocompatibility and non-toxicity.

These constitute the main milestones that a company commercialising the technology would have to achieve, and each has its own subset of tasks that will need to be carried out. Product development should be streamlined as much as possible in order to achieve the three milestones quickly and in a cost-effective manner. However, it is also important to ensure that the right questions are answered at each step. For example, the prototypes used to demonstrate product safety must be internally identical to what would be used on a patient in a clincal setting, as any change to the dimensions or ferromagnetic matrix could cause unanticipated effects. Therefore, before the safety trials can be conducted, the device design and specifications need to be finalised, and further biocompatibility and toxicity tests performed.

5.5.2 Product development

5.5.2.1 Cost estimates

Product development, achieving the milestones above, will be the most expensive aspect of commercialisation. In order to estimate the costs of developing a CE marked instrument and consumables, a medical device prototyping company, EG Technology (Cambridge, UK), was approached and asked for both top-down and bottom-up estimates of the total cost. The top-down estimate was £2 million, while the bottom-up estimate was £1.125 million, as detailed in Table 5.6. It should be noted that these estimates exclude any unforeseen problems or changes.

5.5.2.2 Development strategy

As the milestones will need to be achieved in order to generate any revenue, all expenditure on product development will need to come from investment into the company. The estimates from EG Technology underline the need to streamline the product development process in order to reduce the size of investment needed to get to market. Table 5.6 shows that, of the £1.125 million, about £800,000 is needed to develop the instrument, while around £325,000 is needed for the consumables.

The instrument is expensive as it includes all the safety mechanisms, needs to consist of medically approved parts, required custom electrons and software, etc. However, in the safety and clinical trials, it is the consumables that are being tested (along with the external magnets), and not the instrument, which is essentially a blood pump. The trials could be conducted using medically approved commercial blood pumps with the necessary safety controls (e.g. pressure monitors) already included. The trials therefore only require

Item	Cost	Cumulative
Phase 1: concept study		
Detailed report outlining project	£30,000	
Sub-total	£30,000	**£30,000**
Phase 2: risk reduction		
Initial failure mode and effects analysis	£5,000	
Mitigation or elimination of risks	£10,000	
Outline electrical design	£10,000	
Bill of materials for consumables	£5,000	
Optimise design of consumables	£10,000	
First prototypes of consumables	£10,000	
Sub-total	£50,000	**£80,000**
Phase 3: detailed mechanical design		
Consumables		
Detailed manufacturing plan	£50,000	
Tooling	£50,000	
Pilot set up & protocol writing	£20,000	
Instrument		
Electronics	£150,000	
Tooling	£100,000	
Mechanical design	£80,000	
Software	£200,000	
Assembly test and integration	£100,000	
First 6 made	£150,000	
Regulatory consultancy	£20,000	
Sub-total	£920,000	**£1,000,000**
Phase 4: transfer to manufacture		
Sterilisation validation	£5,000	
ISO-10993 testing	£10,000	
ISO-60601 testing	£20,000	
EEC testing	£20,000	
Pilot parts (200 consumables)	£20,000	
Documentation	£50,000	
Sub-total	£125,000	**£1,125,000**

Table 5.6: Cost estimates for product development (from EG Technologies, Cambridge, UK)

prototypes of the consumables, which could be manufactured in-house, for lower than the price quoted by EG Technology.

5.5.2.3 Prototypes of consumables

The consumables, and more specifically the separation chambers, constitute the most important part of the device. The final design, based on the work described in section 3.2.4, would be checked by dialysis experts, confirming that characteristics such as the flow rates that will be used are appropriate. There will be several different sizes produced so that patients from infants through to adults could be treated, and each size will incur its own tooling costs. They will all undergo the same assay tests as the donated *P. vivax* samples from section 4.4, and any further tests that may be required (e.g. detection of trace metals), before being used in safety and then clinical trials.

The separation chambers would need to be manufactured using medically approved plastic, probably using injection moulding. Moulds would need to be tooled for each chamber size, and each of these would incur a significant cost - one can cost tens of thousands of pounds. However, once the tools are made, the chambers could be manufactured by a professional medical device manufacturer relatively cheaply.

The stainless steel mesh would need to be cut to size and loaded into each chamber, as described in section 3.5. This is a time-consuming process and, to be both rapid and precise, would require specialised machinery. As such, it would probably be more cost effective to outsource the mesh cutting, avoiding the capital expenditure that would be required to purchase the equipment and hire the staff required for in-house cutting.

While the manufacture of the separation chamber cases and the cutting of the mesh would be outsourced, the first prototypes could be assembled in-house. The required

parts, described in Table 5.4, would be purchased from a medical equipment supplier (e.g. The West Group, UK), and the circuits described in Fig. 5.3 assembled. The parts would be sterilised in an autoclave in advance of assembly, which would take place under sterilised conditions. Each consumable would need to be packed into sterilised packaging ready for use in the trials.

5.5.2.4 Development of the instrument

The instrument consists of the following parts:

- Magnetic assembly
- Peristaltic pump to drive the circuit
- Syringe pump for heparin
- Pressure monitor
- Air bubble detector
- UI
- Display panel with information on current filtration

In order to minimise product development costs and focus investment on getting clinical data, commercial equipment will be used where possible, including for example the syringe pump used during this project (see section 4.1.3) and a blood pump such as the Graseby Model 500 Volumetric Infusion Pump (Graseby, Watford, UK), a variable speed peristaltic pump with an occlusion pressure monitor and an air bubble detector included. One of these pumps has generously been donated by Smiths Medical (Watford, UK). The

magnetic assembly, meanwhile, would be the same model used throughout this project (see section 3.3).

In the longer term, it would be desirable to develop an instrument intended for use solely with the consumables, incorporating all the above parts and required features into one piece of equipment. As mentioned in section 5.5.2.1, this would incur significant costs and would require a large investment round which would be sought at the earliest after the safety trials have been completed.

5.5.3 Intellectual property and competition

While there is no magnetic haemofilter currently on the market, there is significant prior art which may limit the scope of any patent that is filed, if indeed a patent could be filed. As discussed in Chapter 2, HGMS is a well-established technology that has existed for over 100 years. There are several biological applications already commercialised, and some patents covering the area of magnetic haemofiltration, including one that proposes using it as a method to treat malaria[106, 131, 132, 243, 244, 136, 245, 200, 139, 202].

While the prior art does not necessarily rule out the filing of a patent - the magnetic haemofilter described in this project is significantly different to other technologies in its design and implementation - this creates a challenging IP environment. There are several strategies that could be adopted and different avenues of IP protection available despite these challenges. There is, for example, significant know-how and protectable design rights associated with the technology. The company commercialising the filter could also 'black box' the technology, maintaining its operation as a trade secret.

However, regardless of the chosen IP strategy, the device, by its nature, will remain

easy to reverse engineer, opening up the possibility of counterfeits appearing on the market. This is likely to be particularly problematic in malaria endemic countries, in which it is typically more challenging for a foreign company to protect its innovations and technologies. One advantage is the high barriers to entry that need to be overcome in the healthcare industry - competing products will need regulatory approval, which may discourage counterfeits and their use.

One method of remaining competitive is to build a strong brand around the company and the product. People need to know and trust the product, so that they are suspicious of, and reluctant to try, alternatives. As such, marketing will be very important for the company commercialising the haemofilter.

5.6 Funding

5.6.1 Funding rounds

Several investment rounds will be required to get to market. Each round has specific targets to achieve, with the aim of eliminating risks sufficiently to enable the next stage of funding. The targets for each round are:

1. Seed round: ready for clinical trials
    (a) Working prototypes of consumables with the ability to manufacture more
    (b) Safety trials completed with permission for clinical trials
2. 'A' round: first customer
    (a) Clinical data proving reduction in mortality

(b) Working prototypes of instrument

(c) CE marking

(d) First customer

3. 'B' round: profitability

(a) Clinical trials proving benefits for non-severe patients

(b) Grow into other markets

(c) Product optimisation

(d) Company profitable

The seed and A rounds could consist of both private capital, for example from angel investors, and grants, for example the innovateUK biomedical catalyst or the EU SME instrument. It is estimated that £500,000 would be needed to achieve the goal of being ready to conduct the first large clinical trials. This includes development of prototype consumables, benchtop biocompatibility tests, animal and human safety trials, salaries for two staff members, legal costs and operating costs (e.g. office, telecoms, website building).

The 'A' round provides the investment needed to get the first customer, with most of the funds being spent on clinical trials and product development, getting to CE marking and a saleable product. It is estimated that £2.5 million would be required to get the first paying customers. Of this, £2 million would be spent on product development and trials, while £500,000 would be spent on salaries and operating costs. As with the seed round, this investment could be a mixture of private capital, probably from a venture capital fund, and grants. Partnerships with companies, institutions, charities or governments could also be a valuable source of funding. The cost of the clinical trials, for example,

could be shared between the company, the Indian government, and a charity like the Bill and Melinda Gates Foundation.

At this stage, it is not possible to provide a reliable estimate for how much would be needed at the B round, since it depends on the results of the clinical trials and what the objectives of the company are at that time. However, since the goal would be to grow the company new markets as quickly as possible, it is likely that a large investment round would be required. This could come either from venture capital or as part of a trade sale, and could be a logical exit point for the founders.

5.6.2 Estimated spending

Table 5.7 shows the estimated spending on various items up to the B round investment. These were assembled after discussions with business experts, prototyping companies, and healthcare companies. The product development costs include designing, tooling and testing, amongst other activities as described in section 5.5.2. They do not include the cost of manufacturing or assembling prototypes, which were estimated to cost on average £100 each for the consumables, and £20,000 each for the instruments, including sterilisation and packaging. The total was based on an estimated number of prototypes that would be needed for benchtop testing, optimisation and the safety and clinical trials.

The costs of safety trials were estimated after talking to experts in the area. It was estimated that the animal safety trials, with seven pigs, would cost £30,000, while the human safety trials, with 10 healthy subjects, would cost £50,000. The clinical trials, involving at least 50 patients, were estimated to cost £400,000. This is the same as the cost of clinical trials quoted by a European healthcare company at a similar stage, and

may in fact be smaller if the trials are carried out in India.

	2014	2015				2016				2017	
Item	*Q4*	*Q1*	*Q2*	*Q3*	*Q4*	*Q1*	*Q2*	*Q3*	*Q4*	*Q1*	*Q2*
Product development	£10,000	£20,000	£20,000	£20,000		£100,000	£250,000	£200,000	£100,000	£100,000	£50,000
Prototypes/manufacturing		£20,000	£5,000	£5,000	£10,000	£15,000	£15,000	£50,000	£50,000	£100,000	£100,000
Safety/clinical trials			£15,000	£15,000	£25,000	£25,000		£200,000	£200,000		
Salaries	£15,000	£32,500	£32,500	£52,500	£52,500	£87,500	£87,500	£95,000	£95,000	£95,000	£95,000
Operating & legal costs	£25,000	£15,000	£15,000	£20,000	£20,000	£30,000	£30,000	£40,000	£40,000	£50,000	£50,000
Sub-total	£50,000	£87,500	£87,500	£112,500	£107,500	£257,500	£382,500	£585,000	£485,000	£345,000	£295,000
Cumulative total	**£50,000**	**£137,500**	**£225,000**	**£337,500**	**£445,000**	**£702,500**	**£1,085,000**	**£1,670,000**	**£2,155,000**	**£2,500,000**	**£2,795,000**

Table 5.7: Estimated company expenditure until B round

		2014	2015				2016				2017	
Staff member	Salary	*Q4*	*Q1*	*Q2*	*Q3*	*Q4*	*Q1*	*Q2*	*Q3*	*Q4*	*Q1*	*Q2*
CEO	£60,000	£15,000	£15,000	£15,000	£15,000	£15,000	£15,000	£15,000	£15,000	£15,000	£15,000	£15,000
COO	£80,000				£20,000	£20,000	£20,000	£20,000	£20,000	£20,000	£20,000	£20,000
CTO/engineer	£50,000		£12,500	£12,500	£12,500	£12,500	£25,000	£25,000	£25,000	£25,000	£25,000	£25,000
Product manager	£40,000						£10,000	£10,000	£10,000	£10,000	£10,000	£10,000
Technician	£20,000		£5,000	£5,000	£5,000	£5,000	£10,000	£10,000	£10,000	£10,000	£10,000	£10,000
Clinical trials staff	£30,000						£7,500	£7,500	£15,000	£15,000	£15,000	£15,000
Sub-total		£15,000	£32,500	£32,500	£52,500	£52,500	£87,500	£87,500	£95,000	£95,000	£95,000	£95,000
Cumulative total		**£15,000**	**£47,500**	**£80,000**	**£132,500**	**£185,000**	**£272,500**	**£360,000**	**£455,000**	**£550,000**	**£645,000**	**£740,000**

Table 5.8: Company employees up to B round (grey shading indicates two employees)

The salary costs assume a gradually increasing team size, from one employee initially, to nine by Q3 2016 onwards, as can be seen in Table 5.8. The staff required and their salaries were estimated after consultancy with business experts, including prior and current CEOs of medical device start-ups. The operating costs include all the costs associated with running the business (office, telecoms, website maintenance) as well as the cost of business trips and conferences. The figures also include legal fees incurred due to IP protection, contracts and deals.

5.6.3 Required investment

The Gantt chart in Fig. 5.5 summarises the conclusions regarding activities and funding. The analysis in Table 5.7 shows that the company will need approximately £450k in the seed round, and a further £2.35 million in the A round. Allowing for some contingency, and assuming that some unforeseen delays will be encountered, the total funding required to get to the B round (i.e. the first customer) is estimated to be £3 million, consisting of a £500k seed round and £2.5 million B round.

