Transfusion transmitted malaria and bacterial infections in a malaria endemic region

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

by

Alex Kwame Owusu-Ofori

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Dedication

This thesis is dedicated to my lovely wife Shirley and my wonderful kids, Caleb, Chloe and Chantelle who have endured long periods of my absence and who have loved and supported me.

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ABBREVIATIONS

DNA	Deoxyribonucleic acid
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
FBC	Full blood count
GCP	Good Clinical Practice
GCLP	Good Clinical Laboratory Practice
GDBS	Global Database on Blood Safety
HRP-2	Histidine Rich Protein-2
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency virus
ISBT	International Society of Blood Transfusion
KATH	Komfo Anokye Teaching Hospital
LDH	Lactate Dehydrogenase
LSTM	Liverpool School of Tropical Medicine
MSP	Merozoite surface protein
MoH	Ministry of Health
PCR	Polymerase chain Reaction
RDT	Rapid diagnostic test
RPR	Rapid plasma regain
SSA	Sub-Saharan Africa
TMU	Transfusion Medicine Unit
TTI	Transfusion transmitted infections
TTM	Transfusion transmitted malaria
TPHA	*Treponema pallidum* hemagglutination assay
VDRL	Venereal Disease Research Laboratory
WHA	World Health Assembly
WHO	World Health Organization

ABSTRACT

Background and Methods: Blood transfusion saves lives and improves health but the presence of transfusion transmissible infections can have untoward consequences. When undetected, these infections can cause significant morbidity and mortality to transfusion recipients. On the other hand, a high prevalence of transfusion-transmitted infections (TTI) leading to rejection of a large proportion of donated blood can result in blood shortages and subsequent increase in mortality. Malaria and bacterial infections are transfusion transmissible but there is limited data concerning these infections in sub-Saharan Africa. Although the burden of transfusion-transmitted malaria in malaria endemic countries are unknown, it is recommended that all donated blood is screened for malaria parasites and presumptive treatment be given to transfusion recipients. Bacterial contamination in sub-Saharan Africa has been reported to occur in between 8 - 17% of stored blood but the effect of contamination on transfusion recipients has not been determined. Syphilis is currently the only bacterial infection for which routine screening is recommended but screening is not being performed in many blood centres including Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana where this study took place.

This study examined the effects of transfusion-transmitted malaria (TTM) and bacterial infections (including syphilis) on transfusion recipients in a malaria endemic area. Four malaria screening tests were compared to assess their usefulness in the context of African blood banks. Pregnant women, children and immune-compromised transfusion recipients from the Departments of Obstetrics and Gynaecology, Paediatrics, Medicine and Oncology in KATH were enrolled into the study.

Results: Anti-malarial drugs were routinely prescribed with paediatric transfusions. Fifty patients were evaluated after receiving blood transfusions that were positive for *P. falciparum* by PCR and seven recipients developed PCR-detectable parasitaemia. In only one recipient (2%) was TTM confirmed. The prevalence of *P. falciparum* malaria in transfused blood was 4.7% (21/445) by microscopy, 13.7% (60/440) by rapid diagnostic test, 18% (78/436) by polymerase chain reaction and 22.2% (98/442) by enzyme immunoassay.

Bacterial contamination was found in 11.5 %(95% CI 7.0-16.0%) (23/200) of donated blood units but only half of the recipients were observed to developed adverse signs of transfusion related sepsis. The mean duration of storage of blood was 2 days. The prevalence of syphilis sero-positivity in donated blood was 8.0% (95% CI 4.3-11.7%). Seroconversion took place in an 8 year old girl, after receiving a syphilis sero-positive unit of blood.

Conclusions: This thesis has shown that malaria parasites may be commonly detected in donor blood but TTM occurs infrequently in recipients living in malaria endemic areas. The high rate of bacterial contamination and its associated transfusion related sepsis poses a safety risk to transfusion recipients. Transfusion-transmitted syphilis remains a risk for transfusion recipients in blood centres with a high prevalence and short duration of storage of donor blood.

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CHAPTER 1

INTRODUCTION

1.1 Blood transfusion

Blood transfusion is the transfer of blood or blood components from one individual (donor) to another (recipient) (1). This procedure is usually performed by the intravenous route but other uncommon routes such as the intra-osseous can be used for example in very shocked children where venous access is difficult (2). Blood transfusion is an essential part of modern health care and when appropriately administered, transfusions can save lives and improve health.

Overview of blood transfusion history

Blood transfusion has developed over a prolonged period. It can be traced from the time of Hippocrates (~430 BC), when venesection was widely practised, until about 100 years ago when it became part of routine clinical practice (3). The complete description of systemic blood circulation and the functions of the heart and vessels by William Harvey in 1628 was a major breakthrough and this discovery has proved to be the cornerstone of the practice of transfusion (4). The first transfusion; from one dog to another took place in Oxford in 1666 and was performed by Richard Lower (3). James Blundell, an obstetrician at Guy's and St. Thomas' Hospitals in London is credited with the first human to human transfusion in 1818 (5).

Blood transfusion safety

Blood safety encompasses actions aimed at ensuring that everyone has access to blood and blood products that are as safe as possible, available at reasonable cost, adequate to meet the needs of patients, transfused only when necessary and provided as part of a sustainable blood programme within the existing healthcare system (6). In 1975, the 28th World Health Assembly in Geneva passed a resolution to address the issue of blood safety. Resolution WHA28.72 urges member states to promote the development of national blood services based on voluntary non-remunerated donation of blood (7).

Current status of global blood safety

The World Health Organisation Global Database on Blood Safety (GDBS) was set up in 1998 to collect and analyse data from all countries on blood and blood product safety as the basis for effective action to improve blood transfusion services globally (8). The latest report in 2010 is based on data from 173 countries (9). There were 93 million blood donations and 50% of them were collected in developed countries despite being home to only 16% of the world's population. The average donation rate in developed countries is 45.4 donations per 1000 population compared with 3.6 donations in developing countries and 10.1 in transitional countries.

Screening for transfusion-transmitted infections

There are many countries in the developing world that are unable to screen all donated blood for the 4 major transfusion-transmitted infections which are the human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis. The lack of effective screening worldwide results in up to 16 million new infections with hepatitis B, 5 million new infections with hepatitis C and 160,000 cases of HIV infection each year (10).

1.2 Transfusion in sub-Saharan Africa

Blood transfusions are indispensable in sub-Saharan Africa. They play a key role in the correction of anaemia and the prevention of deaths in pregnant women and children. Severe anaemia results in one million deaths each year in children less than 5 yrs old (11) and 26% of maternal haemorrhage deaths were due to lack of blood (12). Blood shortage is therefore an important consideration when transfusion is discussed in sub-Saharan Africa (13).

Blood transfusions are an effective way of transmitting infections and account for an estimated 5-10% of all HIV infections in sub-Saharan Africa (14). Comparing the different routes of transmission for HIV, blood transfusion was more than 90% efficient while maternal-to-child transmission, unsterile injections and heterosexual transmission had efficiencies of 30-35%, 0.30% and 0.01-0.02% (15, 16).

History of blood transfusion in sub-Saharan Africa

It is possible that blood transfusions may have been performed without documentation or publication in the medical literature. The earliest published reports of transfusion in Africa were from Belgian Congo in 1923 and 1940. Belgian Congo and Senegal are the only two colonies in sub-Saharan Africa with published accounts of what could be described as a transfusion service or programme prior to 1950 (16). There was a sharp growth in 1950 to 12 countries and by 1955 there were 19 countries where transfusion was performed (Table 1.1). This sudden increase may have been because transfusion techniques were successfully developed during the World War II and European countries were eager to introduce it into their colonies.

Table 1.1: First reports of blood transfusion in sub-Saharan Africa

Year	Location
1924	Congo (Belgian)
1943	Senegal
1950	Angola, Cameroon, Dahomey, Ivory Coast, Guinea, Mali, Mozambique, Niger, Togo, Upper Volta
1951	Southern Rhodesia (1926 for Europeans only)
1952	Nigeria
1953	Kenya
1954	Gold Coast (Ghana)
1955	Chad, Congo (French), Gabon

This table has been adapted from W. H. Schneider and E. Crucker 2006 (16).

Blood safety in sub-Saharan Africa

Although all 46 member countries in the WHO African Region are signatories to the World Health Assembly resolution WHA28.72 of May 1975, not much progress has been made towards blood safety. A combination of factors has resulted in the subcontinent facing major challenges in its quest for blood safety. These include a high prevalence of blood borne disease agents, poverty, organizational deficits and a lack of resources in the face of poorly developed health infrastructure (17, 18). On the other hand the recommended centralised blood collection from voluntary donors for safe blood, based on the Western model, may not be appropriate for Africa (19). Alternative systems may have to be embraced and used concurrently

(20). Further, open mindedness about innovative ways to improve supply and safety of blood is needed to promote more evidence based approaches in low income countries (21).

Current blood safety indicators in sub-Saharan Africa

Data from the GDBS (22) show that a hundred percent testing for TTI is currently being performed for HIV, HBV and HCV by 95%, 83% and 59% of the reporting member states respectively. Prevalence rates of TTI are difficult to report because of different testing algorithms and different denominators. The rates of blood units being discarded for testing positive to TTI were exceptionally high and ranged between 21.0% and 26.6% for countries such as Sao Tome, Mali and Mauritania.

Limitations of GDBS: The data published by the GDBS is based on responses to a questionnaire sent to national health authorities. This information is provided from the national transfusion services that are often based in urban centres and have good documentation processes. Because family replacement donations usually come from hospital facilities where there are no adequate central collating systems, input from such facilities is not reflected in these reports, and this leads to biased data (19,21). The GDBS data has to be interpreted with caution, especially the data from Africa.

1.3 Transfusion-transmitted infections (TTI) in Ghana and West Africa

The focus in Ghana as in many other places has been on the 4 major TTI namely HIV, Hepatitis B and C and syphilis. A hundred percent of blood banks in the country screen for HIV, HBV and HCV but only 20% screen for syphilis. The average national prevalence rates in donors for HIV, HBV, HCV and syphilis were 1.27%, 10.14%, 0.99% and 1.48% respectively. The national prevalence of all the screened TTI is higher in replacement donors than in voluntary donors (23). In contrast, recent evidence from Kumasi (24), Cameroon (25) and Guinea (26) has shown no difference in prevalence between first time volunteer donors and replacement donors.

In Kumasi, where the current study took place, despite screening for the major viruses, it has been demonstrated that the residual risk is high. The estimated residual risk of HIV is 1 in 2578 and for HCV is 1 in 1450 (27).

The amount of blood discarded varies from region to region across Ghana. Those regions performing pre-donation screening of TTI discard less than those doing post donation screening. The Upper East region discarded 1.42% of blood units collected while Eastern region discarded 15% of the blood collected.

As well as the major TTI some other viruses have been investigated as TTI in Ghanaian donors. These include human erythrovirus B 19, West Nile virus and GB virus C (28-31).

There are a few publications from West Africa that describe the prevalence of the major viruses in their hospitals or in the centre. A mathematical model based on such data has estimated the incidence of TTI in some African countries including 6 from West Africa (32). The incidence in West Africa is generally lower than in East or Southern Africa. Within the sub-region, the highest incidence was for TTI- HIV was 164 and 112 per 100,000 donations in Senegal and Ghana. Senegal had the highest incidence of TTI-HBV and TTI-HCV. With an incidence of 81, 431 and 96 per 100,000 donations for HIV, HBV and HCV, Mali was the country with lowest incidence of all 3 viral TTI.

Transfusion-transmitted malaria in Ghana and West Africa

There has been no study to determine the prevalence of malaria in Ghanaian donors and neither has there been any study to determine the effects of blood transfusion on malaria. In the West African sub region, eleven studies have been conducted, one in Benin and ten in Nigeria to determine the prevalence of malaria in blood donors. The prevalence of malaria in blood donors, determined by microscopic examination of peripheral blood ranged from 4% to 55% (33, 34). These studies were conducted at different malaria endemic areas and transmission seasons.

Determining the prevalence of malaria parasitaemia in blood donors, however, does not provide the actual risk of transfusion-transmitted malaria especially in a

malaria endemic area where majority of recipients may have some immunity that protects them from clinical disease.

Bacterial contamination of donated blood in Ghana and West Africa

Bacterial contamination is not routinely tested for in donated blood. Platelet concentrates are however routinely screened in some developed countries because of the high risk of bacterial contamination (35). In Ghana, two studies (36, 37) have recently been published, one from the northern sector (Tamale) and another from the southern sector (Accra) of Ghana. The studies determined the rates of bacterial contamination of donor blood to be 17.5% in Tamale and 9% in Accra. Neither of these studies looked at the clinical effects on the recipients of the blood. No other studies have been identified across West Africa.

Transfusion-transmitted syphilis in Ghana and West Africa

Transfusion-transmitted syphilis has not been documented to have occurred in Ghana or in the West African sub region. Studies tend to identify sero-positivity among donors but not in recipients. There are 2 published studies on blood donors in Ghana (38, 39). Both studies were conducted in the capital city of Accra and the sero-reactivity was 13.5% and 7.5% respectively. The rates from these published studies are higher than that reported by the blood banks in the annual report. It is uncertain why these discrepancies exists but it may be due to the fact that published studies used particle agglutination assays, which are more sensitive than the VDRL that is used for screening in the national programme. A very low sero-reactivity prevalence of 0.3% among donors has been reported by the National Centre for Blood Transfusion in Mali (40). Although they attribute this low prevalence to widespread use of antibiotics, there must be other reasons including the screening test used which in this case was the - Venereal Disease Research Laboratory (VDRL) non treponemal test which has a low sensitivity. Olokoba et al also found a low sero-positivity rate of 1.3% in donors from North-eastern Nigeria (41).

Transfusion practices in Ghana and West Africa

In Ghana, the national blood policy is silent on transfusion-transmitted malaria. It recommends all donor blood should be screened for HIV, HBV, HCV and syphilis but many blood banks, including the hospital, where this study was carried out, do not screen for syphilis. Regular audits are not carried out and there are insufficient publications to adequately describe what the current practices are within the country. In Nigeria for example, an audit showed inappropriate use of platelet transfusion in 81% of instances and a 36% unnecessary use of fresh frozen plasma (42). Transfusion practices, like any other branch of medicine, should be derived from evidence based guidelines and the documentation of these practices can help evaluate them against any emerging new evidence. A hospital in the Ashanti region of Ghana has recently shown a decrease in the rates of transfusions and of malaria infections following the implementation of malaria control programme in that area (43).

1.4 Study location: Ghana

Ghana is an English speaking West African country that lies along the Gulf of Guinea and shares borders with Burkina Faso, Togo and Cote D'Ivoire (Figure 1.1).