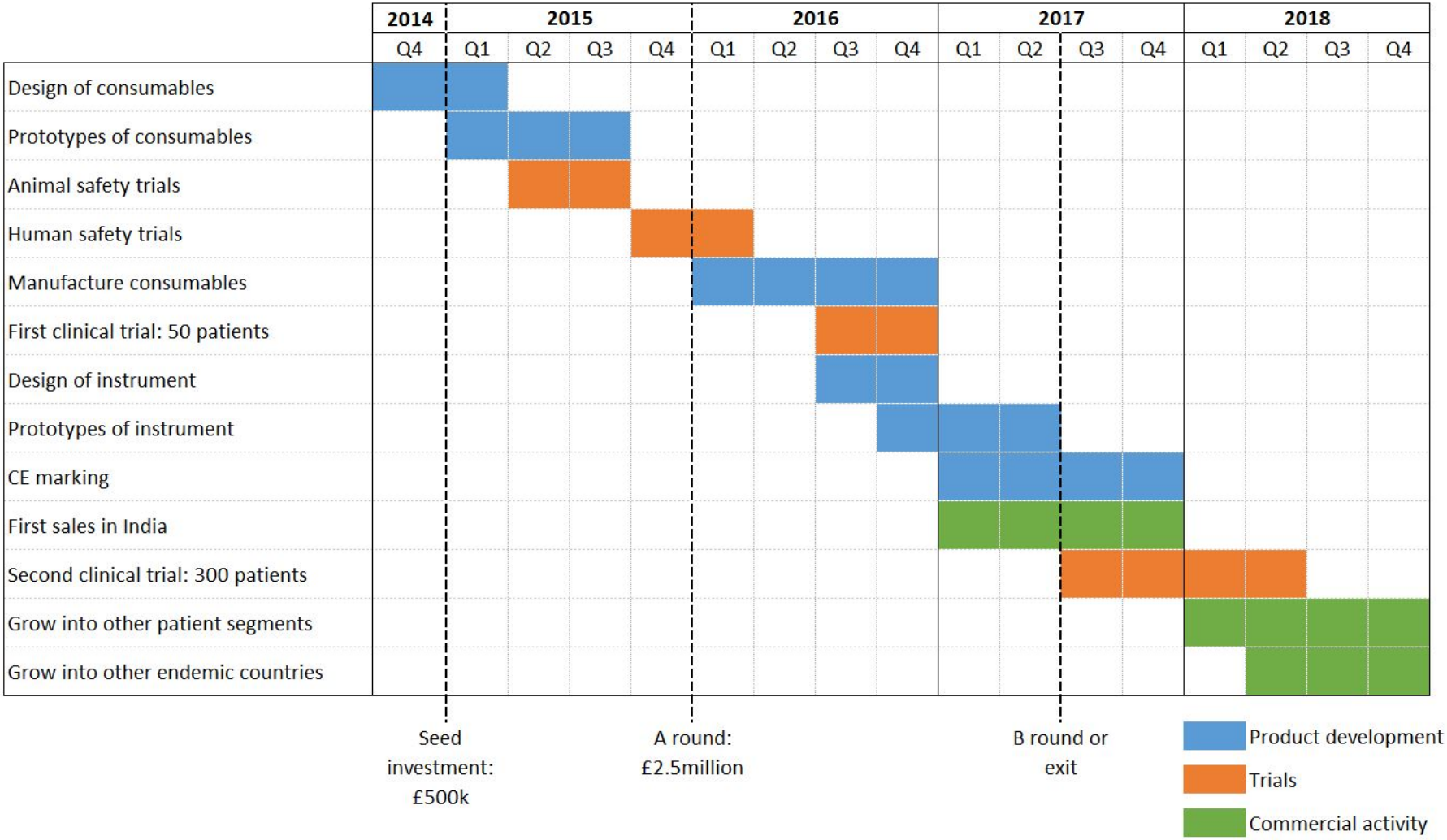


Figure 5.5: Gantt chart outlining commercialisation plan

5.7 Conclusion

This section presents an analysis of the commercial viability of the magnetic haemofilter as a treatment for malaria, as well as a suggestion for how a company might undertake commercialisation. The analysis successfully identified a number of customer segments (i.e. patients who could benefit from the treatment), and, after considering several endemic countries with very high burdens, concluded that the best beachhead market is hospitalised patients in India. This market segment promises high human impact, enables relatively hassle-free clinical trials, drives adoption, and is quite large, with an estimated TAM of over half a million patients. There is significant potential for growth, with a large number of endemic countries to grow into - half the world's population is in an endemic region - and large and growing private healthcare markets in many of those countries.

The analysis suggests that it will be possible for a company to earn a healthy margin on each treatment, by both saving patients' lives and reducing hospitals' costs. However, this analysis is based on the current cost of treating a severe patient in India. The cost in other endemic countries may be significantly lower, and the healthcare system less able to afford the treatment. Any profitable commercial activities in these countries would have to focus on the private healthcare market. Here, there is still a strong value proposition, even for non-severe patients, if the haemofilter is able to significantly decrease recovery time.

The route to market has been identified, a roadmap has been outlined and costs of getting to market have been estimated. The results from this analysis resulted in the conclusion to focus development and funds on the consumables initially, and to eventually adopt a 'razor-and-blades' model. It also demonstrated that product development and

prototypes would account for almost half of the total expenditure, with salaries accounting for a further quarter. The conclusion from this analysis was the two funding rounds would be needed to get to market, consisting of a seed round of £500k and an A round of £2.5 million. Both of these would be sourced from a mixture of private capital and public grants, with the target of using this investment to get the first customer by mid-2017. At that point, a further investment round would be required, which might provide a logical exit point for the company's founders.

In conclusion, the commercialisation of the haemofilter is a viable venture and worth pursuing. However, despite the commercial potential, it is clear from the manufacturing costs estimated in section 5.4.3 that many of the patients who need this treatment will be unable to afford it, and many healthcare systems are likely to be unable to provide it. Any company commercialising this technology therefore needs to carefully consider the ethical implications of the business, as the ultimate goal has to be to enable access to as many people as possible and achieve the maximum possible human impact. One solution to this would be to partner with the organisations who currently provide funding for much of the malaria treatment and prevention in the world, such as the Bill and Melinda Gates Foundation and the Global Fund to Fight AIDS, Tuberculosis and Malaria. The company could provide instruments and consumables to these organisations at-cost.

Chapter 6

Discussion and Conclusions

6.1 Magnetic haemofilter performance

In this project, the centuries old principles of HGMS have been used to design a novel magnetic haemofilter medical device, capable of removing magnetic material directly from a patient's bloodstream. The design is successful in terms of fulfilling the initial requirements set out at the start of the design process (see section 3.1.1):

- *High efficiency*: although not consistently reaching the target efficiency of 90%, the device successfully lowered the parasitaemia in all experiments with an average separation efficiency of about 65%. Variations in the performance were principally due to different initial parasitaemias, which seemed to have a greater effect on the filtered parasitaemia than the flow rate. Efficiencies of close to 90% were achieved in several experiments, and efficiencies of 70-80% were common. The objective should still be to increase these values, but the intial results are very promising and indicate that high separation efficiencies are possible.

- *High selectivity*: the experiments conducted with donated *P. vivax* indicate that there was no false positive capture of uninfected cells. However, this requires further investigation, particularly by analysing the contents of the separation chambers after filtration to ensure that there is no consistent false positive capture. This was only done to a limited degree in this study.

- *High throughput*: the flow rates used in the experiments are orders of magnitude higher than any that have been previously reported in the literature (see section 2.2.1). Scaled up to the clinical-scale designs, the flow rates used enable acceptable treatment times of only a few hours. As with the efficiency, the target should always be to increase the throughput, but these intial results are very promising, and even before optimisation are already sufficient for a clinical device.

- *Able to incorporate different chamber types*: the four chamber sizes, intended for infants, children, youths and adults, all integrate easily into the same magnetic assembly, producing consistent magnetic forces across a range of flow rates. The design is highly flexible, allowing modules to be added or removed for different applications, and could be used on chambers with volumes varying from $<$1ml to $>$250ml. Furthermore, the magnetic force can easily be increased by reducing the separation between the magnetic assemblies, creating a much higher magnetic field in the chamber area to provide even more flexibility.

- *Controlled, consistent and variable flow rates*: the chamber and assembly design easily allow for different flow rates, and bespoke chambers capable of much smaller flow rates could be designed to integrate with the magnetic assembly. In the experiments, the flow was provided by a syringe pump, but in a clinical device would be

provided by a highly accurate peristaltic pump. Future testing of the device should employ an appropriate pump to ensure that the results are repeatable.

- *No harm caused to other blood components*: the full blood counts performed on samples of blood donated by patient infected with *P. vivax* demonstrate that the device does not cause any harm to other blood components. Light microscopy analysis did not reveal any haemolysis, further supporting this conclusion.

- *Intuitive and easy-to-use*: the device has been designed to be 'plug-and-play', with very little user input required. An appropriate UI will need to be designed, but the device will be useable by anyone who can insert a cathetar. In principle this would be a procedure that could be carried out by any trained nurse, although in practice, more senior staff supervision will be required, especially as the treatment first enters the market.

- *Cheap to manufacture*: both the consumables and the magnetic assembly have been designed to be simple and cheap. The consumables will have amanufacturing cost of less than £5 each, and even if each treatment costs over £20, the haemofilter could still reduce the total cost-per-death-averted (see section 5.4.2).

The design is therefore deemed fit for purpose, performing beyond expectations in these first tests. While further improvement would be desirable, and a lot of development work is still required to turn the bench-top prototype into a viable clinical product, the work presented here clearly demonstrates the potential of the design and can be taken as the first step towards a novel medical device for the drug-free treatment of malaria.

6.2 Potential as a medical device

The results in this thesis indicate that the magnetic haemofilter has significant potential as a novel medical device. It has been shown to be both technically and commercially viable, both of which are essential for the success of any medical device. Designed from the start with end-users in mind, the haemofilter is simple, flexible and cost-effective. The consumables and magnetic assembly have low manufacturing costs and can easily be modified to enable different separation chambers and flow rates to be used. The instument is easy-to-use, would have a long lifetime, and will be compatible with any number of different blood circuits and separation chambers intended for different applications.

6.2.1 Malaria

The focus within this thesis has been on the potential use of the haemofilter as a medical device to treat malaria patients. This potential has been demonstrated through the testing and results presented in Chapter 4, which shows that the haemofilter is capable of capturing malaria-infected erythrocytes, and could achieve significant reductions in patient's parasite burdens in a short time period. The device would be the world's first medical device for the treatment of malaria, as well as the first drug-free treatment. It could be used in combination with other treatments or as a standalone therapy, and has the potential to deliver significant benefits to a wide range of patients, improving recovery and saving lives.

However, it should be noted that a lot of work is still required before the goal of using the haemofilter as a drug-free method for treating malaria (and indeed other conditions) can be achieved. The results are positive, but inconsistent (the target of 90% efficiency

was not achieved), and a medical device would need to be able to rapidly, reliably and repeatedly reduce a patient's parasitaemia every time it is used. Furthermore, questions remain as to the safety of the device, notably the potential for false positive capture of healthy cells, causing haemolysis and activation of leukocytes. Some significant engineering challenges have also not beed addressed, specifically methods for priming the blood circuits, ensuring that no air bubbles are formed or returned to the patient and no blood clots are formed. The potential for saturation of the filter has also not been investigated, due to the small samples volumes used in the experiments.

The commercial evaluation presented in Chapter 5 outlines how the haemofilter could be commercialised as a medical device to treat malaria. It shows that there is a market for such a device, particularly thanks to its unique selling point of being the only drug-free treatment. Commercialisation will require significant investment to enable product development and clinical trials, but the haemofilter will be, relatively speaking, cheap to get to market (compared to a new drug, for example). The haemofilter has the potential to have a significant human and economic impact, helping to reduce the overall malaria burden and cost. It could prove an invaluable new tool in the fight against current and future drug resistance.

However, the device's potential needs to be viewed in the light of not only the technical challenges that still need to be faced, but also the commercial and logistical hurdles that will need to be overcome to get the device into the hands of those who could effectively use it. Malaria patients are often in difficult to reach areas and challenging environments, with limited medical facilities and often no electricity. Being a relatively complicated treatment (compared to tablets), the haemofilter will face significant adoption challenges,

particularly as, at first, it will only be possible to use it in a hospital environment.

Furthermore, which the costs have been kept low, they will still represent a barrier to adoption in many malaria endemic countries where neither patients nor the healthcare systems may be able to afford the treatment. Any company commercialising the haemofilter will need to face these difficulties, and will certainly require the assistance of the global anti malaria community, working with and getting support from organisations such as the Global Fund, WHO and Bill and Melinda Gates Foundation.

6.2.2 Other applications

Despite the focus on malaria, the haemofilter has been designed to be used in a wide variety of clinical applications through the use of magnetic labelling. Chapter 2 gives an idea of the type and number of potential applications and wealth of research in the area. It is currently impossible to identify which of these techniques will prove most successful in the long-term, but it is possible, if not likely, that magnetic haemofiltration will become a commonplace treatment for a variety of ailments in the near future. Should some of these applications come to light, the clinical and commercial potential of the haemofilter would increase drastically.

Those applications will all require a high performance magnetic haemofilter capable of removing the magnetically labelled targets with high efficiency and, more importantly, high throughput. The magnetic haemofilter presented in this thesis is unique in terms of the potential flow rates achievable in the scaled-up versions, providing the possibility of filtering several times a patient's TBV in a small time period. Its performance in capturing malaria infected cells is hugely promising when considering other applications

- they are very weakly magnetic compared to the MNP and magnetic beads used in magnetic labelling techniques. The haemofilter should therefore be able to achieve better magnetic particle separation efficiencies at much higher flow rates, potentially reducing total treatment times to minutes.

While the device unquestionably holds the potential to treat a wide variety of diseases and conditions, each will have its own significant challenges in terms of development. The biggest will actually be in the development of the magnetic particles, which will need to able to survive in the bloodstream and be effective at labelling the targets whilst not labelling any other blood components. They will need to go through a stringent and expensive regulatory approval process, and clinical trials will be required for each new application.

6.3 Future work

6.3.1 Optimisation

There are many further tests that could be conducted to improve the design and optimise the performance of the haemofilter. These include:

- Further investigations into the relationship between the flow rate and separation efficiency, especially using whole blood from patients.
- Increased understanding of which infected cell stages are captured, and improved capture of late ring-stage cells.
- Performance variation across different samples, for example with varied initial parasitaemia or different malaria species.

- Optimisation of the ferromagnetic mesh in terms of materials and dimensions.
- Increased homogeneity of flow rates, particularly for the larger clinical versions.

The overall goal should be to maximise first the separation efficiency and then the flow rate, as demonstrated by the analysis in this thesis. Beyond that, investigations into whether the device can capture gametocytes as well as infected erythrocytes could prove very interesting. Their successful capture would imply that the device could have an effect on malaria transmission rates, although this would require its own study. Establishing whether the capture of circulating gametocytes in hospitalised patients has an impact on the spread of drug resistance, for example, would be a challenging research project.

The safety of the device, most importantly any potential affect on blood components, needs to be established beyond doubt. This includes ensuring that there is no false positive capture of uninfected cells. While the experiments detailed in section 4.4 are a good start in terms of testing safety, more work is required, notably continuous flow experiments conducted with the blood at body temperature. These would consist of a relatively large sample of blood (e.g. 500ml) continuously circulated through the haemofilter, with regular monitoring to check for any harmful or undesirable effect. It will be particularly important to monitor for false positive capture, haemolysis and activation of WBC.

Furthermore, the protocol for using the magnetic haemofilter as a medical device needs to be well-defined, with appropriate minimisation of any risks associated with its use. One key issue is the priming of the device, ensuring that no air bubbles are formed within the blood circuit. The current design of the chamber makes guaranteed elimination of all air within it during priming a significant challenge, and this will need to be considered throughout the product development. One solution may be to ship the chambers and

circuits 'pre-primed' with saline, although this could have implications in terms of shelf-life and maintaining integrity during shipping.

Another important consideration is the use of anticoagulant and its dosage, ensuring that any coagulation within the separation chamber can be avoided while utilising dosages which remain safe for the patient.

6.3.2 Medical device development

For the magnetic haemofilter to become a successful medical device, a lot of further development is required. In any country, a medical device needs approval from the relevant regulators before it can be sold, probably with CE marking (EU) and/or FDA approval (USA). Often devices imported into other countries need to be granted approval in the country in which they are going to be sold (e.g. India). This is typically granted on the back of approval from the manufacturer's country. As such, for this device, CE marking is the ultimate goal.

In order to get regulatory approval, the device must be shown to be both safe and effective. This is done through benchtop toxicology studies, followed by safety trials on healthy subjects and finally clinical trials, when the device is shown to deliver some clinical benefit. The magnetic haemofilter will need to go through this process for each of the separation chambers, culminating in a clinical trial on malaria patients with sufficient participants to demonstrate that it increases survivability.

The clinical trials will need to answer a number of questions regarding the clinical benefits of the device. The most important are questions surrounding the device's effect on survival rates in cases of severe malaria:

- Does the device reduce mortality?
- How much does a patient's parasitaemia need to be reduced by to have a clinical benefit?
- If the device is used in combination with antimalarial drugs, are these administered before, during or after treatment with the haemofilter?
- How important is it to reduce parasitaemia quickly?
- Should patients in the ICU be treated constantly with the device, or just given a one-off treatment for a few hours upon arrival?

Other questions surround clinical benefits beyond reducing mortality, such as whether it can be used to treat drug resistant or other untreatable patients:

- Can the device deliver sustained clinical benefits without being used in combination with drugs?
- How often do drug resistant patients need to be treated with the haemofilter?
- Can the haemofilter trigger remission or help the patient build up a natural immunity?

Finally, the clinical benefits of magnetic haemofiltration combined with drug treatments for non-severe patients should be studied:

- Are symptoms eliminated more rapidly?
- Can drug dosages be reduced?
- Are side-effects eliminated?

All of these (and probably more) questions will need to be addressed at various clinical trials in order to demonstrate the device's effectiveness as a treatment for malaria. In order to progress down this road, the clinical-scale versions of the device will need to be manufactured and tested, along with an appropriate instrument comprising the magnetic assembly, peristaltic pump, UI, safety controls etc. These will all need to pass the required standards for medical devices and be part of appropriate trials.

Moving into the longer term, new versions of the device appropriate for use in more challenging environments (i.e. outside of a hospital setting) should be developed. These will need to build on and learn from observations regarding the safety of the device during hospital use. There will be risks when using the device, including infection of the patient through the device or cathetar, the formation of clots that can cause brain aneurysms, or an embolism caused by air bubbles within the blood circuit returning to the patient. In a hospital setting, the risk of these complications can be minimised and if they occur appropriate action can be taken quickly in order to minimise the danger to the patient. Outside of a hospital however, they could be fatal. A 'portable' version of the device will therefore need to be designed ensuring that these and other complications are rare and minimised, and that there is an established strategy for tackling them when/if they occur.

6.3.3 Other applications

The review in Chapter 2 highlights the wide potential clinical applications of magnetic haemofiltration. These range from the treatment of sepsis and viral infections, to blood detoxification and prevention of cancer metastasis. These applications still require a lot of investigation before becoming new clinical techniques, particularly in the development

and testing of the MNP or magnetic beads that enable them. Each of these will need its own clinical trials and regulatory approval.

The haemofilter has been designed to be flexible, allowing for a wide variety of flow rates and separation chamber dimensions and volumes. It should be possible to combine it with any promising new magnetic labelling techniques that emerge. However, it will be important to collaborate with other researchers as early as possible so as to tailor the device to their particular application. In particular, in applications where successful recovery from the separation chamber is more important than net removal from the bloodstream, such as the targeting of stem cells for transplantation or CTC for diagnosis of metastasis, recovery methods and post-processing protocols (e.g. centrifugation) will need to be established.

One of the first tasks will be to test the performance of the haemofilter in removing magnetic particles of various sizes from blood. In many of the potential applications, such as blood detoxification, the targets are much smaller in size than cells, so the effect of the drag force on a magnetic particle will be essentially the same whether it is bound to targets or not. The objectives of these experiments should be to:

1. *achieve very high separation efficiencies*: for some applications, particularly those where the magnetic labelling will take place within the extra-corporeal loop, it will be crucial to remove essentially 100% of the particles to prevent their circulation in the bloodstream.

2. *achieve very high flow rates*: demonstrate that the device could filter several times TBV in short time periods (e.g. less than 30 mins).

3. *test the removal of different targets*: use magnetic particles bound to specific targets

(e.g. toxins or cells) and demonstrate their removal from whole blood. Experiments regarding the magnetic labelling (testing binding efficiencies, binding times, specificity, blood halflife etc.) should be done separately. These experiments could be performed in collabortion with the research groups developing the magnetic particles.

Each new application will need to follow a similar development pathway to the malaria application - benchtop testing, evaluation of the clinical usefulness and commercial evaluation - before clinical trials are performed and regulatory approval sought. If magnetic haemofiltration is to achieve its potential, it is likely that several commercial and research organisations will perform this work on lots of different projects simultaneously.

6.4 Final words

The aim during this PhD was to design and test a magnetic haemofilter which could be used to remove magnetic agents directly from a patient's bloodstream, with a focus on evaluation of the device as a potential drug-free treatment for malaria. In this thesis, a proposed design for a high throughput, high power magnetic separator incorporated into an extra-corporeal loop is presented. The performance of the design was modelled and a scaled down version of the device, in which the relevant forces were the same as in clinical versions, was tested and shown to successfully and consistently remove malaria infected erythrocytes from blood samples.

The device has been designed to be cheap to manufacture and easy to use. It has the potential to deliver significant clinical benefits to a wide variety of malaria patients, saving lives in serious cases, providing a treatment option for currently untreatable patients, and

speeding up recovery in uncomplicated cases, while improving the efficacy and eliminating the side-effects of pharmaceutical drugs. The experiments performed show that it is effective but safe, and does not negatively affect other blood components. The commercial evaluation indicates that there is a gap in the market for such a device, and a large potential client base.

This work is merely the first step in the development of the magnetic haemofilter as a platform technology with a wide variety of clinical applications - a lot of work is still required if the device is ever going to be used to treat patients. The purpose of this thesis is to demonstrate the rich clinical potential of magnetic haemofiltration technology, which could provide valuable solutions and improvements in a huge number of areas. Despite many research groups working on the magnetic separation of biomaterials, this is the first piece of work presenting the development of a platform magnetic haemofilter able to unify these applications with a single medical device.

The objective of the haemofilter in this thesis is to treat patients and save lives. But beyond that, the work presented in this thesis forms the basis of the prediction that a whole new healthcare industry is on the cusp of being created, one that promises revolutionising new treatments and diagnostic techniques. It is a call for researchers to tap into the huge potential created by magnetic labelling technologies. It is a proposal to bring magnetism to the forefront of healthcare, providing a much needed alternative to the pharmaceutical treatments that have dominated for so long.

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Appendix A

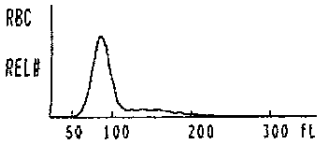
Haemogram results

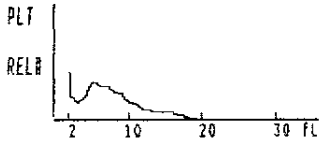
The document below shows the raw data from the complete blood counts discussed in section 4.4. Note that the patient numbering is different, as specified below:

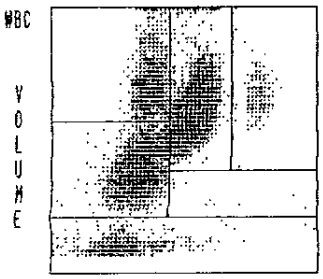
- GF1 →Patient 1 initial
- GF2 →Patient 1 filtered
- 71 →Patient 2 initial
- 73 →Patient 2 filtered
- 75 →Patient 2 recovered from chamber
- 86 →Patient 3 initial
- 88 →Patient 3 filtered
- 99 →Patient 4 initial
- 102 →Patient 4 filtered
- Wilmer Raigosa Restrepo (1) →Patient 5 initial
- Wilmer Raigosa Restrepo (2) →Patient 5 filtered
- Jaider Romero Meza (1) →Patient 6 initial
- Jaider Romero Meza (1) →Patient 6 filtered

The last four analyses were performed in the laboratories on site at El Bagre, and not at the Universidad de Antioquia, accounting for the different formatting and data provided.

05/09/14 15:30:15 MORFOLOGIA CELULAR U. de A. LHA
SN40504 OPR CALLE 64 N. 51D 158
4441333 EXT. 3614 BLOQUE 6 HUSVF







OF 1

Cass/Pos ID# 1 DATE: TIME:
S CBC+Diff FRANCISCO CASTAN 05/09/14 15:20:15

ID# 2 70925476

Location MALARIA
Physician
Date of Birth Sex
Comments

Sequence #
Date & Time
User field 1 MUESTRA GF 1
User field 2
User field 3

Abnormal WBC Pop			Normal RBC Pop			Abnormal PLT Pop		
WBC 7.2		10^3/uL	RBC 5.67		10^6/uL	PLT 81	RL	10^3/uL
NE% 64.5		%	HGB 16.4		g/dL	MPV 8.7	R	fL
LY% 19.7	L	%	HCT 49.4		%	PCT 0.070	R	%
MO% 11.3	H	%	MCV 87.2		fL	PDW 16.0	R	(ratio)
EO% 2.2		%	MCH 29.0		pg			
BA% 2.3	H	%	MCHC 33.3		g/dL			
NE# 4.6		10^3/uL	RDW 12.6		%			
LY# 1.4		10^3/uL				RET%		%
MO# 0.8	H	10^3/uL				RET#		10^6/uL
EO# 0.2		10^3/uL						
BA# 0.2		10^3/uL						

SUSPECT FLAGS:

WBC RBC PLT

DEFINITIVE FLAGS:

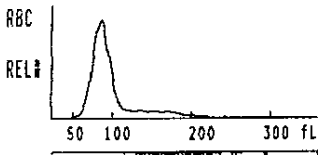
Lymphopenia %
Monocytosis %
Monocytosis #
Basophilia %