Its population, according to provisional results from a census in 2010 is 24,223,431 with 51.3% females (44). The country is administratively divided into 10 regions (Figure 1.1). The capital city of Ghana is Accra, which is located in the Greater Accra region. Vegetation varies across the country, low sandy shores and grassy plains in the south, tropical rain forest in the middle and low bush savannah in the north. The majority of people are farmers and cocoa, timber and gold are the main source of foreign exchange. Some of the demographic, health and economic indicators of Ghana are shown in Table 1 (table derived from UNICEF) (45).

The health sector in Ghana

Ghana has 2189 health facilities, of which 952 are government owned, 181 are owned by religious organisations, 75 are quasi-government and 980 belong to the private sector. Out of the total number of health facilities, 2 are teaching hospitals, 10 regional hospitals, 91 district hospitals, 124 other hospitals, 558 health centres,

1085 clinics and 320 are maternity homes. Ghana operates a National Health Insurance Scheme so regular contributors do not have to make direct payments to the health providers. The Scheme was established in 2003 and currently covers about 60% of the population.

In 2009, malaria accounted for 32.9% of all admissions and was the leading cause of admission across the country. The top 3 causes of death in Ghana were malaria (13.4%), HIV related conditions (7.4%) and anaemia (7.3%). Syphilis prevalence varies depending on the population examined. Among prison officers in Accra sero-prevalence was 7.9% but among prison inmates it was double the figure at 16.5% (46).

Table 1.2: Country statistics for Ghana (table taken from UNICEF website http://www.unicef.org/infobycountry/ghana_statistics.html).

Indicator	
Under-5 mortality rate (per 1000 live births), 2009	69
Infant mortality rate (per 1000 live births), 2009	47
Neonatal mortality rate (per 1000 live births), 2009	27
Annual no. of births (thousands), 2009	766
Annual no. of under-5 deaths (thousands), 2009	50
Gross national income per capita (US$), 2009	700
Life expectancy at birth (years), 2009	57
Total adult literacy rate (%), 2005-2008	66
Estimated adult HIV prevalence rate (aged 15-49), 2009	1.8
Estimated number of people living with HIV (thousands) 2009	260
Malaria 2006-2009, % households owning at least one ITN	33
Malaria 2006-2009, % under-fives sleeping under ITNs	28
Malaria 2006-2009, % under-fives with fever receiving anti-malarial drugs	43

Kumasi

Kumasi is located in and is the capital city of the Ashanti region of Ghana. Ashanti is the region with the highest population in Ghana and has a population of 4,725,046.

There are 530 health facilities in the region including Komfo Anokye Teaching Hospital, the site for this study.

Komfo Anokye Teaching Hospital (KATH)

Komfo Anokye Teaching Hospital (KATH) is the 2nd largest hospital in Ghana (Figure 1.2). It is a 1000 bed hospital and serves as the referral centre for the upper half of Ghana. KATH has 10 clinical directorates that provide specialized services to the public. Blood transfusion services in the hospital are provided by the Transfusion Medicine Unit.

Figure 1.1: Map of Ghana showing its boundaries, regions and proposed centres (shown by red arrows) for the National Transfusion Service

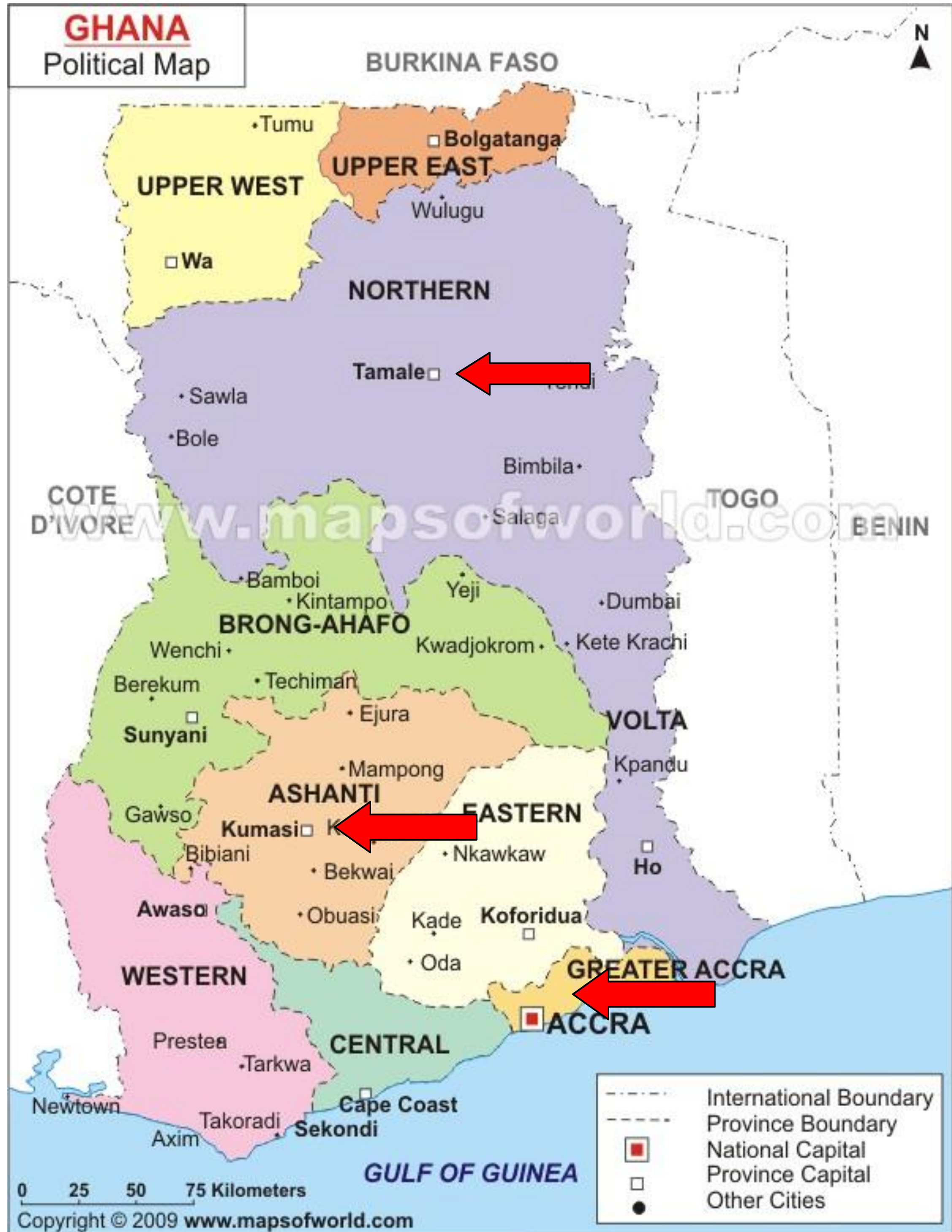


Figure 1.2: The front view of the Komfo Anokye Teaching Hospital (KATH)

Blood transfusion in Ghana

The National Blood Transfusion Service (NBTS) of Ghana has adopted a national blood policy since April 2006 (47). However, the legislative instrument backing the policy is yet to be passed through Parliament for enactment into a law. There is also a national guideline on the clinical use of blood and blood products. Funding has been secured for the NBTS to construct three centres to coordinate the supply of blood and blood products across the country. These will be located in the southern (Accra), middle (Kumasi) and northern (Tamale) parts of Ghana as shown by arrows in the map of Ghana (Figure 1). The national average for the proportion of voluntary blood donations is 38.4%. Blood banks in each hospital screen their own blood for HIV, Hepatitis B and C and syphilis depending on local preferences and availability of test materials.

Blood transfusion in KATH

The Transfusion Medicine Unit (TMU) of the Komfo Anokye Teaching Hospital is responsible for obtaining the blood donations, storage and distributing of blood in the hospital. The TMU is organised into sections including donor organisation, donor care, and laboratory sections. It is currently only able to provide whole blood, concentrated red cells (packed cells) and fresh frozen plasma for transfusion. Each year about 15, 000 transfusions are carried out in KATH.

Blood donors and donation: KATH depends on both voluntary non-remunerated blood donors (VNRBD) and replacement donors (48). Seventy percent of blood used in the hospital is from voluntary donations. The VNRBD are predominantly recruited from secondary schools but there are other sources such as walk in donors and donors from public drives which are held in conjunction with local FM radio stations. The replacement donors come to the hospital to donate and replace blood used for a hospitalized family member or friend. There are no recognised paid blood donors.

Screening of blood: Blood donors are screened for HIV, hepatitis B and hepatitis C prior to donation. This is known as pre-donation screening. When screening is carried out on the blood after the unit of blood has been donated it is referred to as post-donation screening. Donors that are initially positive for any of these viruses are deferred and asked to return a couple of weeks later by which time confirmatory tests will have been performed. Screening is initially by rapid tests and confirmation is by ELISA.

The rates of the transfusion-transmitted infections screened for in KATH for 2011 are as follows: prevalence of HIV is 0.8%, prevalence of HBV is 8.5% and prevalence of HCV is 0.4% (personal communication with head of KATH transfusion unit). Blood donors are not screened for syphilis or malaria and there are no blood cultures done for bacterial contamination.

1.5 Justification for the study

In September 2008, a workshop was held in Mombasa, Kenya for key stakeholders in blood transfusion services in sub-Saharan Africa. The purpose was to develop and prioritize a research agenda and to fill gaps in the evidence needed to improve the

supply and safety of blood for transfusion (49). Key among the priority area's identified was transfusion-transmitted infections and transfusion reactions.

Transfusion researchers, transfusion service directors, representatives of transfusion users including senior obstetricians, anaesthetists and paediatricians and funders, confirmed that there is limited knowledge about the morbidity and mortality due to transfusion-transmitted malaria and the benefits and feasibility of screening for malaria are unknown. There is almost no information about whether there is clinically significant bacterial contamination of blood packs and the patterns and prevalence of transfusion reactions in sub-Saharan Africa.

Transfusion-transmitted malaria

It is important to determine the consequences of blood transfusion if the blood contains malaria parasites. Effects may vary according to age and immunity. Knowledge about adverse effects will give an indication of whether it is important to screen donated blood for malaria parasites.

Malaria is a well known transfusion-transmissible infection but there remains great uncertainty with regards to its effect on those receiving blood, especially in malaria endemic regions. Some national programmes and guidelines recommend routine use of anti malarials. There is little evidence to support or oppose current practices and opinion remains divided as to whether malaria screening should be carried out on all blood donors. The gold standard for diagnosis of malaria is microscopy but this method is time consuming and may miss low levels of parasitaemia. Rapid malaria test kits are gradually becoming available and PCR technology has high sensitivity. A critical evaluation of these tests is needed to determine their usefulness in screening donors.

Bacterial contamination

Bacterial contamination and its effects on transfusion recipients in the developing world is another poorly researched field of transfusion. Bacteria readily proliferate in blood and under poor storage conditions a tiny inoculum of bacteria can proliferate to reach dangerous levels which, when transfused, may cause significant morbidity or mortality. In sub-Saharan Africa, children are major users of blood.

Because paediatric packs are expensive and not readily available, adult units are split into smaller aliquots with the potential risk of bacterial contamination. It is important to identify the presence of contamination and the factors that contribute to it. In developing countries where quality assurance and monitoring systems are not followed, identifying factors that cause or enhance bacterial contamination will be helpful to draw up policies that enhance blood safety.

Transfusion-transmitted syphilis

Syphilis has been known as a transfusion-transmitted infection for more than 100 years but there still remain questions about the value of screening. Some evidence suggests that because the prevalence of syphilis is low and because the *Treponema pallidum* is no longer viable after a few days of refrigeration of the blood, the transmission of syphilis by blood is negligible. In addition, the serological tests used may not necessarily detect infectious donors. There is, however, insufficient evidence to justify stopping screening for syphilis in blood donors and the practice of syphilis screening continues.

This study seeks to provide some evidence of whether these transfusion-transmitted infections are occurring in Ghana and to identify any effects they have on recipients of transfusion.

1.6 Study objectives

Aim

The aim of this study is to determine the prevalence of malaria, bacterial contamination and sero-positive syphilis in donated blood and to determine any effects of these transfusions on recipients living in a malaria endemic area.

Objectives

The specific objectives of the study are:

- To describe the impact of policies relating to malaria transmission and blood transfusion on clinical practice in endemic countries
- To determine the prevalence of malaria parasitaemia, bacterial contamination and syphilis sero-positivity in donated blood and blood products for transfusion.

- To identify any adverse effects on recipients of donated blood/blood products that had malaria parasites or bacterial contamination.
- To compare microscopic, serological and molecular methods for screening blood for malaria parasites

1.7 Thesis outline

Chapter two provides a review of the literature that this thesis is based upon. There are four main sections. A brief summary on general transfusion-transmitted infections is presented followed by a detailed focus on malaria, general bacteria and finally syphilis. The review also examines the applicability of screening methods and tests in sub-Saharan Africa. The study organisation and general methodology is described in chapter three.

In chapters four, five, six and seven, different studies are presented. A study evaluating transfusion practices in relation to policies in transfusion-transmitted malaria is presented in chapter four. Chapter five describes a study which determines the existence of transfusion-transmitted malaria in a malaria endemic country. A study on bacterial contamination and its effects is described in chapter six and one on transfusion-transmitted syphilis is described in chapter seven. In chapter eight, a general discussion of the study findings is presented and in chapter nine the conclusions and recommendations are made.

1.8 Summary

In this chapter an overview of blood transfusion in Africa and the current status of blood transfusion safety in Ghana and West Africa have been presented. The justification and study aims have also been presented. In the next chapter, a review of the literature on transfusion-transmitted infections is presented.

CHAPTER 2

LITERATURE REVIEW

2.1 TRANSFUSION-TRANSMITTED INFECTIONS

Introduction

Different types of organism including viruses, bacteria and parasites can be transmitted by transfusion of blood or blood products although this is not their primary route of transmission. The risk of transmitting a particular infection is related to the prevalence of the infection in the region, but also depends on other factors such as the virulence of the organism and ability to survive storage conditions. Some organisms, such as HIV, are ubiquitous whereas others, such as *Plasmodium*, are endemic in specific regions. With the current globalisation and the ease of travel around the world, transfusion recipients in non endemic countries can still be infected by a non endemic infection like malaria.

Emerging infections

The American Association of Blood Banks has recently identified 68 infectious agents that can be transmitted by transfusion and there may be more yet to be identified (50). Besides the major transfusion-transmitted infections such as HIV, hepatitis B and hepatitis C, novel agents such as prion proteins, responsible for prion diseases or spongiform encephalopathy, have recently been recognised (51,52).

The risk to recipients of the major transfusion-transmitted infections is declining, both in developed and developing countries. This is due largely to a continuing focus on improvements in donor selection and advances in testing for infectious agents (53).