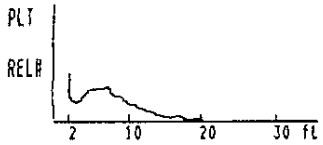
Thrombocytopenia

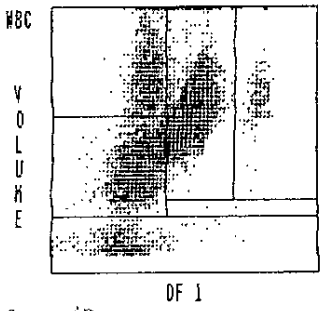
NORMAL RANGES

WBC	MF	4.5- 11.0	RBC	M	4.70- 6.10	F	4.20- 5.40	PLT	MF	150- 400
NE%	MF	43.0- 65.0	HGB	M	13.0- 18.0	F	12.0- 16.0	MPV	MF	7.4- 10.4
LY%	MF	20.5- 45.5	HCT	M	42.0- 52.0	F	37.0- 47.0	PCT	MF	0.000-0.990
MO%	MF	5.5- 11.7	MCV	M	80.0- 94.0	F	81.0- 99.0	PDW	MF	0.0- 99.9
EO%	MF	0.9- 2.9	MCH	MF	27.0- 31.0					
BA%	MF	0.2- 1.0	MCHC	MF	32.0- 36.0					
			RDW	MF	11.5- 15.5					
NE#	MF	2.2- 4.8								
LY#	MF	1.3- 2.9	RET%	M	0.60- 2.60	F	0.60- 2.60			
MO#	MF	0.3- 0.8	RET#	M	0.000-.9990	F	0.000-.9990			
EO#	MF	0.0- 0.2								
BA#	MF	0.0- 0.1								

05/09/14 15:31:39 MORFOLOGIA CELULAR U. de A. LHA
SN40504 OPR CALLE 64 N. 51D 158
4441333 EXT. 3614 BLOQUE 6 HUSVF







Cass/Pos ID# 1 DATE: TIME:
S CBC+Diff FRANCISCO CASTAN 05/09/14 15:21:46

ID# 2 70925476

Location MALARIA
Physician
Date of Birth Sex
Comments

Sequence #
Date & Time
User field 1 MUESTRA GF 2
User field 2
User field 3

Abnormal WBC Pop			Normal RBC Pop			Abnormal PLT Pop		
WBC 6.8		10^3/uL	RBC 5.27		10^6/uL	PLT 92	R	10^3/uL
NE% 61.6		%	HGB 15.4		g/dL	MPV 8.5	R	fL
LY% 25.5		%	HCT 45.3		%	PCT 0.078	R	%
MO% 10.9	H	%	MCV 85.9		fL	PDW 16.3	R	(ratio)
EO% 1.8		%	MCH 29.2		pg			
BA% 0.2		%	MCHC 34.0		g/dL			
NE# 4.3		10^3/uL	RDW 12.4		%			
LY# 1.7		10^3/uL				RET%		%
MO# 0.7		10^3/uL				RET#		10^6/uL
EO# 0.1		10^3/uL						
BA# 0.0		10^3/uL						

SUSPECT FLAGS:

---------WBC----------RBC----------PLT----------
DEFINITIVE FLAGS:
Monocytosis % Thrombocytopenia

NORMAL RANGES

WBC	MF	4.5- 11.0	RBC	M	4.70- 6.10	F	4.20- 5.40	PLT	MF	150- 400
NE%	MF	43.0- 65.0	HGB	M	13.0- 18.0	F	12.0- 16.0	MPV	MF	7.4- 10.4
LY%	MF	20.5- 45.5	HCT	M	42.0- 52.0	F	37.0- 47.0	PCT	MF	0.000-0.990
MO%	MF	5.5- 11.7	MCV	M	80.0- 94.0	F	81.0- 99.0	PDW	MF	0.0- 99.9
EO%	MF	0.9- 2.9	MCH	MF	27.0- 31.0					
BA%	MF	0.2- 1.0	MCHC	MF	32.0- 36.0					
			RDW	MF	11.5- 15.5					
NE#	MF	2.2- 4.8								
LY#	MF	1.3- 2.9	RET%	M	0.60- 2.60	F	0.60- 2.60			
MO#	MF	0.3- 0.8	RET#	M	0.000-.9990	F	0.000-.9990			
EO#	MF	0.0- 0.2								
BA#	MF	0.0- 0.1								

05/12/14 14:46:03 MORFOLOGIA CELULAR U. de A. LHA
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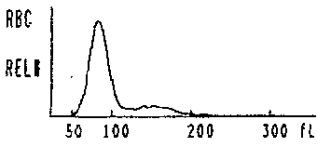
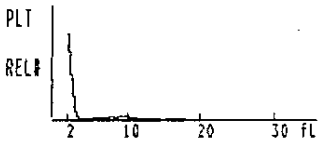
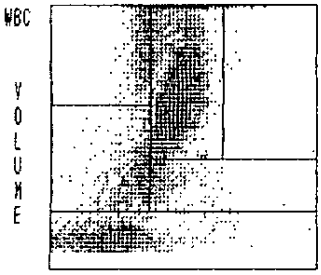
4441333 EXT. 3614 BLOQUE 6 HUSVF







OF 1

Cass/Pos ID# 1 DATE: TIME:
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ID# 2 43754867 (71)

Sequence #
Date & Time
Location MALARIA User field 1 MUESTRA 71
Physician User field 2
Date of Birth Sex F User field 3
Comments

Abnormal WBC Pop			Abnormal RBC Pop			Abnormal PLT Pop		
WBC 5.0	*R	10^3/uL	RBC 4.22		10^6/uL	PLT 36	RL	10^3/uL
NE% 84.0	H	%	HGB 12.1		g/dL	MPV 11.1	RH	fL
LY% 9.8	L	%	HCT 36.1		%	PCT 0.040	R	%
MO% 5.8		%	MCV 85.6		fL	PDW 15.3	R	(ratio)
EO% 0.4	L	%	MCH 28.7		pg			
BA% 0.0		%	MCHC 33.5		g/dL			
NE# 4.2	R	10^3/uL	RDW 13.4		%			
LY# 0.5	RL	10^3/uL				RET%		%
MO# 0.3	R	10^3/uL				RET#		10^6/uL
EO# 0.0	R	10^3/uL						
BA# 0.0	R	10^3/uL						

SUSPECT FLAGS:

Blasts	NRBCs	Giant Platelets
Imm Grans/Bands2	Micro RBCs/ RBC Fragments	

---------WBC----------RBC----------PLT----------

DEFINITIVE FLAGS:

Neutrophilia %		Thrombocytopenia
Lymphopenia %		Large Platelets
Lymphopenia #		

NORMAL RANGES

WBC	MF	4.5- 11.0	RBC	M	4.70- 6.10	F	4.20- 5.40	PLT	MF	150- 400
NE%	MF	43.0- 65.0	HGB	M	13.0- 18.0	F	12.0- 16.0	MPV	MF	7.4- 10.4
LY%	MF	20.5- 45.5	HCT	M	42.0- 52.0	F	37.0- 47.0	PCT	MF	0.000-0.990
MO%	MF	5.5- 11.7	MCV	M	80.0- 94.0	F	81.0- 99.0	PDW	MF	0.0- 99.9
EO%	MF	0.9- 2.9	MCH	MF	27.0- 31.0					
BA%	MF	0.2- 1.0	MCHC	MF	32.0- 36.0					
			RDW	MF	11.5- 15.5					
NE#	MF	2.2- 4.8								
LY#	MF	1.3- 2.9	RET%	M	0.60- 2.60	F	0.60- 2.60			
MO#	MF	0.3- 0.8	RET#	M	0.000-.9990	F	0.000-.9990			
EO#	MF	0.0- 0.2								
BA#	MF	0.0- 0.1								

05/12/14 14:46:55 MORFOLOGIA CELULAR U. de A. LHA
SN40504 OPR CALLE 64 N. 51D 158
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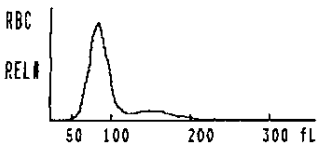

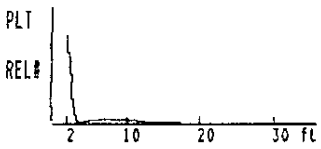

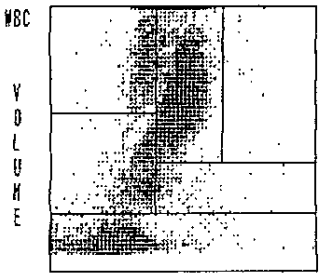

OF 1

Cass/Pos ID# 1 DATE: TIME:
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IDH 2 43745867 (73)

Location MALARIA
Physician
Date of Birth Sex F
Comments

Sequence #
Date & Time
User field 1 MUESTRA 73
User field 2
User field 3

Abnormal WBC Pop				Abnormal RBC Pop				Abnormal PLT Pop			
WBC	5.2	*R	10^3/uL	RBC	3.86		10^6/uL	PLT	43	RL	10^3/uL
NE%	77.8	H	%	HGB	11.2	L	g/dL	MPV	10.2	R	fL
LY%	11.1	L	%	HCT	33.1	L	%	PCT	0.044	R	%
MO%	10.0		%	MCV	85.9		fL	PDW	15.7	R	(ratio)
EO%	0.4	L	%	MCH	28.9		pg				
BA%	0.7		%	MCHC	33.7		g/dL				
NE#	4.1	R	10^3/uL	RDW	13.6		%				
LY#	0.6	RL	10^3/uL					RET%			%
MO#	0.5	R	10^3/uL					RET#			10^6/uL
EO#	0.0	R	10^3/uL								
BA#	0.0	R	10^3/uL								

SUSPECT FLAGS:

Blasts	NRBCs	Giant Platelets
Imm Grans/Bands2	Micro RBCs/ RBC Fragments	

--------WBC----------RBC----------PLT---------

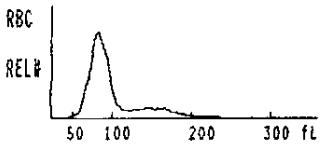
DEFINITIVE FLAGS:

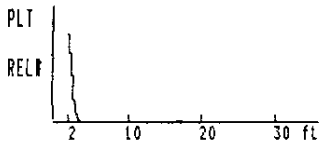
Neutrophilia %	Anemia	Thrombocytopenia
Lymphopenia %		
Lymphopenia #		

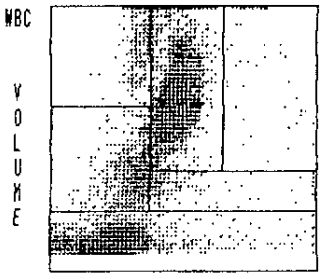
NORMAL RANGES

WBC	MF	4.5- 11.0	RBC	M	4.70- 6.10	F	4.20- 5.40	PLT	MF	150- 400
NE%	MF	43.0- 65.0	HGB	M	13.0- 18.0	F	12.0- 16.0	MPV	MF	7.4- 10.4
LY%	MF	20.5- 45.5	HCT	M	42.0- 52.0	F	37.0- 47.0	PCT	MF	0.000-0.990
MO%	MF	5.5- 11.7	MCV	M	80.0- 94.0	F	81.0- 99.0	PDW	MF	0.0- 99.9
EO%	MF	0.9- 2.9	MCH	MF	27.0- 31.0					
BA%	MF	0.2- 1.0	MCHC	MF	32.0- 36.0					
			RDW	MF	11.5- 15.5					
NE#	MF	2.2- 4.8								
LY#	MF	1.3- 2.9	RET%	M	0.60- 2.60	F	0.60- 2.60			
MO#	MF	0.3- 0.8	RET#	M	0.000-.9990	F	0.000-.9990			
EO#	MF	0.0- 0.2								
BA#	MF	0.0- 0.1								

05/12/14 14:48:41 MORFOLOGIA CELULAR U. de A. LHA
SM40504 OPR CALLE 64 N. 51D 158
4441333 EXT. 3614 BLOQUE 6 HUSVF







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ID# 2 43745867 (75)

Location MALARIA
Physician
Date of Birth Sex F
Comments

Sequence #
Date & Time
User field 1 MUESTRA 75
User field 2
User field 3

Abnormal WBC Pop				Abnormal RBC Pop				Abnormal PLT Pop			
WBC	3.4	*RL	10^3/uL	RBC	6.83	H	10^6/uL	PLT	14	RL	10^3/uL
NE%	80.7	H	%	HGB	19.0	H	g/dL	MPV	-----		fL
LY%	13.1	L	%	HCT	58.1	H	%	PCT	0.012	R	%
MO%	5.2		%	MCV	85.1		fL	PDW	16.0	R	(ratio)
EO%	1.0		%	MCH	27.9		pg				
BA%	0.0		%	MCHC	32.7	L	g/dL				
NE#	2.8	R	10^3/uL	RDW	13.8	H	%				
LY#	0.4	RL	10^3/uL					RET%			%
MO#	0.2	R	10^3/uL					RET#			10^6/uL
EO#	0.0	R	10^3/uL								
BA#	0.0	R	10^3/uL								

SUSPECT FLAGS:

WBC	RBC	PLT
Blasts	NRBCs	
Imm Grans/Bands2		

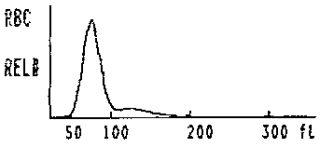
DEFINITIVE FLAGS:

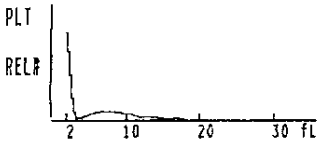
WBC	RBC	PLT
Leukopenia	Erythrocytosis	Thrombocytopenia
Neutrophilia %		
Lymphopenia %		
Lymphopenia #		