Screening of donated blood

Laboratory testing only forms part of donor screening. There are general guidelines available which guide transfusion practices but each country must adapt them to suit their needs. The WHO recommends that at a minimum, blood should be

screened for HIV, hepatitis B (HBV), hepatitis C (HCV) and syphilis; furthermore, and where appropriate, malaria and Chagas disease should be screened for in donated blood (54). One of the ways to prevent transfusion-transmitted infections (TTI) is to ensure that all donated blood is screened for the major transfusion infections. Not all countries are able to screen donated blood units. Out of 162 countries providing worldwide data for the Global Database for Blood Safety, 39 countries are not routinely testing blood donations for one or more of the 4 major TTI (8). The WHO estimates that lack of effective screening results in up to 16 million new infections with HBV, 5 million new infections with HCV and 160,000 cases of HIV infection every year (10).

Because of constantly emerging threats, transfusion-transmitted infections will continue be a challenge in blood safety. Preventive approaches such as comprehensive donor questioning and evaluation, and mandatory screening for HIV, HBV, HCV and syphilis will need to remain in place so that potentially infectious blood will be eliminated. Newer tests for emerging pathogens will have to be quickly introduced when necessary.

Challenges in blood donor screening

Serological screening tests have been developed and validated in developed countries. When used in Africa, higher levels of IgG consistently increase the frequency of falsely reactive tests. This phenomenon has been observed for HIV, HCV and Human erythrovirus B 19 serological testing (55-58).

Confirmatory testing should be performed for all initially reactive screening assays. In syphilis testing, the current recommendation is for initial screening tests to be performed with a non-treponemal assay and confirmed with a treponemal assay (See section2.4.6). However, many of the published studies use a single treponemal or non-treponemal test. It is unclear why this is being done in Africa. Sero-reactivity and sero-positivity rates cannot therefore easily be compared. In the syphilis section of this thesis, sero-reactivity is used when only one test was performed and sero-positivity is used when a second test was performed to confirm initially reactive donor blood.

For malaria in endemic countries, there are no recommended confirmatory tests to detect parasitaemia in blood donors. WHO recommends that screening for parasitaemia be done with microscopy. Alternatively a highly sensitive enzyme immunoassay can be used for evidence of antigenaemia (59). Microscopy also remains the only US Food and Drugs Board approved endpoint for assessing outcomes in malaria vaccine and drug trials (60). In this thesis, reference is not made to a confirmatory test in the sections dealing with malaria.

2.1.1 Transfusion-transmitted viral infections

Some of the important transfusion-transmitted viruses are human immunodeficiency viruses types1 and 2 (HIV-1/2), hepatitis B virus (HBV), hepatitis C virus (HCV), and human T-cell lymphotrophic virus types I and II (HTLV-I/II). Other viruses, including West Nile virus (WNV), Epstein-Barr virus, human parvovirus B19, and GB virus C, can also be transmitted by blood products (61). Transfusion-transmitted infections existed long before HIV emerged. The transmission of a viral agent through blood transfusion was first reported in 1943 but since the recognition of HIV and its potential to infect those who receive a transfusion from an infected blood donation, blood safety has been the subject of strong advocacy with global support from major organisations (62,63).

The risk of transfusion-transmitted viral infections has been reduced to minimal levels in developed countries but in developing countries, the risk is higher. Recent estimates of the risk in Africa of acquiring HIV, HCV and HBV were 1, 2.5 and 4.3 per 1000 units respectively (32). In contrast, the estimated risk per unit in the US of acquiring HIV, HCV and HBV was 1: 1.9 million, 1:1.6 million and 1:180,000 respectively (64)

2.1.2 Transfusion-transmitted parasitic infections

Malaria, Chagas disease, and babesiosis are some of the diseases that can result from transmission of parasites by transfusion. Chagas disease and babesiosis are briefly mentioned here but malaria will be discussed extensively.

Chagas disease

Chagas disease, also called American trypanosomiasis is caused by *Trypanosoma cruzi*. It is widespread in South America, Central America and Mexico (65). Up until 2007, there have been seven documented cases transfusion-transmitted Chagas disease (53), six of which were associated with transfusion of platelet concentrates (Young C 2007). With the continuous decline of the major transfusion infections, more attention is being paid to others like *T. cruzi* (53). An enzyme immunoassay was introduced in 2006 for screening blood to detect antibodies to *T Cruzi*.

Babesiosis

Babesia microti is an intracellular parasite that has similarities with malaria. Its transmission from animal reservoirs to humans is by ticks (66). Transfusion-transmitted babesiosis, has occurred across the world (67) but most cases reported have come from the United States of America (68). Although it is usually asymptomatic, it can also be fatal (65). Twelve of the over 70 cases reported between 1979 and 2008 led to a fatal outcome (69). Tests available include blood smears to detect the parasite but this is not sensitive. Indirect immunofluorescence assay (IFA) is regarded as the gold standard (66).

2.2 TRANSFUSION-TRANSMITTED MALARIA

Introduction to malaria

Malaria is the most important parasitic disease of man (70), with 3.28 billion people living in areas that have some risk of malaria transmission (71). Clinical malaria may be caused by any of 5 *Plasmodium* spp. that are known to infect humans. These include *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi* (72). *P. knowlesi,* a parasite of long tailed macaque monkeys, has since 2004 been identified as causing human disease (73) and it can be rapidly lethal especially when a high parasitaemia is reached (74). The most severe forms of malaria are caused by *P. falciparum* (75).

WHO recently estimated, that there were 225 million cases of malaria worldwide with a majority (78%) of these cases coming from Africa. The global number of

malaria deaths in 2009 was 781,000, of which 91% occurred in the Africa region (76).

Malaria transmission

Transmission of malaria parasites primarily occurs through the bite of the blood feeding female anopheles mosquito. Besides transmission by mosquitoes, direct inoculation is another well documented mode of acquiring malaria. Direct inoculation has resulted in malaria after accidental exposure in healthcare workers, among injecting drug users sharing used needles and in blood transfusion (77,78). Malaria transmission by transfusion is the most common form of direct inoculation and is discussed extensively in this thesis. For transfusion-transmitted malaria, the exact life cycle of *P. falciparum* has not been described.

A review in 2005 found 22 published cases of occupational *P. falciparum* malaria following accidental blood exposure (79). All cases were from countries non endemic for malaria. Accidental exposure to infected blood may be challenging to diagnose in areas where infections are not endemic (80) but with increasing travel to malaria endemic areas and the possibility of more patients being admitted and treated for malaria, this route may become more important in occupational health (79).

Susceptibility to malaria infection

All persons living in malaria endemic areas are susceptible to infection but there are some people who are particularly susceptible to acquiring infection including children and pregnant women (81).

Children are most susceptible to malaria but infants and neonates appear to be protected from malaria for the first 3-6 months (82). This may be due to maternal antibodies. Immunity to malaria is slowly acquired after years of repeated exposure to *Plasmodium*. This is reflected by the decrease of incidence of clinical episodes and a decrease in parasite densities with increasing age, in persons living in malaria endemic areas (83). Despite many control efforts, hospital admissions, severe malaria and deaths due to malaria remain concentrated in children under 5 years (84).

Pregnant women are at a greater risk of infection and of symptomatic malaria disease than the non pregnant adult (85). The ability of pregnant women to limit parasite replication is impaired in pregnancy, resulting in higher parasite densities than in non pregnant adults. This happens in pregnancy because, there is a transient depression of cell-mediated immunity that allows foetal allograft retention and also there is immunosuppression mediated by pregnancy associated hormones and proteins (86,87). Age may also be an independent risk factor as younger pregnant women are more susceptible to malaria in some settings (88).

Malaria has been found to be a complicating factor of febrile neutropenia (89). Patients with malignancies and on chemotherapy are likely to be more susceptible to malaria than healthy persons. The relationship between sickle cell disease (SCD) and malaria can be complex. The presence of haemoglobin S is associated with reduced parasitic invasion of erythrocytes, impaired multiplication and accelerated clearance of parasites by the spleen (90). It is assumed that homozygous SCD would confer resistance to malaria, however co-existence of the two is associated with increased mortality and morbidity and malaria is the most common cause of crises in endemic countries (91). Malaria is a significant problem in people with sickle cell disease (92).

Transfusion-transmitted malaria

Transfusion-transmitted malaria (TTM) occurs when the infection in the patient is caused by the same parasite that was present in the donated blood. The first case of TTM was reported by Woolsey in 1911 (93). This was due to the blood in the syringe flowing back when the direct method of transfusion from artery to vein was used (94).

2.2.1 Epidemiology of transfusion-transmitted malaria

Transfusion-transmitted malaria in non-endemic countries

Most of the published data on TTM are from non endemic countries for malaria. This may be due to the ease of diagnosis in non-endemic countries compared to endemic countries where it cannot be readily proven whether malaria which

develops post-transfusion is from the blood received or from a re-infection or recrudescence.

Excluding deliberate infections for malaria therapy or for experimental purposes, there were about 350 cases of accidentally induced human malaria reported between 1911 and 1950 (94). During the period 1950-1972, 1,756 cases were reported to have occurred in 49 countries. The role of surveillance activities of the global malaria eradication programme from various countries and the reports forwarded to the WHO were of considerable value in providing reliable data (95). There is still the possibility that some cases of TTM were missed or not reported. As transfusion of blood and its components increased, more cases of TTM were expected. However, with an increased awareness of this problem, more interventions have been introduced to reduce its occurrence including screening tests and deferral of donors who have travelled to malaria endemic countries. . Currently, there is about one case of TTM every 3 years in France (96,97). In the UK, only 2 confirmed cases of TTM have been reported since 1996 (98). Three cases of TTM were reported in the US in 2009. Prior to 2009, the last case of TTM was reported in 2007 (99). In Japan, there have been 22 cases of TTM since 1945 (100).

Incidence of transfusion-transmitted malaria in sub-Saharan Africa

There are no published studies on the incidence of TTM in malaria endemic regions. This may be due to the use of malarial prophylaxis in recipients, making it difficult to follow up patients to determine incidence. A study in Sudan found that the prevalence of post transfusion malaria in transfusion recipients was 3.5%, four days after transfusion (101). All patients in the study (twelve) who received malaria positive blood became malaria positive (by microscopy). There were also two patients who did not receive malaria positive blood but still developed malaria parasitaemia 4 days after transfusion. Genotyping was not done to determine if the parasites in the patients were the same as the ones received from the donated blood. In malaria endemic countries post transfusion malaria cannot be equated to TTM because there are other sources of transmission. In non endemic countries however, post transfusion malaria is likely to be the same as TTM because the

transfusion is likely to be the only source of parasitaemia in a recipient who has had no previous exposure to malaria.

Prevalence of malaria in blood donors in sub-Saharan Africa

The prevalence of malaria in blood donors depends on the endemicity in the country or region and the transmission season. For example, during a high transmission season in a high endemic area, the prevalence of asymptomatic parasitaemia is high in all age groups, reaching up to 94% (102). Similarly it is reasonable to expect a high prevalence of asymptomatic donors in endemic countries (103). Our recent review of published prevalence studies shows a wide variability of malaria prevalence across sub-Saharan Africa (104). The prevalence of malaria varied from 0.7% in Nairobi, Kenya (low malaria endemicity) to 55% in Anambra, South-eastern Nigeria (high malaria endemicity). The prevalence of malaria varies across Africa and even within countries.

Asymptomatic donors with parasitaemia may be a source of transmission but it is unknown whether presence of parasitaemia in donors translates into infection or disease for the recipient. The presence of parasitaemia in donated blood raises some important issues including

1. How much parasitaemia in donors translates to infection or disease in recipients
2. Whether blood banks can afford to discard all malaria positive blood that is donated and
3. What is the best screening method to detect malaria parasitaemia?

Incubation period for transfusion-transmitted malaria

The incubation period for TTM is variable (105) but for *falciparum*, it ranges from seven to 27 days. This variability may depend on factors such as inoculum size and the intrinsic factors of the recipient such as age, level of immunity, presence of protective red cell abnormalities such as sickle cell etc. Recent observations from TTM cases indicate that the incubation period may last several months (99). Determination of incubation periods is based on the ability of the patient to recollect exactly when the symptoms started and on knowing the date of the

implicated transfusion. This is not always known. In an observation from non-immune subjects in Yugoslavia, 3 children who received blood from the same source had varying incubation periods of 7, 15 and 23 days. Assuming that they each received the same dose of parasites then, it was the recipient's immunity that played the major role in determining the incubation period (106). Inoculum size in a transfused unit of blood can be huge. When one parasite /µl is found, that translates to about 500,000 parasites in a unit of blood.

Parasite viability

Plasmodium parasites can survive refrigeration for up to 18-20 days (59,93) but a recent study shows that refrigeration is detrimental to their survival (107). There was a 7.1 fold reduction after 14 days but parasites remained detectable by microscopy for 28 days when stored in blood at 4°C. Parasites also did not show in vitro replication when cultured after 14 days storage. This may be an indication of the parasite losing viability after prolonged refrigeration. More studies are needed to authenticate this.

Presentation of transfusion-transmitted malaria

It is not expected that symptoms of TTM should be different from the usual symptoms of malaria which include fever chills, myalgia, headache, vomiting and diarrhoea. In a study by Ali et al who found 14 patients with post transfusion malaria after receiving *P. falciparum* positive blood, there was no clear clinical presentation of TTM except for the presence of fever (101).

2.2.2 Prevention strategies of TTM in sub-Saharan Africa

Preventive strategies targeted against TTM could be

A) Pre-transfusion interventions aimed at the donor. Included in pre-transfusion interventions are

- screening the blood donor for parasitaemia,
- donor deferral and/or pre-treatment
- in vitro treatment of blood with anti-malarials

B) Post-transfusion interventions where recipients receive a full course of anti-malarials.

2.2.3 Screening tests

Introduction

This section of the review focuses on test methods that can be used in the diagnosis of malaria. The two ways whereby malaria can be detected is by direct and indirect detection. Methods for direct detection include light microscopy, antigen detection (both immunochromatography/rapid diagnostic tests and antigen enzyme linked immunoassay) and molecular tests. Indirect detection is by antibody serology.

Light microscopy

For light microscopy, two types of blood film preparations are used, the thick and thin film. The thick film is used for detection of the parasite and the thin film for the identification of the species. For the examination of a thick film, 100 microscope fields should be looked at. In a good standard thick film, this is approximately 0.25µl of blood (108,109). Microscopy has a limit of detection of 20-30 parasites/ µl of blood and low parasitaemia can be missed by microscopists (110,111).

Microscopy enables parasite densities to be determined as this may be important for classification of severity. Accurate determination of parasite densities is done on a thin film by counting parasites against at least 100,000 red blood cells (112). This is time consuming. There are other methods but one that is commonly used is to count the number of parasites per white blood cell in a thick film and multiply by 8000, which is presumed to be the average WBC count per µl (113). This method is used in busy clinics and for field studies but is not very accurate.

The stains that can be used are Giemsa, Fields and Leishman; with Giemsa being the commonly used and the best all round stain (114). The Giemsa stain is stable over time and its staining quality is consistent over a wide range of temperature. Giemsa stains the smear well, even in the hands of inexperienced persons, and is therefore the stain of choice in peripheral health laboratories (114).

Importance of microscopy in malaria

Microscopy remains the gold standard for malaria diagnosis (115). Giemsa microscopy is regarded as a suitable diagnostic instrument for malaria control programmes (116). Microscopy is the only U.S. Food and Drug Administration (FDA)-approved end point for assessing the outcomes of drug and vaccine trials, and for serving as a reference standard in the evaluation of new tools for malaria diagnosis (60).

Microscopy use in blood donor screening

Screening is not performed routinely in blood donors in endemic countries but it is the method most frequently used to screen for malaria in blood donors and recipients in clinical studies in sub-Saharan Africa (104). Table 2.1 shows a summary of advantages and disadvantages of employing microscopy as a screening test for blood donors. Gametocytes cannot directly cause disease in humans so if they are found by microscopy in blood, the blood may still be given.

Table 2.1: A summary of the pros and cons of using microscopy as a screening test in blood donors

Pros	Cons
• Ability to determine parasite density. Donated blood with very low parasite density may be used in times of blood shortage • Allows for identification of all species. It is yet to be established if particular species are most relevant for TTM but if it is, the species identification is important. • Allows differentiation between asexual parasite stages: Donated blood with gametocytes may be acceptable since they do not cause disease.	• Not very sensitive: Inherently unable to detect low parasitaemia such as below 20-50 parasites/µl • Turn-around time is slow (at least 30 minutes per sample) and not suitable for screening large numbers • Experienced personnel required

Malaria rapid diagnostic tests

Test principle: Immunochromatographic or rapid diagnostic tests (RDT) are based on the capture of malarial antigens. There are several formats that are commercially available including dipsticks, cards and cassettes but the principle remains the same. Immunochromatographic procedures utilise monoclonal or polyclonal antibodies, conjugated to either gold particles or liposome's containing selenium dye, to capture the malaria antigen of choice. Migration of liquid across a nitro-cellulose membrane allows the captured malaria antigen to bind to a second immobilised monoclonal antibody, thus producing a visible coloured line (117).

Antigens detected: Two malarial antigens, histidine-rich protein-2 (HRP-2) and parasite lactate dehydrogenase (pLDH) have been studied extensively. Plasmodia aldolase is another antigen that has over the past few years been introduced (118) and it can be found in all species of *Plasmodium* (119).

Histidine Rich Protein-2 (HRP-2): HRP-2 is present only in *P. falciparum* (119). Tests conducted in many field studies across areas with different malaria endemicity, show a varied but consistently good sensitivity and specificity, ranging from 85% to 100% (120-127).

HRP-2 tests have performed well but because the HRP-2 antigen is present only in *P. falciparum,* the test cannot be used for the detection of other human malaria parasites such as *P. vivax, ovale* or *malariae* (128). HRP-2 also persists in the bloodstream after malarial episodes and so the test cannot be used to monitor anti-malarial therapy (129).

In areas where a single species is responsible for malaria infections, an RDT for that specific species may be preferred especially since these have lower costs (130).

Plasmodium Lactate Dehydrogenase (pLDH): The metabolic *Plasmodium* enzyme pLDH is actively produced by the malaria parasite during their growth in red cells. All four human malarial parasites produce a unique isomer of pLDH activity (131). Tests for *P. vivax* based on specific LDH (pLDHv) has recently been developed and evaluated in the Republic of Korea (130) where *P. vivax* accounts for 99.9% of all malaria cases (132,133). Also, pLDH has been found to be a good marker for

following active malarial infections and is therefore useful in monitoring treatment (134). Many authors (135-137) acknowledge the monitoring potential that pLDH provides; since it becomes negative soon after parasite clearance. The OptiMAL® assay is one of the widely used commercial products that measures pLDH by a rapid immunochromatographic test.

Performance of RDTs

Several clinical trials using RDTs report 88-100% sensitivity and 92-95% specificity for the detection of *P. falciparum* (117,138). The sensitivity of RDTs decreases markedly if there are <100 parasites/µl (139). In comparative studies where parasite counts were less that 50 parasites/µl, RDT could not detect 50% of positive sample (140). RDTs are not a replacement for microscopy but where microscopy is not possible, RDTs should be introduced (141).

RDTs have been used since the early 1990s and they can perform with high accuracy. An issue of concern, however, is the variation in product performance in some published trials. There is a need for accurate, evidence based information on the performance for each commercially available RDT (139,142-148). This observed variation may be due to poor conditions under which test materials were transported or kept. Quality assurance for RDTs therefore remains a key focus by the World Health Organisation (149). Since 2006 the WHO has embarked on an evaluation of commercial RDTs and so far, 118 products have been evaluated by 2011 (150). A fourth round of evaluation has just begun (151).

A newer generation of 3 band RDTs now use *Plasmodium* aldolase, a pan malarial antigen, in addition to HRP-2 to identify non-*falciparum* infections (152). This combination will distinguish *P. falciparum* from other species but will not differentiate between the non-*falciparum* species.

RDT as a screening test

RDTs can be performed faster than microscopy and generally do not require more than 15 minutes. They are simpler to perform and there is low subjectivity in the results. RDT can therefore be used to screen many donors or samples quickly.

The main drawback for RDTs as with microscopy is the low sensitivity at lower parasite concentrations because healthy blood donors will likely have low parasitaemia. The tendency of HRP-2 tests to remain positive after treatment will result in false positive results and therefore more blood being rejected that necessary.

RDTs have been used to screen for malaria in donors in Nigeria. An evaluation in donors with 2 RDTs; Optimal (based on HRP2 and Clinotech (based on *msp* and circumsporozoite protein) showed inconsistent results. Prevalence by microscopy was 20.2% and prevalence was 3.8% and 57.8% by Optimal and Clinotech respectively (103). RDTs have also been tried in countries with no or low transmission. In Iran (100 donors) and Turkey (2229 donors) all RDT results were negative compared to PCR and microscopy testing which detected 2 positive samples in Iran (153,154). At a regional blood centre in India, routine screening is done for blood donors using HRP2, pLDH and aldolase based RDTs. Of 11,736 donated units of blood, 3 were found to be positive and they were confirmed with microscopy (155).

Antigen detection ELISA

Enzyme linked immunoassays have been developed that are capable of detecting malaria antigens (156-158). The antigen being detected is also HRP-2 as used for the RDTs. In this technique, wells are coated with monoclonal antibodies against HRP-2 of *P. falciparum*. This method has been evaluated in Thailand and is commercially available (159). Antigen ELISA has been recommended for evaluation in blood banks for screening but surprisingly, there are not many published studies. A recent study conducted among blood donors in Venezuela found that the sensitivity and specificity was very high, 100% and 98% respectively (160).

Antigen ELISA as a screening test

This test has similar attributes as described for RDT but an added advantage is the capacity to test many samples at a go because of the setup in a 96 well plate (159).

Polymerase chain reaction

The development of malaria nucleic acid amplification techniques has been based on the polymerase chain reaction (PCR) method. PCR is a method which is used to amplify nucleic acid sequences of the malaria parasite which may be present in blood. Following PCR the amplification product can be detected using gel electrophoresis followed by ethidium bromide staining. Based on various modifications, different types of PCR methods exist including nested PCR, multiplex PCR and real time PCR.

PCR is the most sensitive and specific method to detect malaria parasites (161). Sensitive PCR techniques have been developed that are capable of detecting parasites at a density of one per microlitre in contrast with 10-50 parasites per microlitre, which is the threshold of an experienced microscopist (162). There are PCR methods that detect less than one parasite per microlitre (0.7 parasites /µl) (163). Due to its superior sensitivity PCR is being proposed as the gold standard for the laboratory diagnosis of malaria (164,165). However, because PCR is not widely available, some authors' (166,167) still maintain that microscopy is the gold standard.

The PCR technique is robust (162). DNA has been extracted from samples and stored for duration of a few months to more than 20 years (83,168,169).

Uses of PCR

PCR now plays an important role in the molecular detection of drug resistant parasites. This is performed by identifying or detecting mutations at specific points in the genome and which are associated with resistance. Mutations in the dihydrofolate reductase *(dhfr)*, dihydropteroate synthetas (*dhps)* and *P. falciparum*

chloroquine resistance transporter *(pfcrt)* genes are some of the well defined molecular markers of resistance for pyrimethamine, sulfadoxine and chloroquine respectively (170).

PCR is able to detect lower levels of mixed infections than was previously thought (171). It has also been used to differentiate between malaria re-infection and recrudescence. In endemic areas, where persons could be bitten by infected mosquitoes on a regular or daily basis, it is possible that parasites may be found in a patient's blood a couple of weeks after treatment, despite the treatment being effective. By using polymorphic loci studies, the presence of the same parasite remaining in a specimen can identified. The most frequently used antigens for genotyping are merozoite specific protein 1 and 2 (MSP1 and MSP2) and glutamate-rich protein (GLURP) (83,172-174). Methods for genotyping parasites include restriction fragment length polymorphism, amplified fragment length polymorphism, isoenzyme electrophoresis, microsatellite and single nucleotide polymorphism. To help standardize genotyping methods and reporting, a document, Recommended Genotyping Procedures to identify parasites population (RGP) has been produced by a collaboration of WHO and Medicines for Malaria Venture (172).

PCR as a screening test

PCR is currently the most sensitive method for detecting parasites and can detect parasites below the threshold for microscopic detection (165). Such a highly sensitive and specific test would be ideal especially as low parasitaemia is expected in donors but PCR requires an extensive capital investment. Advanced infrastructure is required for training personnel, quality control, maintaining equipment and preventing contamination (152). Such a high operational cost will be unaffordable for many malaria endemic countries that are also developing countries. It is unlikely that PCR will be suitable for routine purposes in the near future (165) although it may become more accessible in the longer term.

PCR has been compared with microscopy and HRP2 rapid detection test to screen blood donors in a study in Sudan (175). Using PCR as the gold standard, the sensitivity of microscopy and the RDT was 61.9% and 66.7% respectively and the specificity was 100% and 94.9% for microscopy and RDT respectively.

Serology

Serological testing is an indirect way of determining malaria infection. It involves the detection and measurement of *Plasmodium* antibodies. The indirect fluorescent antibody testing (IFAT) and Enzyme linked immunosorbent assay (ELISA) are the most commonly used methods to measure *Plasmodium* antibodies titres with IFAT being the "gold standard" (176,177).

In malaria endemic regions, most of the adult population have had frequent exposure to malaria parasites and will have antibodies to *Plasmodium*. The prevalence of *Plasmodium* antibodies among blood donors in countries such Senegal and Nigeria range from 65.3% to 100% (178,179). The high detection rate seen in these studies would imply the exclusion of all these donors if it were used as a screening test. Since the presence of antibodies does not indicate a current infection and does not indicate infectivity, serological testing is not an appropriate screening test for donors in malaria endemic areas.

Antibody detection as a screening test

Screening by antibody serology has no place in malaria endemic countries. However in non-endemic countries there are some advantages and disadvantages. IFAT has been a reliable serological test for malaria for decades. It has good sensitivity and specificity (180). On the other hand, the process of slides manipulation is time consuming (181,182) and reading the result is subjective (180). The ELISA method is available in automated formats and therefore allows large volumes of samples to be processed quickly. There is no subjectivity with ELISA unlike in IFAT which is subjective especially in samples with low antibody levels (177).

Serological screening tests have been used in non-endemic malaria countries because the presence of antibodies in a donor indicates a prior exposure in the donor, who may then be excluded for a period depending on the guidelines in that country (93,183). Countries that currently screen at risk donors include the UK, Denmark, Finland and New Zealand (184). The enzyme immunoassays currently available use recombinant antigens that provide a higher sensitivity (180).

2.2.4 Deferral and pre-treatment of donors

The WHO recommends that donor selection criteria should be developed in endemic countries to identify and collect blood from donors at the lowest risk of infection, both during the malaria season and during the rest of the year (59). It also recommends pre-treatment of the donors with anti-malarials if indicated (185). These measures are not used in most of Africa (186). There may be several reasons why screening is not being done. Indeed, screening and deferral of malaria infected donors is believed to be impossible to implement in countries where prevalence of malaria is high, and where the deferral of donors will reduce by a third, the available amount of blood (18).

Donor selection is difficult with healthy adults who are asymptomatic carriers as they will not present with fever and questioning does not identify such donors as carriers. There are a few countries that defer donors in Africa. In Zimbabwe, which has low malaria endemicity, the approach is to question donors if they have visited a malaria area within the previous 21 days (187). If they have, and if they did not take prophylaxis, they are deferred for 6 weeks. If they have visited a malaria endemic area, and contracted malaria, they are deferred for 6 months after they have received a full course of treatment and have been clinically symptom free of malaria. There is no evidence of how successful this strategy has been in Africa. In non-endemic countries, stringent deferral policies are in place for travellers to malaria endemic countries. Currently in the US, there is little risk even with the increased travel, and the incidence of TTM is less than 1 case per year. However the down side is that more than 100,000 donors are deferred, many of whom may never return to give blood (53). Cost effectiveness studies from Canada has shown

that compared to standard screening questionnaire, the addition of donor screening by PCR to the standard screening questionnaire is economically attractive (188).

Pre-treatment as a strategy is impractical and is not commonly used. Most donors in malaria endemic countries are replacement donors and they go to donate blood for relatives who urgently need blood. Effective treatment will require about 72 hours and both the donor and recipient cannot realistically wait for that duration. It will therefore not be feasible for such donors to be treated for malaria.

2.2.5 Anti-malarials in blood bag

The in vitro processing of donor blood with anti-malarials in blood bags to kill parasites before the blood is transfused has been suggested as an additional measure to prevent TTM. Two anti-malarials, quinine and sulfadoxine-pyrimethamine (SP) have been used experimentally and found to effective against parasites in blood bags (189,190). In a study in Sudan, SP for example at a dose of 179.65 µg/L killed 99% of all parasites within 24 hours parasites. There are however concern that such an intervention can contaminate the blood bag and has the potential to increase the spread of resistance to the particular anti-malarial (191). In vitro processing of donor blood with anti-malarials has remained an experimental intervention and has not been used in blood for patients.

2.2.6 Presumptive medication of blood recipients

Presumptive treatment of transfusion recipients in malaria endemic countries is recommended by the WHO (185) and countries such as Senegal and Kenya have implemented this recommendation (192). There is little data on the extent to which anti-malarials are currently being used as presumptive treatment. In the past, administration of anti-malarials such as chloroquine to recipients of blood transfusions was a widespread practice. Resistance to chloroquine means that it has largely been replaced by the more expensive artemisinin-based therapies. This has

increased 5-7 fold the cost of treating all transfusion recipients with anti-malarials making this practice unaffordable on a wide scale (192,193).