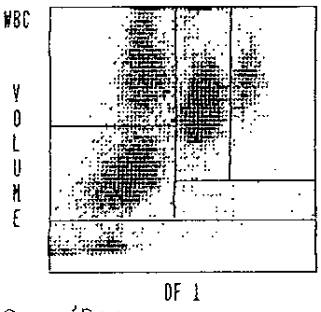
NORMAL RANGES

WBC	MF	4.5- 11.0	RBC	M	4.70- 6.10	F	4.20- 5.40	PLT	MF	150- 400
NE%	MF	43.0- 65.0	HGB	M	13.0- 18.0	F	12.0- 16.0	MPV	MF	7.4- 10.4
LY%	MF	20.5- 45.5	HCT	M	42.0- 52.0	F	37.0- 47.0	PCT	MF	0.000-0.990
MO%	MF	5.5- 11.7	MCV	M	80.0- 94.0	F	81.0- 99.0	PDW	MF	0.0- 99.9
EO%	MF	0.9- 2.9	MCH	MF	27.0- 31.0					
BA%	MF	0.2- 1.0	MCHC	MF	32.0- 36.0					
			RDW	MF	11.5- 15.5					
NE#	MF	2.2- 4.8								
LY#	MF	1.3- 2.9	RET%	M	0.60- 2.60	F	0.60- 2.60			
MO#	MF	0.3- 0.8	RET#	M	0.000-.9990	F	0.000-.9990			
EO#	MF	0.0- 0.2								
BA#	MF	0.0- 0.1								

05/12/14 14:49:22 MORFOLOGIA CELULAR U. de A. LHA
SN40504 OPR CALLE 64 N. 51D 158
4441333 EXT. 3614 BLOQUE 6 HUSVF







Cass/Pos ID# 1 DATE: TIME:
S CBC+Diff JESUS D. DIAZ C. 05/12/14 14:43:15

ID# 2 1007824393 (86)
Location MALARIA
Physician
Date of Birth Sex M
Comments

Sequence #
Date & Time
User field 1 MUESTRA 86
User field 2
User field 3

Abnormal WBC Pop			Abnormal RBC Pop			Abnormal PLT Pop		
WBC 8.1		10^3/uL	RBC 4.34		10^6/uL	PLT 135	RL	10^3/uL
NE% 45.7		%	HGB 10.8	L	g/dL	MPV 9.7	R	fL
LY% 29.0		%	HCT 32.6	L	%	PCT 0.131	R	%
MO% 20.9	H	%	MCV 75.0	L	fL	PDW 15.2	R	(ratio)
EO% 4.0		%	MCH 24.9	L	pg			
BA% 0.4		%	MCHC 33.1		g/dL			
NE# 3.8		10^3/uL	RDW 14.3	H	%			
LY# 2.3		10^3/uL				RET%		%
MO# 1.7	H	10^3/uL				RET#		10^6/uL
EO# 0.3		10^3/uL						
BA# 0.0		10^3/uL						

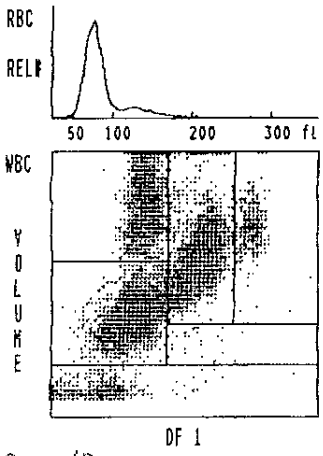
SUSPECT FLAGS:
Blasts

---------WBC----------RBC----------PLT---------
DEFINITIVE FLAGS:
Monocytosis % Anemia Thrombocytopenia
Monocytosis # 2+ Microcytosis
2+ Hypochromia

NORMAL RANGES

WBC	MF	4.5- 11.0	RBC	M	4.70- 6.10	F	4.20- 5.40	PLT	MF	150- 400
NE%	MF	43.0- 65.0	HGB	M	13.0- 18.0	F	12.0- 16.0	MPV	MF	7.4- 10.4
LY%	MF	20.5- 45.5	HCT	M	42.0- 52.0	F	37.0- 47.0	PCT	MF	0.000-0.990
MO%	MF	5.5- 11.7	MCV	M	80.0- 94.0	F	81.0- 99.0	PDW	MF	0.0- 99.9
EO%	MF	0.9- 2.9	MCH	MF	27.0- 31.0					
BA%	MF	0.2- 1.0	MCHC	MF	32.0- 36.0					
			RDW	MF	11.5- 15.5					
NE#	MF	2.2- 4.8								
LY#	MF	1.3- 2.9	RET%	M	0.60- 2.60	F	0.60- 2.60			
MO#	MF	0.3- 0.8	RET#	M	0.000-.9990	F	0.000-.9990			
EO#	MF	0.0- 0.2								
BA#	MF	0.0- 0.1								

05/12/14 14:50:03 MORFOLOGIA CELULAR U. de A. LHA
SN40504 OPR CALLE 64 N. 51D 158
4441333 EXT. 3614 BLOQUE 6 HUSVF



Cass/Pos ID# 1 DATE: TIME:
S CBC+Diff JESUS D. DIAZ C. 05/12/14 14:44:52

ID# 2 1007824393 (88)
Sequence #
Date & Time
Location MALARIA User field 1 MUESTRA 88
Physician User field 2
Date of Birth Sex M User field 3
Comments

Abnormal WBC Pop			Abnormal RBC Pop			Abnormal PLT Pop		
WBC 8.1		10^3/uL	RBC 4.36		10^6/uL	PLT 138	RL	10^3/uL
NE% 45.2		%	HGB 10.9	L	g/dL	MPV 9.9	R	fL
LY% 30.5		%	HCT 33.1	L	%	PCT 0.136	R	%
MO% 19.8	H	%	MCV 75.8	L	fL	PDW 15.2	R	(ratio)
EO% 4.2		%	MCH 25.0	L	pg			
BA% 0.3		%	MCHC 33.0		g/dL			
NE# 3.7		10^3/uL	RDW 14.1	H	%			
LY# 2.5		10^3/uL				RET%		%
MO# 1.6	H	10^3/uL				RET#		10^6/uL
EO# 0.3		10^3/uL						
BA# 0.0		10^3/uL						

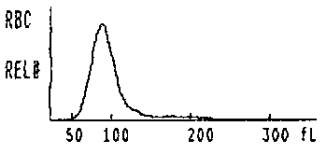
SUSPECT FLAGS:
Variant Lymphs

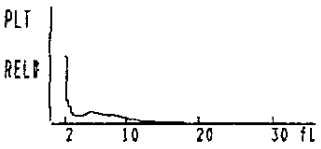
--------WBC----------RBC----------PLT----------
DEFINITIVE FLAGS:
Monocytosis % Anemia Thrombocytopenia
Monocytosis # 2+ Microcytosis
2+ Hypochromia

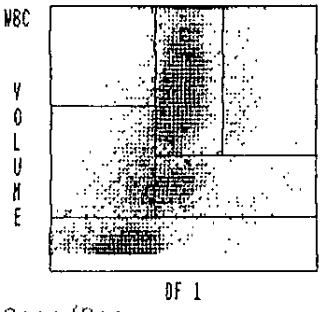
NORMAL RANGES

WBC	MF	4.5- 11.0	RBC	M	4.70- 6.10	F	4.20- 5.40	PLT	MF	150- 400
NE%	MF	43.0- 65.0	HGB	M	13.0- 18.0	F	12.0- 16.0	MPV	MF	7.4- 10.4
LY%	MF	20.5- 45.5	HCT	M	42.0- 52.0	F	37.0- 47.0	PCT	MF	0.000-0.990
MO%	MF	5.5- 11.7	MCV	M	80.0- 94.0	F	81.0- 99.0	PDW	MF	0.0- 99.9
EO%	MF	0.9- 2.9	MCH	MF	27.0- 31.0					
BA%	MF	0.2- 1.0	MCHC	MF	32.0- 36.0					
			RDW	MF	11.5- 15.5					
NE#	MF	2.2- 4.8								
LY#	MF	1.3- 2.9	RET%	M	0.60- 2.60	F	0.60- 2.60			
MO#	MF	0.3- 0.8	RET#	M	0.000-.9990	F	0.000-.9990			
EO#	MF	0.0- 0.2								
BA#	MF	0.0- 0.1								

05/15/14 15:14:43 MORFOLOGIA CELULAR U. de A. LHA
SN40504 OPR CALLE 64 N. 51D 158
4441333 EXT. 3614 BLOQUE 6 HUSVF







Cass/Pos ID# 1 DATE: TIME:
S CBC+Diff JORGE GONZALEZ 0 05/15/14 12:42:38

ID# 2 78672575 (99)

Location MALARIA
Physician
Date of Birth Sex M
Comments

Sequence #
Date & Time
User field 1 MUESTRA 99
User field 2
User field 3

Abnormal WBC Pop				Abnormal RBC Pop				Abnormal PLT Pop			
WBC	5.6	*R	10^3/uL	RBC	4.38		10^6/uL	PLT	72	RL	10^3/uL
NE%	78.5	H	%	HGB	12.1		g/dL	MPV	8.8	R	fL
LY%	19.3	L	%	HCT	37.2		%	PCT	0.063	R	%
MO%	0.7	L	%	MCV	85.0		fL	PDW	15.5	R	(ratio)
EO%	1.5		%	MCH	27.6		pg				
BA%	0.0		%	MCHC	32.5	L	g/dL				
NE#	4.4	R	10^3/uL	RDW	15.8	H	%				
LY#	1.1	RL	10^3/uL					RET%			%
MO#	0.0	R	10^3/uL					RET#			10^6/uL
EO#	0.1	R	10^3/uL								
BA#	0.0	R	10^3/uL								

SUSPECT FLAGS:

Blasts	NRBCs	Giant Platelets
Imm Grans/Bands2	Micro RBCs/ RBC Fragments	

--------WBC----------RBC----------PLT---------

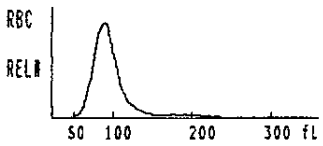
DEFINITIVE FLAGS:

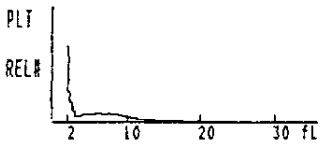
Neutrophilia %	Thrombocytopenia
Lymphopenia %	
Lymphopenia #	

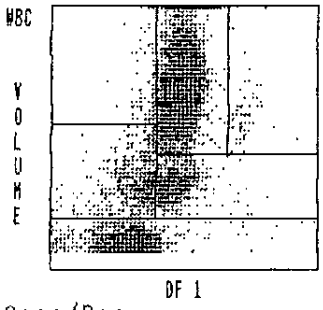
NORMAL RANGES

WBC	MF	4.5- 11.0	RBC	M	4.70- 6.10	F	4.20- 5.40	PLT	MF	150- 400
NE%	MF	43.0- 65.0	HGB	M	13.0- 18.0	F	12.0- 16.0	MPV	MF	7.4- 10.4
LY%	MF	20.5- 45.5	HCT	M	42.0- 52.0	F	37.0- 47.0	PCT	MF	0.000-0.990
MO%	MF	5.5- 11.7	MCV	M	80.0- 94.0	F	81.0- 99.0	PDW	MF	0.0- 99.9
EO%	MF	0.9- 2.9	MCH	MF	27.0- 31.0					
BA%	MF	0.2- 1.0	MCHC	MF	32.0- 36.0					
			RDW	MF	11.5- 15.5					
NE#	MF	2.2- 4.8								
LY#	MF	1.3- 2.9	RET%	M	0.60- 2.60	F	0.60- 2.60			
MO#	MF	0.3- 0.8	RET#	M	0.000-.9990	F	0.000-.9990			
EO#	MF	0.0- 0.2								
BA#	MF	0.0- 0.1								

05/15/14 15:14:26 MORFOLOGIA CELULAR U. de A. LHA
SN40504 OPR 4441333 EXT. 3614 CALLE 64 N. 51D 158
BLOQUE 6 HUSVF







Cass/Pos ID# 1 DATE: TIME:
S CBC+Diff JORGE GONZALEZ O 05/15/14 12:41:04

ID# 2 78672575 (102)

Location MALARIA
Physician
Date of Birth Sex
Comments

Sequence #
Date & Time
User field 1 MUESTRA 102
User field 2
User field 3

Abnormal WBC Pop				Abnormal RBC Pop				Abnormal PLT Pop			
WBC	4.4	*R	10^3/uL	RBC	4.52		10^6/uL	PLT	64	RL	10^3/uL
NE%	78.8	H	%	HGB	12.4		g/dL	MPV	8.9	R	fL
LY%	18.4	L	%	HCT	38.8		%	PCT	0.057	R	%
MO%	1.5	L	%	MCV	85.9		fL	PDW	15.5	R	(ratio)
EO%	1.3		%	MCH	27.5	L	pg				
BA%	0.0		%	MCHC	32.0	L	g/dL				
NE#	3.4	R	10^3/uL	RDW	15.7	H	%				
LY#	0.8	RL	10^3/uL					RET%			%
MO#	0.1	R	10^3/uL					RET#			10^6/uL
EO#	0.1	R	10^3/uL								
BA#	0.0	R	10^3/uL								

SUSPECT FLAGS:

Blasts	NRBCs	Giant Platelets
Imm Grans/Bands2	Micro RBCs/ RBC Fragments	

--------WBC----------RBC----------PLT---------

DEFINITIVE FLAGS:

Neutrophilia %	Thrombocytopenia
Lymphopenia %	
Lymphopenia #	

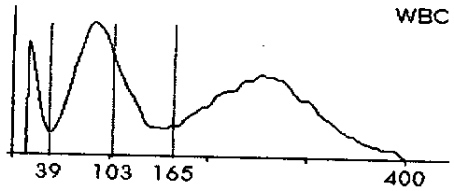
NORMAL RANGES

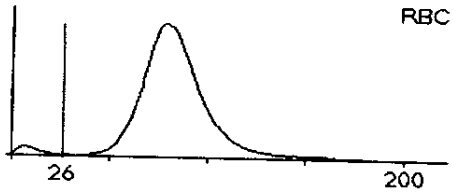
WBC	MF	4.5- 11.0	RBC	M	4.70- 6.10	F	4.20- 5.40	PLT	MF	150- 400
NE%	MF	43.0- 65.0	HGB	M	13.0- 18.0	F	12.0- 16.0	MPV	MF	7.4- 10.4
LY%	MF	20.5- 45.5	HCT	M	42.0- 52.0	F	37.0- 47.0	PCT	MF	0.000-0.990
MO%	MF	5.5- 11.7	MCV	M	80.0- 94.0	F	81.0- 99.0	PDW	MF	0.0- 99.9
EO%	MF	0.9- 2.9	MCH	MF	27.0- 31.0					
BA%	MF	0.2- 1.0	MCHC	MF	32.0- 36.0					
			RDW	MF	11.5- 15.5					
NE#	MF	2.2- 4.8								
LY#	MF	1.3- 2.9	RET%	M	0.60- 2.60	F	0.60- 2.60			
MO#	MF	0.3- 0.8	RET#	M	0.000-.9990	F	0.000-.9990			
EO#	MF	0.0- 0.2								
BA#	MF	0.0- 0.1								

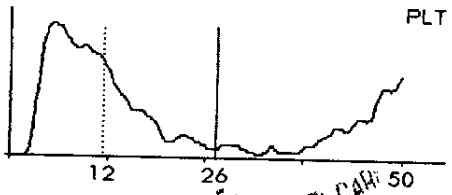
Abacus 380
ESE HNSC

Identificación de muestra	00	Nombre	WILMER RAIGOSA RESTREPO
ID paciente	1040512091	Fecha de Nacimiento	00/00/0000
Tipo	Masculino	Sexo	Masculino
Fecha de análisis	18/11/2014 03:22 PM	No. De serie:	640699
Fecha de reporte	18/11/2014 03:22 PM	Doctor	

WBC	5.90	10³/µl	5.00	10.00
LYM	2.28	10³/µl	1.30	4.00
MID	0.44	10³/µl	0.15	0.70
GRA	3.19	10³/µl	2.50	7.50
LYM%	38.6	%	25.0	40.0
MID%	7.5	%	3.0	7.0
GRA%	54.0	%	50.0	75.0
RBC	4.96	10⁶/µl	4.50	5.60
HGB	14.4	g/dl	14.0	17.4
HCT	41.71	%	42.00	50.00
MCV	84	fl	84	96
MCH	29.1	pg	27.0	32.0
MCHC	34.6	g/dl	30.0	35.0
RDWc	16.4	%		
PLT	73	10³/µl	150	400
PCT	0.08	%		
MPV	10.9	fl	8.0	15.0
PDWc	41.0	%		
P-LCC	25	10³/µl		
P-LCR	34.74	%		







Comentario:

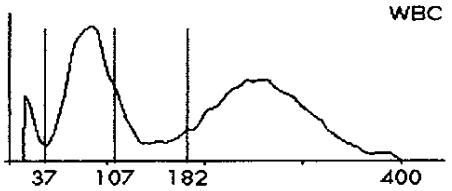
E.S.E. NUESTRA SEÑORA DEL CARMEN SAGRADA ANTIOQUIA

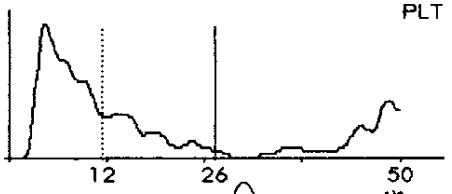
Abacus 380
ESE HNSC

Identificación de muestra	00	Nombre	WILMER RAIGOSA RESTREPO
ID paciente	1040512091	Fecha de Nacimiento	00/00/0000
Tipo	Masculino	Sexo	Masculino

Fecha de análisis	18/11/2014 05:14 PM	No. De serie:	640699
Fecha de reporte	18/11/2014 05:16 PM	Doctor	

WBC	4.91 -	10*/µl	5.00	10.00
LYM	2.01	10*/µl	1.30	4.00
MID	0.20	10*/µl	0.15	0.70
GRA	2.70	10*/µl	2.50	7.50
LYM%	40.9 +	%	25.0	40.0
MID%	4.1	%	3.0	7.0
GRA%	55.0	%	50.0	75.0
RBC	4.19 -	10*/µl	4.50	5.60
HGB	12.5 -	g/dl	14.0	17.4
HCT	35.35 -	%	42.00	50.00
MCV	84	fl	84	96
MCH	29.9	pg	27.0	32.0
MCHC	35.5 +	g/dl	30.0	35.0
RDWc	16.2	%		
PLT	50 -	10*/µl	150	400
PCT	0.05	%		
MPV	10.4	fl	8.0	15.0
PDWc	39.2	%		
P-LCC	15	10*/µl		
P-LCR	30.94	%		





Comentario:

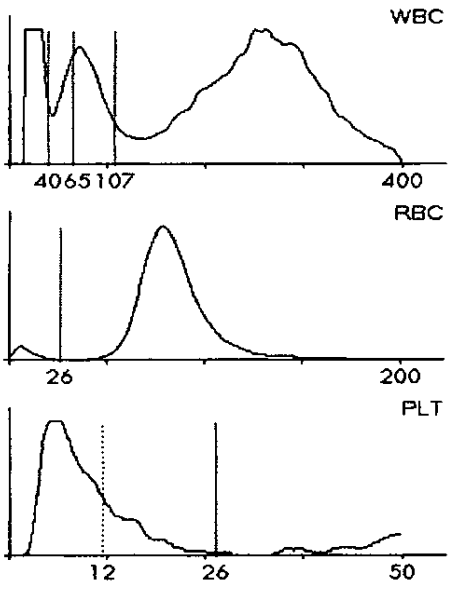
E.S.E. HOSPITAL NUESTRA SEÑORA DEL CARMEN
LABORATORIO

Abacus 380
ESE HNSC

Identificación de muestra	00	Nombre	JAIDER ROMERO MEZA
ID paciente	1049318682	Fecha de Nacimiento	00/00/0000
Tipo	Masculino	Sexo	Masculino

Fecha de análisis	18/11/2014 03:25 PM	No. De serie:	640699
Fecha de reporte	18/11/2014 03:26 PM	Doctor	

WBC	6.04	10*/µl	5.00	10.00
LYM	0.67 -	10*/µl	1.30	4.00
MID	0.75 +	10*/µl	0.15	0.70
GRA	4.62	10*/µl	2.50	7.50
LYM%	11.1 -	%	25.0	40.0
MID%	12.4 +	%	3.0	7.0
GRA%	76.5 +	%	50.0	75.0
RBC	4.38 -	10*/µl	4.50	5.60
HGB	13.0 -	g/dl	14.0	17.4
HCT	37.12 -	%	42.00	50.00
MCV	85	fl	84	96
MCH	29.6	pg	27.0	32.0
MCHC	34.9	g/dl	30.0	35.0
RDWc	15.4	%		
PLT	98 -	10*/µl	150	400
PCT	0.09	%		
MPV	9.6	fl	8.0	15.0
PDWc	38.5	%		
P-LCC	24	10*/µl		
P-LCR	24.49	%		



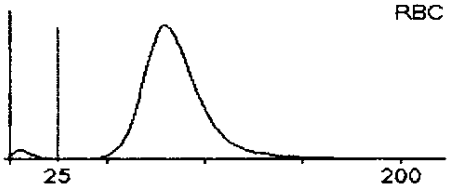
Comentario:

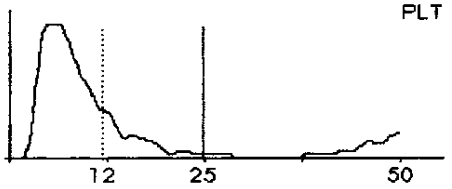
Abacus 380
ESE HNSC

Identificación de muestra	00	Nombre	JAIDER ROMERO MEZA
ID paciente	1049318682	Fecha de Nacimiento	00/00/0000
Tipo	Masculino	Sexo	Masculino

Fecha de análisis	18/11/2014 05:18 PM	No. De serie:	640699
Fecha de reporte	18/11/2014 05:19 PM	Doctor	

WBC	4.75 -	10*/µl	5.00	10.00
LYM	1.14 -	10*/µl	1.30	4.00
MID	0.27	10*/µl	0.15	0.70
GRA	3.34	10*/µl	2.50	7.50
LYM%	24.0 -	%	25.0	40.0
MID%	5.7	%	3.0	7.0
GRA%	70.3	%	50.0	75.0
RBC	4.58	10*/µl	4.50	5.60
HGB	13.6 -	g/dl	14.0	17.4
HCT	38.90 -	%	42.00	50.00
MCV	85	fl	84	96
MCH	29.7	pg	27.0	32.0
MCHC	34.9	g/dl	30.0	35.0
RDWc	15.6	%		
PLT	57 -	10*/µl	150	400
PCT	0.05	%		
MPV	8.9	fl	8.0	15.0
PDWc	36.4	%		
P-LCC	11	10*/µl		
P-LCR	18.76	%		





Comentario:

Appendix B

Survey of malaria patients

B.1 Summary

- *Respondents*: 45
- *Questions*: 14
- *Date of survey*: August 2014
- *Method*: Online, using Survey Money (`https://www.surveymonkey.com/s/MalariaPatients`)

B.2 Questions

1. Are you answering for yourself or on behalf of someone else?
2. Are you/they male or female?
3. At what age did you/they most recently have malaria?
4. In which country did you/they most recently:
   (a) Contract malaria?
   (b) Get treated?
5. What type of malaria did you/they have?
6. Was this your/their first case of malaria?
7. In total, for how long did you/they feel unwell?
8. Please tick all symptoms that you/they had (please specify others)
9. Did you/they take any time off work/school (please specify how many days)?
10. How long after the first symptoms did you/they get treatment?
11. After getting treatment, when were you able to resume normal activities (like return to work or school)?

12. Where did you/they go to get treatment?
13. Please describe the treatment in as much detail as possible.
14. In total, approximately how much did you/they pay for the treatment (please input amount and currency)?

B.3 Responses

	Q1	Q2	Q3	Q4 (a)	Q4 (b)	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14
#1	Myself	M	21-29	Togo	Togo	Don't know	No, 7 or 8 times	>7 days	Fever, headache, dizziness, cold sweats	Yes, 1 week	1-2 days	>7 days	Private clinic	2/3 sessions with IV drip	DNA
#2	Myself	M	21-29	Togo	Togo	Don't know	No, 8 times	>7 days	Headache, vomiting, fever, breathing difficulties, exhaustion	Yes, 1 week	<24 hours	3-4 days	Private clinic	Daily IV drip	$110 (£72)
#3	Myself	F	13-17	Togo	Germany	Don't know	Yes	>7 days	Fever, vomiting	Yes, 5 days	<24 hours	>7 days	Public clinic	DNA	Insurance
#4	Myself	F	6-12	Nigeria	Nigeria	Falciparum	No, 10 times	3-4 days	Fever, vomiting, high temperature, loss of appetite, body ache	5 days	3-4 days	4-7 days	Private clinic	Injections (novajine, naloxone, chloroquine), with paracetamol and supplements	500 Naira (£1.80)
#5	Myself	F	6-12	Côte d'Ivoire	Côte d'Ivoire	Don't know	No, 2 times	3-4 days	Headache, fever	No	<24 hours	1-2 days	At home	Flavoquine and aspirin	DNA
#6	Myself	F	5 or younger	Togo	Togo	Don't know	No, 2-3 times/year	3-4 days	Headache, fever, breathing difficulties	Yes, 1 day	<24 hours	3-4 days	DNA	African herbs from Togo	DNA
#7	Someone else	F	21-29	Togo	Togo	Don't know	Yes	>7 days	Headache, vomiting, fever, breathing difficulties, haematuria	Yes, 2 weeks	DNA	DNA	DNA	DNA	DNA
#8	Myself	F	6-12	Togo	Togo	Don't know	No, 10 times	3-4 days	Headache, vomiting, fever	Yes, 1 week	1-2 days	3-4 days	Private hospital	IV and malaria pills	CFA 50,000 (£60)
#9	Someone else	F	13-17	Tanzania	Tanzania	Vivax	No, many times	3-4 days	Fever	No	3-4 days	3-4 days	Public hospital	Malafin	DNA
#10	Myself	M	30-39	Togo	Togo	Falciparum	No, many times	5-7 days	Headache, fever, bitter taste, shivering	Yes, 3 days	<24 hours	1-2 days	Private hospital	Quinimax or artesunate	CFA 10,000 (£12)
#11	Myself	M	18-20	Nigeria	Nigeria	Falciparum	No, 5 or more	3-4 days	Headache, vomiting, fever	Yes, DNA	1-2 days	1-2 days	Private clinic	Regular injections	350 Naira (£1.25)
#12	Myself	F	21-29	Togo	Togo	Don't know	No, twice	5-7 days	Headache, fever, joint pain	Yes, 4 days	1-2 days	1-2 days	Private clinic	Tablets	Pro-bono
#13	Myself	F	21-29	Togo	Togo	Falciparum	No, many times	5-7 days	Headache, vomiting, fever	Yes, 7 days	1-2 days	4-7 days	Private clinic	Lufanter cac 1000	CFA 5,000 (£6)
#14	Myself	F	5 or younger	Tanzania	Tanzania	Don't know	Yes	1-2 days	Fever	No	1-2 days	1-2 days	Father is doctor	Tablets	Nothing
#15	Myself	M	21-29	South Sudan	South Sudan	Vivax	No, twice	>7 days	Headache, vomiting, fever	Yes, 1 month	4-7 days	4-7 days	Private clinic	Artesunate	Insurance
#16	Myself	F	13-17	Benin	Benin	Don't know	No, 5-6 times	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA
#17	Myself	F	21-29	Zambia	Zambia	Falciparum	Yes	3-4 days	Headache, fever, fainting, tiredness	Yes, 4 days	1-2 days	3-4 days	Public hospital	Tablets	Nothing
#18	Myself	M	13-17	Togo	Togo	Falciparum	No, 3-4 times	5-7 days	Headache, fever	Yes, DNA	3-4 days	3-4 days	Private clinic	Perfusion, coartem, maloxine	DNA
#19	Myself	F	40-49	Ghana	Ghana	Don't know	No, many times	5-7 days	Headache, vomiting, fever, anaemia	Yes, two weeks total	<24 hours	3-4 days	Self-treat	Artesunate, ACT, doxycycline, iron supplements	£2 on average
#20	Myself	M	40-49	Nigeria	Nigeria	Falciparum	No, 25 times	5-7 days	Headache, fever, high temperature, loss of appetite, shivering	Yes, 1 week	3-4 days	>7 days	Private hospital	Chloroquine injection, paracetamol, aspirin, natural herbs (donoyaro/neem tree)	100 Naira (£0.35)
#21	Myself	F	13-17	Benin	France	Don't know	No, 3 times	>7 days	Vomiting, fever	Yes, 2 weeks	<24 hours	>7 days	Public hospital	Tablets	DNA
#22	Myself	F	21-29	Senegal	Senegal	Don't know	Yes	3-4 days	Headache, vomiting, fever, skin ache	Yes, 5 days	<24 hours	4-7 days	Private hospital	Tablets	DNA
#23	Myself	M	21-29	India	UK	Don't know	No, many times	>7 days	Vomiting, fever	Yes, 7-10 days	1-2 days	>7 days	Public hospital	Nothing	Nothing (NHS)
#24	Myself	M	40-49	Nigeria	Nigeria	Don't know	Yes	>7 days	Headache, fever, hypertension, coughing, chest pains	Yes, 3 days	1-2 days	3-4 days	Private hospital	Tablets, IV drip, painkillers	8,000 Naira (£28.50)
#25	Myself	F	21-29	Tanzania	Tanzania	Don't know	No, 3 times	3-4 days	Headache, dizziness, joint ache	Yes, 1 day	1-2 days	1-2 days	Public hospital	DNA	DNA
#26	Myself	M	40-49	The Gambia	The Gambia	Other	No, >10 times	5-7 days	Headache, vomiting, fever	Yes, 3 days	1-2 days	1-2 days	Private clinic	Nivaquine	$30 (£19.65)