Opinion is divided on who should receive anti-malarial prophylaxis. While some follow the WHO recommendations (194-197) others in contrast recommend restricting malaria treatment to selected 'at risk' transfusion recipients such as neonates (34,179,198).

Unfortunately all these suggestions are only based on expert opinion but without sufficient evidence.

2.3 BACTERIAL CONTAMINATION AND TRANSFUSION RELATED SEPSIS

Introduction

Bacterial contamination of refrigerated whole blood has been a recognised complication of blood transfusion since the 1950's (199). With the introduction of sterile, single use plastic collection sets and component therapy, the frequency of septic reactions decreased dramatically (64). Despite this decrease, the risk of bacterial contamination has remained a major cause of transfusion related morbidity and mortality (200-202). Among the blood components, it is recognised that platelet concentrates, which are stored at room temperature, provide a more hospitable environment for the contamination and growth of a wide range of organisms compared to red blood cells (RBC) which are stored at +4^{0}C (203). The majority of published data considers blood component transfusion with few studies about whole blood because the developed world now predominantly uses blood components and products. In sub-Saharan Africa, where whole blood continues to be used widely, septic transfusion reactions due to bacterial contamination have been described as a major but under-reported and unappreciated risk of transfusion (204,205).

Definition and classification

The Serious Hazards of Transfusion (SHOT) scheme in the UK was one of the first haemovigilance systems and was launched in 1996. Transfusion-transmitted infection (206) were defined as a post transfusion infection in which

- there was no evidence of infection prior to transfusion, and no evidence of an alternate source of infection and either
- at least one component was donated by a donor who had evidence of the same transmissible infection or
- at least one component received by the infected recipient was shown to contain the agent of infection

Transfusion associated bacterial contamination is identified based on the results of culture of the blood of the recipient and/or the transfused unit, genotyping of the isolated organisms, the presence of symptoms, and the use of an antimicrobial. At present there is a lack of uniformity in definitions used worldwide as individual haemovigilance systems from different countries have different reporting systems and investigation requirements (207-209). Confirmation of cases from haemovigilance systems are usually retrospective as the transfusion reaction which has occurred must be investigated before a final confirmation can be made. A classification by the Canadian Haemovigilance system (210) based on confirmation of a set of criteria is shown in table 2.2.

Consensus definitions for all bacterial events are being developed jointly by the International Society of Blood Transfusion Working Parties on transfusion-transmitted infectious diseases and haemovigilance to harmonise the current situation (211).

Table 2.2: A classification system for transfusion-transmitted bacterial contamination

Possible Bacterial contamination is considered "Possible" if it meets the following criteria: • The recipient's blood culture is positive. • Contamination of the blood sample or laboratory contamination is not suspected. • The recipient presents signs and symptoms of sepsis (nothing else explains it). • A blood, blood component, or blood product (plasma derivative) cultures was not done. ➢ No specimen was available ➢ A blood culture was not ordered
Probable • Bacterial contamination is considered "Probable" if it meets the following criteria: • Positive blood, blood component, or blood product (plasma derivative) culture. • Contamination of the blood sample or laboratory contamination is not suspected. • The recipient presents signs and symptoms of sepsis (nothing else explains it). • The recipient's blood culture was not done. ➢ No specimen was available. ➢ A blood culture was not ordered. • The recipient's blood culture is negative. ➢ The recipient is already taking antibiotics.
Definite Bacterial contamination is considered "Definite" if it meets ALL of the following criteria: • The same bacteria are found in the recipient and the blood, blood component, or blood product (plasma derivative). • Contamination of the blood sample or laboratory contamination is not suspected.

2.3.1 Epidemiology of bacterial contamination

The precise incidence of bacterial contamination of blood and blood products is unknown. This is because different studies use variable methods of detection and apply different case definitions (212). In addition, the number of confirmed and reported cases of bacterial sepsis can be considered the tip of the iceberg because there are many more cases which could not be confirmed and others that may have had no apparent harm and therefore not reported (211). Despite these underestimations, bacterial contamination has become the most common infectious risk from transfusion (213), in part due to the success with reducing viral transmission (214).

In the US, bacterial contamination poses a greater infectious threat in transfusion medicine than combined risks of receiving a blood product or component contaminated with HIV-1 or 2, hepatitis C virus, hepatitis B virus, and human T-cell lymphotrophic virus I or II (215). Surveillance reports from the UK between October 1996 and December 2009 also show that bacteria are the commonest cause of transfusion-transmitted infections. Forty out of 66 reported incidents (60%) of transfusion-transmitted infection within the period were due to bacteria compared to 17 (25%) that were due to viruses including HBV, HCV, HEV, HIV and human T-cell lymphotrophic virus (HTLV) (98). Similarly, from 1997 and 2007 in Germany, 71 cases of confirmed transfusion related bacterial infections compared to 46 confirmed cases of transfusion associated virus infections were reported (216).

Studies across Europe and North America show that the rate of bacterial contamination in platelets is about one per 1-3,000 units, while clinical sepsis or septic transfusion reactions is 1 per 5-20,000 transfusions (208,217-219). Blood and blood component haemovigilance data from France shows that the risk of bacterial transfusion reaction in apheresis platelet concentrates is 69.1 per 1,000,000 units and for RBC's is 10.4 per 1,000,000 units. The risk for whole blood is 12.9 per 1,000,000 units. The risk of a fatal outcome per 1,000,000 units is 12.0, 0.7 and 1.1 for platelets, RBCs and whole blood respectively (220).

Few studies have investigated bacterial contamination in blood transfusions in sub-Saharan Africa. There are two studies from Ghana and one from Kenya. The frequency of bacterial contamination of paediatric whole blood in Kenya was 8.8% (205) and that for whole blood in Northern Ghana was 17.5% (36). Another study in Southern Ghana (37) found a 9% overall contamination rate in blood and blood components. Contamination in whole blood alone was 13% (24/192), surprisingly higher than bacterial contamination of platelets which was 9% (2/22) although the number of platelets screened was small (37). All three studies in Africa did not follow up the transfusion recipients to determine the occurrence of any septic reactions or adverse events. The contamination frequency reported in Kenya is 2500 times greater than that of bacterial contamination RBCs in developed countries (205).

Organism and the sources of contamination

Organisms

A wide spectrum of organisms has been associated with transfusion-transmitted bacterial infection including skin, enteric and environmental organisms. *Staphylococcus epidermidis, S. aureus*, other coagulase-negative staphylococci and *Bacillus* spp are the common organisms which contaminate platelets. *Yersinia enterocolitica* and *Pseudomonas* spp commonly contaminate RBC (221). A summary of the reported organisms are shown in table 2.3 with *Y. enterocolitica* being the most common. Most of the bacteria associated with transfusion reactions are aerobic or facultative species but a few anaerobes such as *Propionebacterium acnes* and *Clostridium perfringes* have been associated with septic reaction (222).

Table 2.3: Distribution of organisms responsible for bacterial contamination of blood components

RBCs	Platelets
Yersinia enterocolitica – 46%	*Staphylococcus* spp – 42%
*Pseudomona*s spp – 25%	*Streptococci* spp – 12%
Serratia spp – 11%	*Escherichia coli* – 9%
Others – 18%	*Bacillus* spp – 9%
	Salmonella spp – 9%
	Serratia spp – 8%
	Enterobacter – 7%
	Others – 4%

(Adopted from Wagner SJ 2004 (223))

Sources

Organisms that contaminate blood or blood components will generally come from four main areas (213). These are:

1. Donor bacteraemia

2. Donor arm contamination

3. Contaminated collection equipment

4. Contamination during processing and storage.

These four areas from which bacterial contamination occurs can be grouped into two sources; endogenous (donor bacteraemia) and exogenous (donor arm contamination, contaminated collection equipment and contamination during processing and storage) sources of infection.

Endogenous sources

Endogenous sources occur when the organisms are circulating in the donor blood and are then taken into the blood pack. It is expected that donors who are unwell will not be allowed to donate. Donors however may feel well but have bacteraemia (asymptomatic) or may have undergone a minor surgical or manipulative procedure which will cause a transient bacteraemia. Examples of organisms which have been implicated with endogenous sources are *Yersinia enterocolitica, Salmonella spp, Bacteroides* spp and streptococci (224,225). *Y. enterocolitica* causes enterocolitis, characterised by diarrhoea, low grade fever and abdominal pain in the donor and about 75% of implicated blood donors recalled having had diarrhoea in the days preceding or following their blood donation (226).

Exogenous sources

Exogenous sources refer to organisms which contaminate blood from outside the body (i.e. from skin, collection equipment or during processing and storage (224). Contamination during collection is the major cause of bacterial contamination of platelet units and the majority of the organisms are normal skin flora (227). In an interesting report, *Serratia marcescens* was found to have contaminated transfused blood. A follow up investigation identified 0.73% of 1515 blood bags were contaminated (228). Bacteria implicated in transfusion-associated sepsis are

typically endotoxin-producing Gram negative bacilli that are found in soil, water and faeces, such as *Y. enterocolitica*, *Pseudomonas* spp, *Escherichia coli* and *Salmonella* spp (61,229) .

2.3.3 Pathogenesis

Bacteria may undergo three general phases when in contaminated blood.

Lag phase

Initially, low numbers of bacteria (between 10-100) may be present in blood/blood components but non-specific host defence mechanisms including complement activation in the presence of plasma or phagocytosis by leukocytes after opsonisation can eliminate or inactivate the bacteria (230). This can be described as the self limiting phase. There are instances when bacteria may survive in the blood but will not multiply. Those that survive begin to multiply and enter the exponential phase (231). Different bacteria exhibit variable lag phases and this adds to the uncertainty of a reliable detection time (232).

Exponential phase

This phase may also be referred to as phase of continuous growth. A bacterial load of less than 20 in the component of blood can grow up to 10^{8-9}/ml and this accounts for life threatening complications of transfusion (233). The exponential phase ends when available nutrients are exhausted or due to accumulation of toxic products of metabolism.

Death phase

This phase occurs because of depletion of cellular reserves. In this phase there may not be bacteria alive but sufficient endotoxin may have been produced to cause severe morbidity and mortality (231).

Severe sepsis and shock are the consequences of a generalised and uncontrolled inflammatory response to infection, rather than the direct effect of microorganisms (234,235). The severity of the clinical presentation depends on both inoculum and bacterial virulence (236). Higher bacterial concentration and more virulent organism present with severe reactions.

2.3.4 Factors promoting bacterial contamination

Inadequate skin disinfection

Contamination, especially from the skin is most common during blood collection (237). This happens because even after disinfection, some bacteria remain on the skin and the hypodermic needle used for phlebotomy becomes inadvertently contaminated. Bacteria can remain deep in the skin sweat glands and contribute to contamination (66). Skin that has several folds, has been scarred or dimpled provides an environment where skin bacteria can grow and are protected from disinfectant (238). This has been observed in frequent donors who have been scarred from repeated needle pricks.

Storage conditions of blood

Platelets are the components of blood that have the highest frequency of bacterial contamination (202). Whereas red cells and whole blood are stored refrigerated, platelets are stored at room temperature and thus provide a suitable environment for bacterial proliferation. For most bacterial species, growth in platelet units can occur, rapidly reaching the log phase within 24-48 hours. An exception is *Staphylococcus epidermidis* which is a slower growing organism and reaches the log phase in 48-72 hours.

In blood banks of countries with unstable economies, erratic or no electricity supply, whole blood may not be stored under optimum refrigeration conditions (205) and therefore may be subjected to similar risk as platelets for bacterial contamination and proliferation. Certain bacteria such as *Yersinia spp* and

Pseudomonas spp can survive in temperatures of 2-8°C and may therefore cause bacterial contamination and sepsis even when blood is stored under refrigerated conditions (239).

Paediatric transfusion practices

A further risk factor for bacterial contamination is the practice where, for lack of pre-prepared small volumes of blood for paediatric transfusions, small volumes are drawn from the standard larger ones for individual patients. This results in a breach of the integrity of a closed system (205).

2.3.5 Presentation of transfusion related sepsis

Clinical presentation of transfusion induced sepsis may vary depending on the state of the patient and also on the type of organism causing the bacterial contamination. Potential signs and symptoms observed are listed in Table 2.4 (240).

Table 2.4: Signs and symptoms of transfusion related sepsis

- Fever (temperature 38.0^{o}C or higher) or an increase in temperature of more than 1.0^{o}C
- Tachycardia (heart rate ≥ 120/min) or an increase in heart rate of more than 30/min
- Rigors/chills
- Hypotension (drop in systolic blood pressure of more than 30mm Hg)
- Nausea, vomiting, diarrhoea, dyspnoea, bleeding, oliguria or other symptoms of shock

The onset of clinical signs is usually rapid and occurs during or shortly after transfusion (241). Fever and chills will typically present first, within 2 hours of transfusion. Subsequently, nausea and vomiting follows and patient can rapidly progress into haemodynamic shock.

Blood heavily contaminated with Gram negative bacteria introduces large amounts of both the microorganism and endotoxin into the circulatory system, leading to rapidly developing severe sepsis or shock (225).

In recipients who are ill prior to transfusion, signs may be missed or misconstrued to be previously existing , leading to misdiagnosis and death (242,243).

Mortality

Reported case fatality rates in transfusion associated septic reactions range from 17.4 to 31.3% for platelet transfusions and 24 to 60% for RBCs (207,208,218) . Transfusion associated sepsis is the most frequent cause of death from infectious agents of transfusion, representing 17-22% of all fatalities (223). In the US, transfusion-transmitted sepsis is the third most reported transfusion related fatalities reported to the Food and Drug Administration (FDA) and 68% of the deaths were due to Gram negative organisms, *Klebsiella pneumoniae* being the commonest isolated organism (244).

Gram negative bacilli are associated with a particularly poor outcome. *Yersinia enterocolitica* sepsis for example has an overall fatality rate of 54.5% (225).

Providencia rettgeri is an opportunistic pathogen usually associated with severe burns or long term indwelling catheters but it is a rare cause of transfusion fatality (245). Coagulase negative staphylococci have also been associated with death (246).

2.3.6 Detection of transfusion associated bacterial contamination

The introduction of better detection methods for viruses and the subsequent successful reduction of the risk of viral transmission via blood components, has

focused attention on the risk of transfusion associated bacterial sepsis (241). However recovery of bacteria from blood/blood products may depend on the number of bacteria present and the provision of appropriate conditions for growth.

Strategies to detect the presence of bacteria in blood components include

- Quality control testing
- Routine surveillance screening or other pre-release testing
- Visual inspection of components prior to issue from the blood centre, at the hospital transfusion laboratory, and at the bedside prior to administration
- Careful monitoring of patients during and after transfusion

Some countries now have routine pre-release surveillance screening of platelets, because of the high risk of contamination associated with platelets. The introduction of routine culture of platelets in the US and Canada has reduced the risk of septic transfusions by 50% (247).

Surveillance can be active or passive. In active surveillance, units are tested prior to transfusion, and any units found to be contaminated are either discarded (if the results are available prior to transfusion) or tracked to determine any adverse outcomes (if results are available only after transfusion). With passive surveillance clinically apparent transfusion reactions following a transfusion are examined retrospectively and testing of units is performed if samples are available. Passive surveillance greatly underestimates incidence of contamination (248). Studies have shown that active surveillance detected 32-fold more bacterially contaminated platelet units and 10.6-fold more septic reactions than passive surveillance (236).

Detection systems

Various methods exist for bacterial detection. There are two broad categories for detection of contaminated platelet components; initial release tests and point of issue tests which are performed immediately prior to transfusion to prevent the transfusion of platelet components contaminated with high levels of bacteria that may cause severe clinical septic events. The technologies available for detection

include: visual inspection; measurement of glucose and pH levels; Gram stain; oxygen consumption; carbon dioxide production; detection of bacterial nucleic acid sequences; fluorescent flow cytometric detection of bacteria; endotoxin detection; bacteria-specific antigen detection; and automated bacterial culture (249). None of these tests are used routinely for whole blood.

Bacterial culture: Bacterial culture is used as an initial release test for platelet components. Two culture systems in use are the automated liquid culture system (BacT/ALERT) and the Pall enhanced Bacterial Detection system (eBDS) (250). The Bactec and VersaTREK systems have also been validated and are sometimes used. The eBDS is used to detect contamination in platelet units using measurement of reduction in oxygen concentration resulting from bacterial growth. Consisting of a disposable sample pouch, an incubator and an oxygen analyser, the system is incubated for 24 hours after the sample is introduced. The oxygen levels are then analysed to obtain a result. In a study comparing detection of low bacteria (1-10CFU) at 24 hours and 30 hours, eBDS had detection rates of 95% and 97% compared to Bactec detection rates of 89% and 94% (251).

The BacT/Alert system is considered to be the gold standard for detection of bacterial contamination of platelet concentrates (252). The assay is validated to detect common contaminants at concentrations as low as 1 to 10 colony forming units (253). Although BacT/Alert has reduced the risk of transfusion of bacterially contaminated platelets, the method is not ideal because of false negative results (254).

Other detection systems

Flow cytometry is less sensitive than culture but it is suitable for testing platelets prior to transfusion. It is fast and relatively simple and uses equipment and techniques widely available in many developed country laboratories (255). Molecular methods such as real-time PCR show high sensitivity and specificity, with results available within hours. Such methods are now being recommended as a suitable alternative to culture methods (256).

Other methods of detection which are used prior to transfusion are measurement of pH and glucose (223,257).

Monitoring of patients

With the exception of platelets, blood or blood components are not routinely screened for bacteria. Monitoring of transfusions for septic reactions is a way of identifying bacterial contamination. Look-back investigations reveal that recipients may display signs indicative of a possible acute transfusion reaction which are not reported or investigated (258). Early recognition and reporting of signs with subsequent investigations will improve diagnosis.

2.3.7 Prevention of transfusion-transmitted bacterial infection

A combination of measures is needed to prevent or reduce bacterial contamination of blood. This is because there are multiple ways in which contamination can occur and therefore all efforts must be made to address each potential source.

General measures

Continuous training and efficient supervision is required to ensure optimal product processing, handling and storage of blood.

General infection control measures such maintaining clean fridges, blood warmers or water baths and containers for transporting blood. Monitoring of fridges for a consistent storage temperature 4^0C for whole blood is important to ensure product integrity.

Donor deferral

It is helpful to ask donors about recent dental procedures or minor procedures which could lead to transient or asymptomatic bacteraemia. Other specific questions to identify osteomyelitis are useful (241). Donors with such risk factors should be deferred. Although about half of donors implicated in *Yersinia*

enterocolitis have had gastrointestinal symptoms in the 30 days before donation, about 13% of all donors have had gastrointestinal symptoms in the previous 30 days (226,259,260). Gastrointestinal symptoms in the 30 days before donation are therefore neither sensitive nor specific for Yersinia bacteraemia. Gastrointestinal symptoms should not be used as a donor question because it will screen out an unacceptable number of healthy donors.

Donor skin disinfection

Universally applied effective donor skin cleansing is an obligatory requirement to minimise the incidence of post transfusion septicaemia (224). A fatal case of transfusion-transmitted *Clostridium perfringens* sepsis (261) was attributed to faecal contamination of the skin of the donor and this highlights the importance of skin cleansing. Improving donor skin disinfection is a critical target for prevention (243). Skin cleansing does not totally eliminate contamination but just minimises the risk. This is because skin disinfection only reduces the load of bacteria present on the skin (262,263).

Diversion of donor blood

Diversion is based on the principle that contamination from the donor's skin will be present in the initial flow of blood and therefore a redirection of the initial flow of blood from the collection bag into a pouch reduces the contamination. Diversion of blood alone reduces contamination with skin bacteria in both whole blood and platelets by 50% (264). When diversion of blood is performed in combination with improved donor skin cleansing, there is a reduction of bacterial contamination by 47-77% (265).

Bacterial detection

Culture based pre-release screening of platelet concentrate with early sampling has been proven to detect bacteria and to prevent transfusion of some but not all

contaminated units (266). In a study of one million apheresis platelet concentrates, the rate of septic reactions decreased from 18 to 5.4 reactions per million transfused units but the fatality rate did not decrease (247). Current bacterial detection systems are unable to completely eliminate the risks associated with bacterial contamination and none of the currently available laboratory techniques are ideal (217)

2.3.8 The challenges in transfusion-transmitted bacterial contamination

Detecting bacterial contamination before it causes harm in a transfusion recipient poses particular challenges. Unlike the situation for viruses where the donor is the source of infection and therefore pre-donation testing can be performed, bacterial contamination can occur during collection or storage and therefore the ideal time is to screen just before the blood is used. Blood culture results are not immediately available, and therefore recipients will receive the blood before it is determined whether the blood is contaminated or not. Other quicker methods such as glucose or pH determination are insufficiently sensitive.

The major challenge is to use tests that provide high sensitivity and specific results at or near the time of blood unit issue (222). Another dilemma is whether to accept all coagulase negative staphylococci as pathogens capable of producing harm in the recipient since they are usually skin contaminants (221)

Pathogen reduction

Pathogen reduction offers a pro-active approach to further reduce the risk of transfusion infections (230). Many techniques exist for pathogen reduction. Pathogen inactivation techniques such as methylene blue and solvent-detergent have been used for treatment of plasma intended for transfusion. Photochemical treatment with amotosalen and ultraviolet A light have also been used for fresh frozen plasma and platelets (169,202). Using log reduction assays, the efficacy of these procedures to reduce bacterial contamination has been demonstrated (267,268). Pathogen inactivation techniques have been used widely and

successfully in Europe but not in North America (184,269). Reasons for slow acceptance of these methods include concerns about safety (including carcinogenicity), cost effectiveness and the inability to inactivate certain pathogens such as spore forming bacteria, (202,269,270).

An advantage of pathogen reduction techniques is that they are not limited to bacteria but can be applied to many other pathogens. In addition, these techniques can be effective in window periods where regular screening methods may be unable to detect pathogens. The use of pathogen inactivation techniques in whole blood or RBCs has been a challenge because of the absorption of light by haemoglobin. The Mirasol pathogen reduction technology system which uses riboflavin (vitamin B2) and UV light has recently been used successfully in whole blood against various bacteria and other pathogens (271,272), So far, no treatment related toxicity has been identified with the Mirasol system (273). More evaluation is however needed.

Multi-component pathogen reduction implementation may lead to potential savings. Elimination or reduction of many donor screening assays, including bacterial testing of platelets, and reduction of donor exclusions based on geography, may help to offset the cost of the process (274). When pathogen reduction becomes available for all components and is adopted by the transfusion medicine community, then blood supply may finally reach the goal of near zero risk (66).

2.4 TRANSFUSION-TRANSMITTED SYPHILIS

Introduction

Syphilis is an infectious disease caused by the spirochete *Treponema pallidum* subspecies *pallidum.* Transmission is by direct contact with lesions or body fluids (275) but it can also be transmitted vertically from a mother to her baby (276), or through receiving a blood transfusion (277). When syphilis is acquired through the transfusion of blood or blood product, it is referred to as transfusion-transmitted

syphilis. Transfusion-transmitted syphilis is one of the oldest recognised infectious risks of blood transfusion (278).

2.4.1 Epidemiology

Syphilis in the general population

Syphilis remains a major public health problem in the world (279). The WHO estimates that 12 million new cases of syphilis occur worldwide each year, mostly in developing countries where access to sexually transmitted disease laboratory services are limited (280,281). In South Africa, a syphilis sero-prevalence of 10% for women attending antenatal and family planning clinics and between 24%-42% in high risk groups has been reported (282). Studies in Ghana have found the prevalence to vary from 4.9% to 16.5% ((46,283,284). It is important to know the prevalence of syphilis in the general population because such information provides clues of disease patterns or outbreaks and may reflect the situation in the blood donor population In Ghana for example, the high sero-prevalence reported for syphilis in parts of the Central region led to the identification of yaws.

Re-emergence of syphilis

In recent years there has been an increase in cases of syphilis in some parts of the developed world such as in Russia, Australia and the UK (285-287). New cases in the UK are reported to have increased eight fold over a five year period, from 1997 to 2002 (288). The increases are due to outbreaks in particular communities (289-291).

Syphilis in North America and Western Europe, and particularly in the UK, has re-emerged mainly as a disease in injecting drug users and men who have sex with men (292,293). The re-emergence of syphilis in the Russian Federation, Eastern Europe and China has been associated with social upheaval and is a potential contributor to burgeoning HIV epidemics in these countries (285,294).

An increase in recently acquired syphilis in the blood donor population in the UK, as observed by Brant et al indicates that risky sexual behaviours are increasing, with implications for the microbiological safety of blood (295). In general, a resurgence of sexually transmitted diseases in a community may represent an increase in risk in the blood donor population.

Africa does not appear to have seen such epidemics although the data is very limited. In the Gambia, for example, the prevalence of syphilis has decreased significantly from 11.2 % in 1994 to 1.5% in 2007 (296)

Markers of syphilis in blood donors

Syphilis sero-positivity in blood donors varies across the world (297) but some countries such as the UK have traditionally had low sero-positive rates. The current prevalence of syphilis sero-positivity in the UK is 3.7 per 100,000 (298). Among donors in Albania, sero-positivity is 70 per 100,000 (299) and that for Italy is 7 per 100,000 (300).

In sub-Saharan Africa, the sero-reactivity among donors is generally higher than that in developed countries but rates vary from country to country. Sero-reactivity has been reported to be as low as 0.05% in Egypt, (301) 0.8% in Senegal (178) and 1.2% in Mozambique (302) compared to higher rates such as 9.1% in Cameroon (303) and 8% in Nigeria (304). The prevalence of syphilis sero-reactivity in Ghanaian donors is high, ranging between 7.5% and 13.5% (38,39).

The differences in reactivity reported in specific regions may relate to variation in high risk behaviour but may also be accounted for by the kind of donors that are screened. Statistics from the UK (298) shows that first time or new donors have a higher sero-positivity (25.4 per 100,000 donations) than repeat donors (0.9 per 100,000 donations). It was previously thought that voluntary non-remunerated blood donors were safer than family/replacement donors but current evidence shows that first time VNRD are no safer than family or replacement donors. Rather, repeat donors, whether VNRD or replacement are the safest donors (13). This has been seen in Africa; and also in the US where syphilis sero-positivity in repeat

donors and first time donors in the US was 5.7 and 135.2 per 100,000 respectively (305). Sero-reactivity in Nigeria is also higher in commercial (7.5%) than in non-commercial donors (0.5%) (304).

The use of different screening tests and/or screenings algorithms may be another reason for the different sero-reactivity between countries. Adjei et al determined the sero-positivity of syphilis in blood donor to be 7.5% using a combination of Venereal Disease Research Laboratory (VDRL) and *Treponema pallidum* particle agglutination (TPPA) assays (38) whereas in a similar population, a sero-reactivity of 13.5% was reported when TPPA was used alone (39).

Syphilis in transfusion recipients

Over 200 cases of transfusion-transmitted syphilis have been published in the English literature (306) but over the past 40 years, there have been only 3 reported cases of transfusion-transmitted syphilis (307-309). These cases occurred in the US, Indonesia and the Netherlands respectively and in all the 3 cases, initial serological screening of donated blood was negative for syphilis and fresh blood was transfused.

The reduction in the risk of acquiring syphilis by transfusion may be due to a number of factors including the improved donor selection processes, universal serological screening of all blood donors and the shift to usage of refrigerated blood components (278).

There may also be the possibility that clinicians do not diagnose or misdiagnose transfusion-transmitted syphilis. This is because when a disease is reported as rarely as transfusion-transmitted syphilis, clinicians are unlikely to recognise a case when it occurs. It has been suggested that a patient who has received a blood transfusion and develops unexplained symptoms even months after the transfusion, should be checked for syphilis (309). Also, because it is difficult to exclude sexually transmitted syphilis, transfusion-transmitted cases may be excluded erroneously (277).

2.4.2 Clinical presentations

Clinical manifestation of venereal syphilis

<u>*Staging and presentation:*</u> Syphilis is a chronic infection with diverse clinical manifestations that occur in distinct stages. These stages include the incubating, primary, secondary, latent and the tertiary stage. Primary syphilis is characterised by a chancre which is painless and occurs at the site of inoculation, most commonly the genitalia (310) but can also appear in the mouth and anal mucosa. *T. Pallidum* then disseminates from the primary chancre through the blood till secondary syphilis becomes apparent. Secondary syphilis is characterised by systemic symptoms such as fever, malaise, weight loss and widespread erythematous rash (311).

Symptoms of primary syphilis occur between 10 and 90 days following exposure and secondary syphilis 4-10 weeks following the appearance of the primary chancre (312). If untreated, the rash gradually fades and the disease enters a latent phase during which the patient is sero-positive but asymptomatic and this phase can last from a few months to many years (277). Tertiary syphilis is the tissue-destructive phase that appears 10 to 25 years after the initial infection in up to 35% of untreated patients (313).

<u>*Complications:*</u> Complications of syphilis include central nervous system disease, cardiovascular abnormalities, eye disease and granuloma-like lesions called gummas that can occur in any organ. Untreated syphilis during pregnancy may have serious consequences for the child including spontaneous abortion, stillbirth, premature birth or perinatal death (314).