#27	Myself	M	30-39	Kenya	Kenya	Don't know	No, 4 times	5-7 days	Headache, fever, joint pain	Yes, DNA	1-2 days	1-2 days	Private clinic	Antibiotics	Insurance
#28	Myself	F	21-29	Nigeria	Nigeria	Don't know	No, 4 times	1-2 days	Headache, fever	No	1-2 days	24 hours	Pharmacy	Tablets	200 Naira (£0.70)
#29	Myself	M	21-29	Malawi	Malawi	Falciparum	Yes	>7 days	Headache, fever, fatigue, seizures	Yes, 3 weeks	4-7 days	>7 days	Private hospital	Quinine drip, then tablets	£50
#30	Myself	M	30-39	Nigeria	Nigeria	Falciparum	No, 4-6 times/year	3-4 days	Headache, fever, Joint pain, quaesiness, loss of appetite	Yes, 2 days	<24 hours	3-4 days	Pharmacy	Artesunate	500 Naira (£1.80)
#31	Someone else	M	30-39	Mozambique	Switzerland	Falciparum	Yes	3-4 days	Headache, vomiting, fever	Yes, 1 week	<24 hours	3-4 days	Private hospital	DNA	DNA
#32	Myself	M	21-29	Angola	Angola	Falciparum	Yes	3-4 days	Headache, vomiting, fever, fainting	Yes, 2 days	1-2 days	3-4 days	Private clinic	Coartem	$500 (£328)
#33	Someone else	M	30-39	USA	USA	Don't know	Yes	3-4 days	Headache, vomiting, fever	No	1-2 days	<24 hours	Public hospital	DNA	DNA
#34	Someone else	M	5 or younger	Nigeria	Nigeria	Don't know	Yes	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA
#35	Myself	M	40-49	Nigeria	Nigeria	Don't know	No, 4 times/year	5-7 days	Headache, fever, muscle pain	Yes, <1 week	<24 hours	<24 hours	Pharmacy	Coartem	€17 (£13.30)
#36	Myself	F	30-39	Pakistan	Pakistan	Don't know	Yes	5-7 days	Headache, fever	Yes, 3 days	<24 hours	>7 days	Private hospital	Tablets	PKR 200 (£1.30)
#37	Myself	M	6-12	Nigeria	DNA	Vivax	Yes	5-7 days	Vomiting, fever	No	3-4 days	<24 hours	Private hospital	DNA	DNA
#38	Myself	F	21-29	India	India	Other	Yes	5-7 days	Fever, shivering	No	<24 hours	<24 hours	Private clinic	Quinine then other	INR 5,000 (£51.50)
#39	Myself	M	6-12	India	India	Don't know	Yes	>7 days	Fever, bronchitis	Yes, 3 weeks	1-2 days	>7 days	Home	Injections and tablets	DNA
#40	Myself	M	5 or younger	Nigeria	DNA	Falciparum	Yes	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA
#41	Myself	F	30-39	Côte d'Ivoire	Germany	Falciparum	Yes	>7 days	Fever	Yes, 2 weeks	3-4 days	>7 days	Public hospital	Lariam	Nothing
#42	Myself	M	6-12	Papua New Guinea	Australia	Don't know	Yes	>7 days	Headache, vomiting, fever	Yes, 6 weeks	3-4 days	>7 days	Public hospital	DNA	Nothing
#43	Myself	M	21-29	Ghana	Ghana	Vivax	Yes	>7 days	Headache, vomiting, fever, stiff joints	Yes, 10 days	1-2 days	4-7 days	Public clinic	DNA	GHS 10 (£2)
#44	Myself	M	21-29	India	India	Vivax	No, once	3-4 days	Fever	Yes, 2 days	<24 hours	3-4 days	Private hospital	Tablets	INR 600 (£6.20)
#45	Someone else	M	30-39	Ghana	Ghana	Falciparum	No, 3 times	5-7 days	Headache, vomiting, fever, weakness, sharp pains	Yes, 5-10 days	1-2 days	1-2 days	Hospital at gold mine	Quinine	$100 (£65)
#46	Myself	F	30-39	Madagascar	Madagascar	Don't know	No, 4 times	3-4 days	Headache, fever	No	<24 hours	<24 hours	Public hospital	Antibiotics	$250 (£162.50)

Appendix C

Ethics committee approval

	ACTA DE APROBACIÓN DE PROYECTOS COMITÉ DE BIOÉTICA INSTITUTO DE INVESTIGACIONES MÉDICAS FACULTAD DE MEDICINA UNIVERSIDAD DE ANTIOQUIA	FACULTAD DE MEDICINA	
UNIVERSIDAD DE ANTIOQUIA		CÓDIGO	BE-IIM
		VERSIÓN	01

Acta de aprobación No. 006

Nombre del proyecto: "Studying magnetic separation of malaria infected red blood cells from a patient´s bloodstream".

Investigador Principal: Cesar Segura

Versión No. 1

Enmienda revisada: NO

Fecha de aprobación: **24 de abril de 2014.**

El comité de ética del Instituto de investigaciones médicas se constituyó mediante resolución del Consejo de Facultad de 30 de mayo de 2008, acta 177 y está regido por los principios éticos vigentes en la Resolución 8430 de 1993, la Declaración de Helsinki de 2013 y la Resolución 2378 del 2008. En ellos se delinean las normas científicas, técnicas y administrativas para la investigación en seres humanos.

El Instituto de Investigaciones médicas certifica que:

1. Se revisaron los siguientes documentos en el presente proyecto:
   a. Resumen del proyecto (NO)
   b. Protocolo de investigación (SI)
   c. Formato de recolección de datos (SI)
   d. Formato de consentimiento informado (SI)
   e. Manual del investigador (NO)
   f. Evaluaciones de otros comités de ética (NO)

2. El proyecto fue aprobado por los siguientes miembros: Dr. Gabriel Jaime Montoya Montoya, Dr. José Antonio García P., Dr. Julio Cesar Bueno Sánchez, Dr. Carlos Julio Montoya Guarín y Sonia del Pilar Agudelo López.
3. El comité considera que el proyecto no contiene tensiones éticas que vulnere los derechos y el bienestar de los participantes. El riesgo involucrado en el estudio es:
   a) Sin riesgo ( )
   b) Riesgo mínimo (X)
   c) Riesgo mayor que el mínimo ( )

4. El comité considera que tanto la forma de obtención del consentimiento cuando aplica como las medidas tomadas para proteger el bienestar y los derechos de los participantes son adecuadas. No aplica
5. El comité se reserva el derecho de hacer nuevas revisiones del proyecto a solicitud de alguno o algunos de sus miembros o de las directivas institucionales con el fin de revisar lo relacionado con el bienestar y los derechos de los participantes en la investigación.
6. El comité deberá informar a las directivas institucionales correspondientes cualquier evento tocante con faltas de cumplimiento de las obligaciones del investigador en el desarrollo del proyecto, de las solicitudes del comité o suspensiones del proyecto por razones de tipo ético.
7. Se informara a la dirección del Instituto de Investigaciones sobre situaciones como: 1) efectos dañinos que se ocasionen a los participantes de esta investigación; 2) situaciones que signifiquen riesgos para los participantes o para personas independientes; 3) cambios ocurridos en el proyecto que fueran aprobados por el comité; y 4) situaciones distintas que de alguna manera puedan influenciar negativamente el buen desarrollo de la investigación.
8. La aprobación de este proyecto tendrá una duración de un año a partir de la fecha de aprobación; si se debe continuar por más tiempo, deberá someterse a aprobaciones anuales hasta la finalización del mismo. El investigador deberá anexar la documentación pertinente para cada nueva revisión del proyecto por parte del comité.

El investigador deberá informar al comité y al Instituto sobre los siguientes eventos:

a. Cambios que se realicen en el proyecto, los cuales deberán ser aprobados en una nueva sesión del comité.
b. Situaciones imprevistas que puedan implicar riesgos para los participantes.
c. Efectos adversos que ocurran en los participantes, en las 24 horas siguientes a su ocurrencia.
d. Alteraciones del rumbo de la investigación que alteren la adecuada proporción entre riesgos y beneficios.
e. Las decisiones tomadas por comités de ética de otras instituciones que participen en el proyecto.
f. Los informes parciales, finales o de suspensión temporal o permanente del proyecto, con las debidas razones que los justifiquen.

El investigador deberá presentar informes parciales del estudio cada (6) meses.

UNIVERSIDAD DE ANTIOQUIA	**ACTA DE APROBACIÓN DE PROYECTOS** **COMITÉ DE BIOÉTICA** **INSTITUTO DE INVESTIGACIONES MÉDICAS** **FACULTAD DE MEDICINA** **UNIVERSIDAD DE ANTIOQUIA**	**FACULTAD DE MEDICINA** CÓDIGO BE-IIM VERSIÓN 01

En este proyecto no se encontraron conflictos de interés por parte de los investigadores.

Sugerencias y comentarios: Los investigadores se acogieron a las recomendaciones de los evaluadores.

Nota: Para efectos de la investigación sólo podrá utilizarse el Consentimiento Informado avalado, con el sello del Comité de Bioética.

COMITÉ DE BIOMEDICA IIM
24 ABR 2014
Dr. Gabriel Jaime Montoya
Presidente

GABRIEL JAIME MONTOYA MONTOYA
Presidente
Comité de Bioética

Appendix D

Patient consent form

COMITE DE BIOETICA
I I M.
24 ABR 2014
APROBADO

Instituto de investigaciones Médicas. Facultad de Medicina. Universidad de Antioquia.

Título del proyecto. ***Estudio de la separación magnética de glóbulos rojos infectados con malaria a partir del torrente sanguíneo del paciente. (Studying magnetic separation of malaria infected red blood cells from patient´s bloodstream)***

Identificación de los investigadores.

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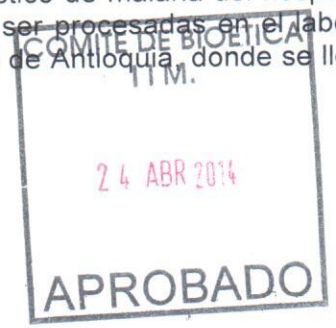
T: 020 3108 4127 M: 07962 232 340
E: q.pankhurst@ucl.ac.uk
W: www.ibme.ucl.ac.uk

Sitio donde se llevará a cabo el estudio.
Las muestras de sangre serán obtenidas en los puestos de diagnóstico de malaria del hospital local del municipios de el Bagre (Antioquia) y serán transportadas para ser procesadas en el laboratorio del Grupo Malaria, Sede de Investigación Universitaria, Universidad de Antioquia, donde se llevará a cabo el procedimiento.



Entidades que respaldan la investigación.
Universidad de Antioquia
Institute of Biomedical Engineering, University College of London.