Clinical manifestation of transfusion-transmitted syphilis

The incubation period for transfusion-related syphilis is between 4 and 14 weeks (6-8 weeks on the average) and is inversely proportional to the number of live treponemes transfused (306). In contrast to sexually transmitted syphilis, there is no primary stage for transfusion-transmitted syphilis. The disease progress immediately to the secondary stage with a generalised rash (307),

lymphadenopathy (308) and systemic symptoms and then follows the natural course of the sexually transmitted disease if left untreated (315).

2.4.3 Diagnosis of syphilis

The diagnosis of syphilis depends on clinical findings, examination of lesion material and serological tests (316). This section will focus on laboratory diagnosis. Diagnosis may be by direct detection or by serological testing (treponemal or non-treponemal). Serological testing has been recommended for screening blood donors (317).

Microbiology of Treponema pallidum

Treponema pallidum is a fastidious, micro-aerophilic spirochete that has 4 subspecies; subspecies *pallidum* causes venereal syphilis, subspecies *endemicum* causes endemic syphilis, subspecies *pertenue* causes yaws and subspecies *carateum* causes pinta (311). *Treponema pallidum* stain poorly with Gram's or Giemsa's stain *and* cannot be cultured in vitro (318).

In humans, anti-treponemal IgM antibodies are detectable within two to three weeks after infection and IgG detectable about two weeks later (319). The detection of these antibodies forms the basis of serological tests and diagnosis, especially given the inability to grow the organism *in vitro*.

Direct detection

The identification of the spirochete *T. pallidum* by direct detection is most productive during primary, secondary, infectious relapsing and early congenital syphilis, when moist lesions containing large numbers of treponemes are present. Direct detection can be by animal inoculation, by microscopy or by using nucleic acid amplification methods such as the polymerase chain reaction (PCR).

Animal inoculation

Numerous animal species from hamsters to chimpanzee have been used to either maintain treponemes or determine infectivity but not all develop visible signs of infection or reactive serological tests (320). The rabbit model has been shown to be the best and subsequently, Rabbit Infectivity Testing (RIT) has become the gold standard for syphilis infectivity testing (321). Briefly, inter-testicular or intradermal inoculation of the rabbit with the infected fluid causes formation of a localised tissue lesion that remains infective for life. Blood may be transferred to another animal and cause a positive serological test for syphilis. Results from RIT can take up to between 3 to 6 months (318), making it impractical to use for screening blood donors or for clinical management.

RIT is used as the gold standard to measure the sensitivity of methods such as PCR (322,323). Because this test is impractical and expensive, it is used only in research settings and as a sensitivity reference for PCR testing (324).

Microscopy

The association between syphilis and *T. pallidum* was first demonstrated by Shaudinn and Hoffman on Giemsa-stained smears of fluid from secondary syphilitic lesions in 1905 (316). The dark field examination or immunofluorescent staining of specimens from muco-cutaneous lesions is the quickest and most direct laboratory method of establishing a diagnosis (324,325). The sensitivity of the dark-field examination approaches 80% (326)and that for the fluorescent testing approaches 100% when fresh lesions are examined (327). The presence of motile spirochetes may be seen using low power microscopy but to verify their long and tightly coiled to spiral shape, oil immersion with a 1000X magnification should be used. Although the dark field examination depends greatly on technical expertise and the number of organisms in the lesion, it can be highly specific when performed on genital lesions (311). The concentration of treponemes in blood is too low to be detected by microscopy (275) and therefore microscopy cannot be used for blood donors.

Polymerase chain reaction

Polymerase chain reaction (PCR) allows direct detection of *T pallidum* (328). Sensitivity and specificity of 94.7% and 98.6% respectively has been reported for PCR in samples from lesions in early or primary syphilis (329) but in blood samples of donors exhibiting IgM reactivity, PCR is only able to confirm about 25% of infections (330). This suggests a low specificity of IgM antibody assays. PCR is not used routinely to screen donors for syphilis. A recent evaluation of *T. pallidum* real-time PCR found it to have no added diagnostic value for secondary syphilis (331). The disadvantage of PCR is that it is expensive and is not readily available to many centres.

Serology

Serological tests remain the method of choice for diagnosing syphilis (332). Serological test for syphilis can be divided into two main groups; the non treponemal and treponemal tests.

2.4.4 Non treponemal tests

Non treponemal (Reagin) test measure immunoglobulin M (IgM) and G (IgG) antibodies to lipoidal antigen released from damaged host cells as well cardiolipin released by the treponemes (333,334). This release occurs between 3 to 6 weeks after infection making non treponemal tests most sensitive at that time (335).

Venereal Disease Research Laboratory (VDRL) /Rapid Plasma Reagin (RPR)

The 2 most commonly used non treponemal tests are Venereal Disease Research Laboratory (VDRL) and Rapid Plasma Reagin (RPR) test. Each of these tests is a flocculation test, in which soluble antigen particles are coalesced to form larger particles that are visible as clumps when they are aggregated by antibodies (311). Both tests have the same sensitivity and specificity but their reactivity levels may differ because of the variation in antigen preparation (324)

RPR and VDRL titres are raised in patients with acute syphilis infection, re-infection or reactivation of a past infection that has not been treated (336). Nontreponemal tests are therefore useful not only in identifying active infection but also in monitoring the effectiveness of treatment (332). Nontreponemal tests can be used as qualitative or quantitative tests. They are qualitative when used as initial screening tests and they are quantitative test when used to follow treatment. The quantitative VDRL/RPR test should become nonreactive 1 year after successful therapy in primary syphilis and 2 years after successful therapy in secondary syphilis (336,337).

General advantages and disadvantages: Nontreponemal tests have the advantage of being widely available, with good sensitivity, are simple to perform, and inexpensive (324). They are also convenient to perform on large numbers of specimen and are necessary for determining the efficacy of treatment. However, they require cold storage for reagents and samples, and electricity to operate a centrifuge (338) and so these tests have not been used often at the primary health care settings (332). Other limitations are false positives and the possibility of a prozone reaction, which is a false negative test results due to very high concentrations of antibody (339). Biological false positives may occur in as many as 25% of pregnant women (340).

2.4.5 Treponemal tests

Treponema pallidum immobilisation (TPI), *Treponema pallidum* particle agglutination test (TPPA), *Treponema pallidum* hemagglutination assay (TPHA), Microhemagglutination assay (MHA-TP), Enzyme immunoassay (EIA) and Fluorescent treponemal antibody absorption test (FTA-ABS) are examples of treponemal tests. These tests in general cannot differentiate between syphilis and other treponemes such as yaws and pinta (341). This is because they detect antibodies directed against treponemal cellular antigens. The major disadvantage of the treponemal test is that once positive, they have limited usefulness because; they tend to yield positive results for the rest of the patient’s life.

Treponema pallidum immobilisation

TPI was the first treponemal antibody test (324) but because it is complicated, technically difficult, time consuming and expensive to perform, it is rarely used today. Only a few research laboratories have maintained the capability to perform the test. It determines the ability of antibody plus complement to immobilize live *T. pallidum* as visualised under a dark field microscope (333). It is less sensitive and specific than the other treponemal tests (342).

Fluorescent treponemal antibody absorption test

The fluorescent treponemal antibody absorption test (FTA-ABS) detects two antibodies; the first called a group antibody reacts with antigens shared with other treponemes. This antibody is present in low titres in most normal non-syphilitic sera and may be a biological balance between natural and immune antibody, or it may be produced after exposure to other commensal treponemes in the body (343). The removal of the group antibody by adsorption, allows for the detection of the 2nd *T pallidum*-specific antibody. The FTA-ABS test is performed by overlaying whole treponemes that are fixed to a slide with serum from patients suspected of having syphilis because of a previous positive VDRL or RPR. The patient's serum is first absorbed with non-*T. pallidum* treponemal antigens to reduce nonspecific cross-reactivity. Fluorescein-conjugated antihuman antibody reagent is then applied as a marker for specific anti-treponemal antibodies in the patient's serum (311). The FTA-ABS test is considered as the gold standard for treponemal tests (344) but it has a number of limitations. The disadvantage of FTA-ABS is that it is a subjective test to read and is difficult to standardise (345).

Treponema pallidum hemagglutination assay

The T. pallidum hemagglutination assay (TPHA) is a micro-hemagglutination assay for IgM and IgG anti-treponemal antibodies (346). It is commonly used as a confirmatory test, especially when non treponemal tests are used for initial screening of blood. TPHA tests have detectable reactivity approximately four

weeks after exposure (347). It is simple to perform and does not take much time. A major advantage of TPHA is the application of automation (344). Another advantage compared with other treponemal tests such as TPI and FTA-ABS include the use of standardised reagents in commercial kits. Like the other treponemal tests, it cannot distinguish between antibodies from past or present infection.

Enzyme immunoassay

T. pallidum enzyme-linked immunosorbent assays (EIA) can detect IgG, IgM or both. EIA's that use recombinant treponemal antigens to detect IgM and IgG antibodies in a 96-well plate have equivalent sensitivity to non-treponemal tests and specificity equivalent to TPPA, FTA-ABS tests (348,349). Guidelines in the UK were published in 2000, in which EIA is recommended as the appropriate alternative to the combined VDRL/RPR and TPHA screen (345). In a recent audit of laboratory diagnostic methods for syphilis in England and Wales, 94% of them were performing syphilis EIA (350). For large scale screening, the use of EIA is cost effective and less labour intensive (332). The other advantage is that they are automated (or semi-automated) and therefore less subject to bias than conventional tests (332,348).

Rapid tests

Treponemal based rapid test kits have recently been developed but have not been widely evaluated in blood donors. Most rapid tests detect IgM, IgG and IgA antibodies and involve immunochromatographic strips in which one or more *T pallidum* recombinant antigens are applied as capture antigens. They were developed as point of care tests for resource poor countries and their reported sensitivities range from 84.5% - 97.7% (346).

Western blot

Testing by Western blot assay allows for the detection of both IgM and IgG antibodies, with detection of IgM antibody suggesting recent or active infection

(351-353). With clinically confirmed samples, the sensitivity of the assay is 93.8% and the specificity is 100% (321). The Western blot method has been used to identify syphilis in cases with doubtful serological results (354)and is has been suggested as a confirmatory test in blood donors for reactive samples of other treponemal test (355)

2.4.6 Syphilis screening in blood donors

Serological tests for syphilis were the first infectious disease marker tests applied to blood for transfusion and was the first test for transfusion-transmitted diseases. Transfusion-transmitted syphilis was first described in 1915 (321) and serological tests for syphilis have been routinely carried out for more than a half a century (356).

Screening algorithms

Different screening algorithms can be used in different countries to test for syphilis. The classical algorithm (figure 2.1) has been used for several years and is recommended by the WHO (357). In reality, especially in developing countries, single screening tests rather than the algorithms has been used ((39,40,304). The Centre for Disease Control and Prevention (CDC) in the US has released an alternative algorithm (Figure 2.2) based on the EIA ((358). The major difference between the 2 algorithms is that while the classical algorithm begins screening with a non treponemal test, in the newer algorithm a treponemal EIA is used for initial screening. It has been shown that EIA with treponemal IgG gives comparable results to the VDRL and TPHA combination (359,360) and the recombinant antigen-based treponemal IgG and IgM EIA is most suitable for screening (361).

Egglestone et al (345) proposed an algorithm which is a modification of the alternative algorithm described in Figure 2. In this algorithm, EIA is the test method used for initial screening. However when EIA is used as a screening test, an alternative treponemal test such as TPHA should be used as a confirmatory test

(345). This algorithm was used for screening donor blood samples in this study and further details are provided in chapter 7.

It is important for clinicians and laboratory personnel to familiarise themselves with whatever algorithm they decide to use and be able to interpret their results appropriately. Each approach has its advantages in screening. EIA has a practical advantage in laboratories with large workloads. When RPR/VDRL is used as the initial screen, it will miss previously treated cases and may miss infections from many years previously. This can be an advantage for blood banks in Africa because less blood will be discarded. A cost effectiveness analysis has showed that though both algorithms yield similar positive results; the classical algorithm is more cost effective in a low prevalence setting and more cost saving in the high prevalence setting (362).

Figure 2.1: Classical algorithm used for syphilis screening

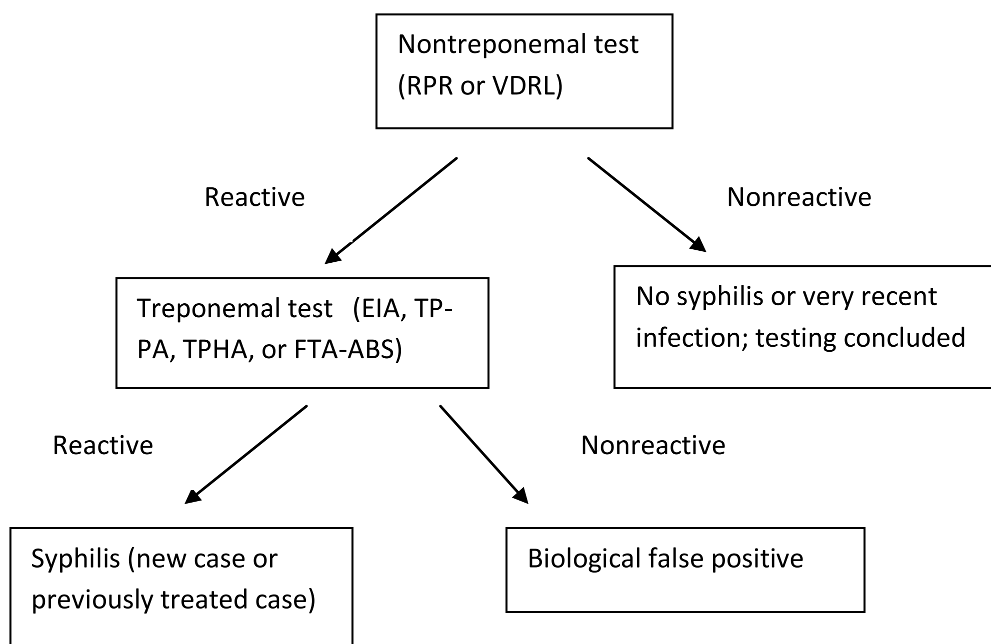
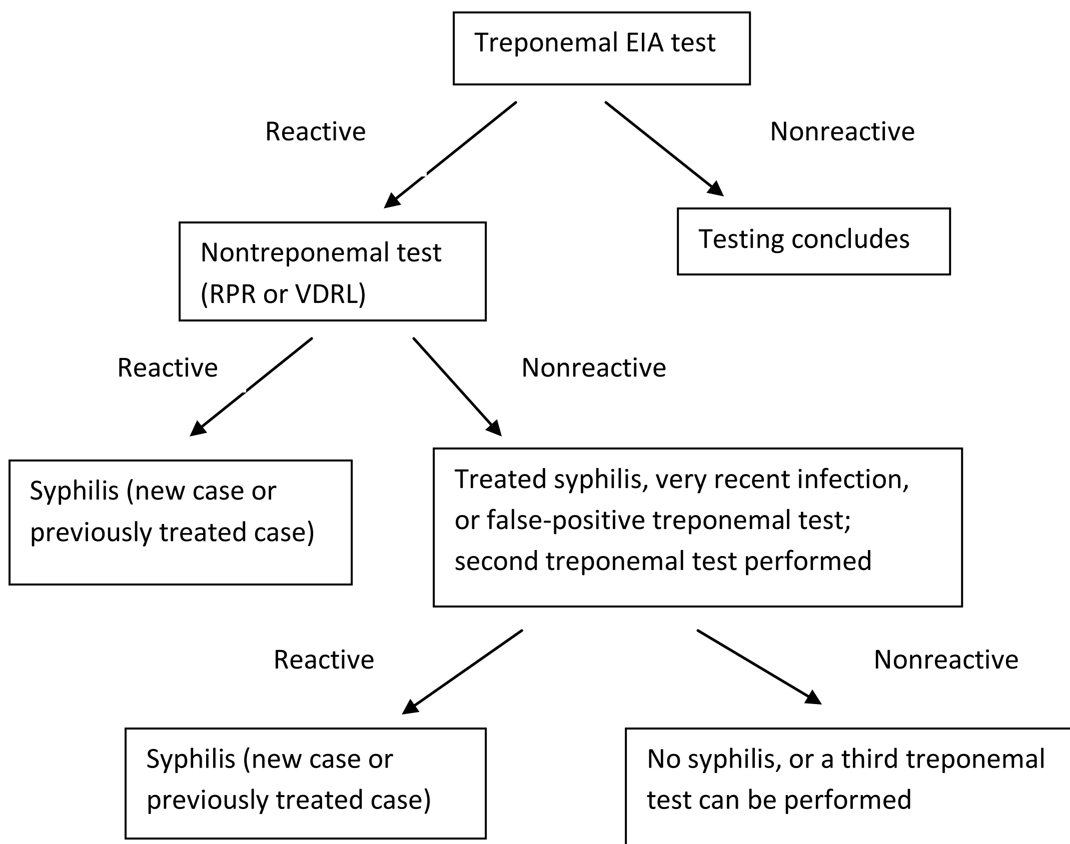