Entidades que patrocinan la investigación.
Universidad de Antioquia, Grupo Malaria (Colombia)
Rosetress Trust (http://www.rosetreestrust.co.uk) and University College of London. Institute of Biomedical Engineering (United Kindom)

Información para el paciente

La malaria o paludismo es una enfermedad parasitaria que afecta la salud de la población en Colombia y que puede llegar a ser mortal si no se diagnostica y trata a tiempo. En Antioquia muchos pacientes consultan varias veces en el mismo año por presentar episodios de malaria. El Grupo Malaria de la Facultad de Medicina de la Universidad de Antioquia en colaboración con el Instituto de Ingeniería Biomédica de la Universidad College de Londres y mediante la investigación, "***Estudio de la separación magnética de glóbulos rojos infectados con malaria a partir del torrente sanguíneo del paciente***" desean conocer si un nuevo aparato es eficiente en remover los parásitos de la sangre sin necesidad de drogas antimaláricas o tratamientos químicos. La investigación se llevará a cabo en Medellín, Departamento de Antioquia, las muestras de sangre serán colectadas del municipio de El Bagre y se colectará una muestra de 50 mL de sangre venosa a 20 pacientes.

Usted ha sido diagnosticado con malaria y cumple con los requisitos para ser incluido en esta investigación, por esta razón lo estamos invitando a participar de ella. Su participación será de gran importancia para su comunidad y en general para los pacientes con malaria porque puede servir para el diseño de un tratamiento para pacientes con altas cantidades de parásitos de la malaria quienes pueden presentar una malaria grave; ello permitiría mejorar el proceso de recuperación de los pacientes. Antes de otorgar su consentimiento para participar, es importante que usted lea y comprenda la siguiente explicación acerca de los procedimientos del estudio y que nos dé una información básica para diligenciar una encuesta.

La participación en este estudio es libre y voluntaria, y usted tiene derecho a retirarse en cualquier momento. En caso de que no acepte participar su atención no se verá afectada.

Procedimiento.
Usted ingresará al estudio el día que se diagnostique con malaria. Un profesional del equipo investigador verificará que Usted no presenta signos de peligro o que no presenta una malaria grave; se le tomará una muestra de sangre del dedo para establecer que no tenga anemia. Si cumple que no tiene estas condiciones que impiden participar en el estudio, le tomará una única muestra de sangre por punción venosa. Una vez Ud. haya donado la sangre, se le administrará el tratamiento correspondiente para la malaria aprobado por el Ministerio de Salud y Protección Social y podrá regresar al examen de control el día 3 posterior al inicio del tratamiento, como lo indica la norma de atención del gobierno, para verificar que se ha curado.

COMITE DE BIOETICA
I I M.
24 ABR 2014
APROBADO

La muestra que se le tomará será de 50mL de sangre mediante punción venosa en uno de los brazos con sistema al vacío. A partir de estas muestras de sangre se prepara una muestra en una lámina de vidrio para confirmar el diagnóstico con microscopio y se depositarán 4 gotas de sangre sobre un papel especial para identificar genéticamente los parásitos. El resto de la sangre será aplicada para verificar si el aparato es eficiente en remover los parásitos. Esta muestra de sangre también será empleada para confirmar el diagnóstico de malaria con una prueba rápida que hará el personal del Grupo Malaria.

No se requerirán muestras de sangre por duplicado sin embargo las láminas de vidrio y la gotas sobre papel serán conservadas por 10 años en el centro responsable del estudio y se marcarán con un código que permite la identificación futura del caso (tipo de malaria, tratamiento dado). Después de este periodo de tiempo las muestras sobrantes serán destruidas. Las muestras obtenidas NO se podrán utilizar en el futuro para propósitos diferentes a los especificados en este trabajo. No se realizarán pruebas genéticas de los pacientes.

La toma de la muestra tiene riesgos mínimos para usted y consisten en un dolor leve en el sitio de punción de la vena o el dedo y enrojecimiento. Las personas que le tomarán las muestras de sangre han sido entrenadas para evitar en lo posible que sienta dolor y evitar infecciones o morados en el sitio de la punción. Para la toma de la muestra se usarán tubos al vacío nuevos y agujas o lancetas desechables y estériles.

Si presenta alguna complicación clínica por el procedimiento de toma de muestra, usted será remitido al hospital del municipio para su atención y el investigador del Grupo Malaria se asegurará de que reciba la atenciónadecuada.

Los gastos de la toma de la muestra y los análisis de las mismas son totalmente gratuitos para los participantes y serán pagados en su totalidad por el Grupo Malaria. El aparato que se aplicará a su sangre fue diseñado en la Universidad Colegio de Londres y será empleado por el Grupo Malaria gracias a un convenio que tiene con esta institución que le permite usarlo. Su participación en la investigación no le generará ningún gasto adicional.

La muestra será enviada a Medellín al Grupo Malaria de la Universidad de Antioquia donde se realizará el procedimiento con la sangre. El objetivo del procedimiento es remover de la sangre los parásitos usando un filtro magnético. El aparato atrae las células infectadas gracias a que estas son atraídas por un imán, mientras las que están sanas no se atraen. El procedimiento con la sangre será similar para todos las muestras y se recolectarán 20 muestras de sangre; este consiste en: pasar la muestra de sangre a través del aparato magnético, lo que será realizado en el laboratorio del Grupo Malaria ya que es importante asegurarse de realizar el ensayo en un ambiente de trabajo apropiado, limpio y libre de obstáculos como el que ofrece el laboratorio del Grupo Malaria de la SIU. Igualmente se tomarán todas las medidas preventivas como uso de guantes, toallas de papel sobre el mesón, alcohol a la mano para la manipulación de la sangre y evitar la contaminación con sangre de los mesones. Los sobrantes de sangre se dispondrán siguiendo los protocolos de la SIU para el manejo de residuos biológicos.

Este proyecto es importante porque en el largo plazo puede ofrecer a los pacientes con malaria una alternativa al tratamiento sin el uso antimaláricos y posiblemente permitirá disminuir los parásitos que están en la sangre de manera casi instantánea.

Confidencialidad.

Toda la información que usted nos suministre en la encuesta y los resultados de los análisis, será guardada de manera confidencial por los investigadores del Grupo Malaria en las instalaciones de la Sede de Investigación Universitaria de la Universidad de Antioquia, ubicada en Medellín, en donde se realizarán el procedimiento y el análisis de los datos. La información de todos los pacientes se manejará de manera anónima y solo se utilizará un código que se asigna al inicio del estudio. Únicamente los responsables del estudio tendrán acceso a esta información. En ningún momento su nombre o el de sus familiares aparecerán en ninguno de los documentos que se generan de esta investigación.

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Alternativas del estudio.
Es importante que usted recuerde que el tratamiento para la malaria que damos en esta investigación es el único disponible en el país para cuando usted presenta una malaria por *P. vivax* y es el mismo tratamiento que recibirá en caso de que no desee participar. La diferencia es que su participación en esta investigación permitirá obtener una muestra de sangre que será sometida a un filtro magnético para probar la capacidad de remover parásitos de la sangre, lo que posiblemente servirá para evitar que los pacientes con muchos parásitos en la sangre presenten complicaciones por la enfermedad.
Beneficios para el participante. Los participantes de este estudio no tendrán beneficios adicionales diferentes de los determinados por ley. Se les realizará un diagnóstico oportuno y en caso de ser positivos, se les suministrará el esquema de tratamiento recomendado por el Ministerio de Salud y Protección Social de Colombia. En caso de que no se cure con el tratamiento, podrá de regresar al puesto de malaria y se le suministrará un nuevo tratamiento de acuerdo con las normas del Ministerio de la Protección Social y en caso de ser necesario será remitido al hospital municipal, para que allí lo sigan atendiendo.

Resultados esperados.
Los resultados de esta investigación permitirán conocer cuál es el porcentaje de parásitos eliminados de las muestras de pacientes con malaria. Si los resultados son positivos y promisorios este dispositivo podria ser aplicado en nuevos estudios donde se pruebe su efectividad en el campo directamente sobre mas pacientes. En caso de obtenerse buenos resultados, serán discutidos con las entidades gubernamentales de salud y podrán ser empleados para tomar decisiones terapéuticas para la infección por malaria complicada, esto podría evitar que más personas con malaria se compliquen al reducir la carga parasitaria.

Personas a contactar para información.
Usted tiene derecho a poseer una copia del presente documento y a que se le respondan satisfactoriamente todas las preguntas respecto al estudio que se le ocurran ahora o en cualquier momento. Para esto podrá contactar al personal del Grupo Malaria en su municipio (Nombre y teléfono) o a los responsables del estudio en Medellín, **Cesar Segura y Alberto Tobón,** en los teléfonos **2196490, 2196486 o en el fax 2196487.**

Esta propuesta ha sido revisada y aprobada por un Comité de Revisión de la Universidad de Antioquia. Este es un comité que se asegura de que los participantes del estudio están protegidos de cualquier daño. Si desea conocer algo más acerca de este comité, puede ponerse en contacto con **Gabriel Jaime Montoya M.** Instituto de Investigaciones Médicas. Facultad de Medicina – Universidad de Antioquia. Carrera 51D No. 62-29, **Teléfonos: 2196060 – 2196090.**

Aceptación de la participación.
Yo he sido invitado a participar en un estudio de prueba de la eficiencia de un dispositivo magnético para remover los parásitos de la malaria de una muestra de sangre. Entiendo que yo voy a recibir el tratamiento correspondiente para la malaria por tener la enfermedad y debo asistir al control rutinario

al centro de salud. Se me ha informado que los riesgos son mínimos y pueden incluir dolor en el dedo o el brazo. Soy consciente de que no habrá ningún beneficio personal. Se me ha proporcionado el nombre de un investigador que puede ser fácilmente contactado usando el número que me dieron.

He leído la información anterior, o se me ha leído a mí. He tenido la oportunidad de hacer preguntas al respecto y cualquier pregunta que he hecho, me ha sido contestada a mi satisfacción. Doy mi consentimiento voluntariamente para participar en este estudio de prueba con mi sangre y entiendo que tengo el derecho a no donar la sangre para el estudio sin afectar de ninguna manera mi atención médica.

Manifiesto que no he recibido presiones verbales, escritas y/o mímicas para participar en el estudio; que dicha decisión la tomo en pleno uso de mis facultades mentales, sin encontrarme bajo efectos de medicamentos, drogas o bebidas alcohólicas, consciente y libremente.

Nombre del participante: _____

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Firma: _____ Fecha: _____

Huella del participante (si no sabe firmar):

Testigo de la firma: He sido testigo de la lectura exacta del documento de consentimiento al candidato de participante, y el individuo ha tenido la oportunidad de hacer preguntas. Confirmo que el individuo ha dado consentimiento libremente.

Nombre del testigo:_____

Firma del testigo:_____

Responsable Grupo Malaria

Nombre:_____ Firma:_____

Fuentes:

Declaración de Helsinki 2002, Resolución 008430 de 1993 del Ministerio Nacional de Salud, Normas éticas internacionales para la investigación en humanos. Decreto 2378 de 2008.

Appendix E

Patient registration form

UNIVERSIDAD DE ANTIOQUIA / GRUPO MALARIA
Studying magnetic separation of malaria infected red blood cells from patient´s bloodstream

País	**Colombia**	**Medellín, Antioquia**	**Cesar Segura, Alberto Tobón**	**Centros de investigación**	**U de A**

Hoja 1: REGISTRO DEL PACIENTE

Municipio _____	Iniciales* _____	Código Paciente (CC, TI, RC) :

***Iniciales de los dos apellidos y del primer nombre.**

DATOS DEMOGRÁFICOS

Edad:	_____ años	Sexo	1 ( ) Masculino 2 ( ) Femenino	Teléfono _____
Etnia		1( )Afro descendiente	2( )Blanco 3( )Indígena	4( )Mestizo
Actividad económica/ oficio		1( ) Ama de casa 2( ) Agricultura 3( ) Minería 4( ) Pesca 5( ) Oficina 6( ) Comercio 7( ) Artesanía 8( ) Estudiante 9( ) Otro _____		

ANTECEDENTES

Lugar de residencia (Barrio o vereda)_____	1( ) Urbano 2( ) Rural Tiempo de Residencia : _____
Lugar de procedencia (Dpto./Mpio/barrio o vereda)_____	1 ( ) Urbano 2( ) Rural
Le ha dado malaria en el último 6 meses, además de esta: 1( ) Si 2( ) No. Fecha ultimo dx ____/____/____	
Por cual especie de *Plasmodium*?	1 ( ) *P. falciparum* 2 ( ) *P. Vivax* 3 ( ) No sabe
Que tratamiento recibió en la última malaria?	1 ( ) Cloroquina 2 ( ) Primaquina 3 ( ) Mefloquina 4 ( ) Amodiaquina 5 ( ) Sulfadoxina/Pirimetamina 6 ( ) Quinina 7 ( ) Artemeter/Lumefantrine 8 ( )Otro _____
Ha tomado algún medicamento en los últimos 30 días? 1( ) Si 2 ( ) No Cual?_____	
Fiebre no diagnosticada en los últimos 3 meses: 1( ) Si 2( ) No	

INFORMACIÓN CONFIDENCIAL

Nombre completo del paciente (sobrenombre)	
Nombre de uno de los padres, familiar o amigo (escribir parentesco)	
Teléfono, dirección de la casa y señales para ubicar la vivienda	

Hoja 2: REGISTRO DEL PACIENTE

INGRESO

Gota gruesa de ingreso (microscopista). Dx_____ Recuento:_____ /µL. Anillos:_____ /µL
Resultado RDT: _____
PCR: Se tomo la muestra (Si/No): _____ Resultado:_____

Nombre de quien llena la hoja		**Firma**		**Fecha**	