Figure 2.2: An alternate algorithm used for syphilis screening



2.4.7 Risk of Transfusion-Transmitted Syphilis

The risk of transfusion-transmitted syphilis (TTS) though real, is most likely an event of low probability. The risk of *T pallidum* in blood being transmitted to cause syphilis in the recipient, will depend on factors such as infectivity, its presence and detection in the blood donor, storage conditions of the blood and the recipient factors (363,364). The validity and relative contribution of each of the factors has not been determined (310).

Factors affecting transmission

Infectivity of positive serological blood

Blood donors that have positive tests for syphilis by any of the serological tests may not necessarily be infectious since the current screening tests are unable to determine infectivity. The limitation for screening tests for syphilis is that the gold standard for infectivity, which is the RIT, is impractical for screening blood donors (321). The other limitation is the persistence of reactivity associated with treponemal tests so that even after treatment, samples remain positive and cannot be differentiated from untreated persons.

Presence in blood

The presence of *T pallidum* in blood is difficult to establish with the current screening tests for donors as they do not directly detect the DNA, antigen or the bacterium. A confirmed reactive test may indicate a recent infection but it is not indicative of the presence of *T pallidum.* Data from a pilot study of 169 serologically positive blood donor samples in the US (365) showed that no *T. pallidum* DNA or RNA was detectable. This lack of demonstrable *T. pallidum* circulating DNA or RNA suggests that blood donors with confirmed positive results are unlikely to have circulating *T. pallidum* in their blood and is unlikely to be infectious.

T pallidum could be present in blood but missed on screening when blood is donated in the window period i.e. the time between acquiring the infection and the

identification by serological tests (297). *T. pallidum* reaches the lymphatics and the blood within a few hours of infection during early primary syphilis, and transmission of syphilis through blood transfusion is possible before the appearance of serum antibodies detected by serological tests (306,309). Transfusion-transmitted syphilis has occurred in the Netherlands, when fresh blood was used for an exchange transfusion, although the blood donor had negative serological tests for syphilis (309). A similar case had been reported by Soendjojo et al where the donor was negative at the time of donation (308). Later investigations in both cases showed both donors had later sero-converted. In the report from the Netherlands, the donor later admitted to sexual contact with a prostitute 10 days prior to his blood donation (308).

In the US, without serological screening of volunteer donors, it has been calculated that potentially infectious blood from about 100 persons with early syphilis might enter the blood supply system each year (278).

Storage conditions of blood

The storage temperature and duration of storage of blood are important in affecting viability of treponemes. *T. pallidum* is fragile and at 4°C, does not survive beyond 120 hours (366). The use of refrigerated blood for transfusion has been an important factor in reducing the risk of transfusion-transmitted syphilis (297). In conditions of inadequate blood supply and excessive demand for blood as seen in many developing countries, blood will not be stored for long periods, thus the risk of transmitting syphilis will remain.

Host factors

Immunity: Sexually transmitted syphilis may present with non typical features in the HIV positive patient (367) but no specific relationship has been established between transfusion-transmitted syphilis and recipients with lowered immunity. The HIV/AIDS epidemic has not been associated with an increase in cases of transfusion-transmitted syphilis.

Antibiotic use in recipients: *T pallidum* has remained susceptible to the penicillin's and the extensive use of antibiotics among transfusion recipients is one of the reasons advanced for the decrease in incidence of transfusion-transmitted syphilis (310,368).

2.4.8 Should screening of syphilis be continued?

In the developed world, especially in the US, the value of syphilis screening has been questioned and it has been suggested that testing continues for emotional rather than scientific reasons (368). Regulators such as the FDA maintain that there is insufficient data to warrant discontinuation of testing blood donors (278). The risk for transmission of syphilis may depend on the prevalence of the infection and other non-epidemiological factors in that region but considerable disagreement exist as to whether testing blood donors for syphilis serves any useful purpose(66) .

Reasons why screening should start or continue

Identifying high risk donors by means of a detailed donor questionnaire and deferring such donors is the first step in screening and it is helpful. However, it has been shown that relatively few blood donors with infectious syphilis reported risk factors for syphilis and donor –history questionnaire did not result in deferral of these donors (278). Some centres do not also question donors at all to identify the high risk donors. Serological screening is crucial under such circumstances to detect those positive for syphilis.

An increase in syphilis prevalence in the community may mean an increase in the high risk sexual behaviour of potential donors and could lead to less safe blood because though testing is done for many pathogens; there is a risk that an infectious donation may enter the blood supply (369). Continued vigilance by screening is therefore required by blood services as the risk of syphilis increases in the general population (295).

In developing countries, a multiplicity of factors combines to make the continued screening of blood syphilis important. These include: inability to store refrigerated blood for prolonged periods (because of the high demand for blood); absent or poor supply of electricity leading to inadequate refrigeration; and the major source of blood being family replacement donors (usually first time donors) with a higher prevalence of transmissible diseases.

Reasons why screening should discontinue

The absence of any published report of transfusion-transmitted syphilis since 1983 and the inability of *T. pallidum* to survive refrigeration implies that transfusion-transmitted syphilis is nonexistent and therefore screening is not required in blood donors (368).

The significance of a confirmed positive test result is not clear (Orton S 2001). Discarding blood which does not contain infectious *T pallidum* in a community where blood is scarce may not be appropriate. The inability of serological tests to identify the true infectivity of the blood means many previously exposed donors who have been treated are identified as positive though they are not infectious.

Syphilis testing has previously been used as a surrogate test for HIV, hepatitis B, hepatitis C (370). A correlation between sero-positivity for syphilis and tests for hepatitis B and C and HIV in both high and low risk groups, including prostitutes, drug users, homosexual men, blood donors and the general population was observed in the early 1990's (371-376). There are now more sensitive tests for these viruses than for those for syphilis. Evidence now shows that syphilis testing does not to provide surrogate value for other pathogens such as HIV, HCV and HBV (377).

Syphilis policies

Despite the uncertainty of the infectivity of positive serological sample, syphilis screening of blood donations remains a legal requirement in most countries (378).

The World Health Organisation recommends that at the minimum, all donated blood to be used for transfusion should be screened for HIV, hepatitis B, hepatitis C and syphilis (54).

Syphilis policies in Ghana

There is a national blood policy in Ghana that proposes mandatory syphilis screening for blood donors (379). Though such a policy exists, the National Blood Service has not been legally mandated by the parliament of Ghana. Each hospital/ blood bank works independently of the NBS and their policy and only screens blood with whatever is provided by the individual hospital managements. This is reflected by the fact that only 20% of blood in Ghana is being screened for syphilis (23). Furthermore, there is no recommended test algorithm to be used for screening.

Research gap in transfusion-transmitted syphilis

The follow-up of transfusion recipients for evidence of transfusion-transmitted syphilis is urgently required and would address the question of whether transfusion-transmitted syphilis exists today (321). This is a crucial gap in current knowledge that needs answers. This study seeks to provide evidence of transfusion-transmitted syphilis.

2.5 Summary

The review of literature in this chapter has shown the paucity of data in Africa and the challenges associated with the existing screening methods or tests. These shortfalls support the urgent need for studies in these areas. Some of these studies were conducted and are presented in this thesis. The next chapter (chapter three) gives a general methodology and how the studies were set up in Kumasi, Ghana.

CHAPTER 3

STUDY ORGANISATION AND GENERAL METHODOLOGY

3.1 Introduction

The study presented in this thesis has several components and therefore had to involve detailed planning including choosing an appropriate study design to answer the study questions, the site and the correct staff for the study. This chapter describes the planning that went into this study, how the study was set up, coordinated and conducted, and the challenges encountered. Background information of the study site and a general methodology of the study are also provided in this chapter.

Study overview

The execution of this project was in three phases:

Phase 1: This phase involved proposal development, writing up the protocol for the study and obtaining ethical approval for the study. These activities took place in Liverpool, UK.

Phase 2: This phase was in Ghana. It involved identifying study staff, setting up and conducting the study.

Phase 3: This phase involved molecular analysis in Liverpool including performing malaria PCR and genotyping, data analysis and write up of the thesis.

Study oversight in Ghana

My supervisors provided study oversight in 2 ways.

1. Two working site visits were done by my supervisor to Ghana. The first was done very early in the study to ensure the set up was appropriate, and working well, that the protocol was being followed, that study procedures were being followed and samples were being collected and stored

appropriately. The second was completed a couple of months prior to the study end.

2. We established a monthly reporting system where I provided a written update on study progress and activities including number of enrolled subjects, default rate for follow up, challenges and forecasts. On a few occasions, a follow up teleconference was held to discuss issues and to re-plan where necessary.

These measures ensured that problems and issues were identified and solved early, assuring the overall quality of the study.

3.2 Sample size calculation

Sample size for transfusion-transmitted malaria

As the prevalence of plasmodium parasitaemia in the general population in the study area is approximately 5%, it is assumed that the same proportion of blood donations (5%) will contain malaria parasites. Data available from 2007 suggest that approximately 4000 patients in the three high-risk groups of interest to this study receive a blood transfusion per year; 200 (i.e. 5%) of these patients can be expected to be transfused malaria parasite infected blood. Although equipoise exists, in that there is no evidence available to indicate the effect giving malaria parasite infected blood transfusions has on post-transfusion malaria rate, it is anticipated that giving infected transfusions will increase this rate; thus, the study has been powered on the basis of non-inferiority (i.e. will assume one-sided significance testing at the conventional alpha = 0.05 level).

The study was to recruit 200 high-risk patients found to have been transfused malaria parasite infected blood (exposed cohort); this was to take a maximum of 12 months to achieve. Patients in the exposed cohort would receive intensive post-transfusion monitoring for 72 hours and with further review at days 7 and 14 in parallel with standard clinical care.

Each participant in the exposed cohort was to be matched with two participants from the same high-risk sub-group (child/immune compromised/pregnant) found

to have been transfused non-infected blood (unexposed cohort). The unexposed participants would be the two participants from the same high-risk sub-group transfused with non-infected blood closest in time to the date on which the matched exposed participant received their infected blood transfusion.

The total sample size for this study was thus 600, of whom 200 were to be in the exposed and 400 in the unexposed cohort. These numbers were to provide 80% power to detect a non-inferiority difference of 5.7% in the post-transfusion malaria rate (i.e. the hypothesis of non-inferiority will be accepted if the rate increases from 5.0% to no more than 10.7%) – and 90% power to detect a non-inferiority difference of 7.0% in the post-transfusion malaria rate (i.e. the hypothesis of non-inferiority will be accepted if the rate increases from 5.0% to no more than 12.0%). [Clinically, non-inferiority would be accepted in this context if the rate increases from 5.0% to no more than 15.0%.]. This study design was therefore to allow for a clinically relevant increased rate of malaria in the exposed group of up to 15% to be detected.

The proposed sample size of 600 patients was not achieved. It became obvious early in the study that the frequency of identifying parasitaemia in the blood was too low to enable the required sample size to be achieved and for the study design to be carried out. It would have taken several years to complete the study if matching was to be done. Screening of blood was carried out for a year without any matching of cases. In all, a total of 372 recipients of transfusion were enrolled into the study.

Sample size calculation for bacterial contamination study

The rationale for this sample size calculation was to determine an adequate sample size to estimate the prevalence of contamination among donors with a good precision. Based on two previous studies in Ghana (36,37), the prevalence of bacterial contamination was assumed to be 15%. Taking a confidence interval of 95% the sample size for this study was calculated using the formula $n = Z^2 P \frac{(1-P)}{d^2}$ (380) where:

n = sample size

Z = Z statistic for a level of confidence = 1.96 (for 95% CI)

P = expected prevalence or proportion = 0.15 (15%)

d = precision = 0.05 (5%)

$n=Z^2P\ (1-P)/d^2$

= 1.96 x 1.96 x 0.15 x 0.85/.05x.05

= 192

200 patients were recruited into the study instead of the calculated 192.

Sample size for transfusion-transmitted syphilis study

Using an expected sero-positivity prevalence of 12% for syphilis, the sample size for this study was calculated using the formula $n=Z^2P\ (1-P)/d^2$ (380) where:

n = sample size

Z = Z statistic for a level of confidence = 1.96 (for 95% CI)

P = expected prevalence or proportion = 0.12 (12%)

d = precision =.05 (5%)

The calculated size n= (1.96 x 1.96 x 0.12 x 0.88/ 0.05 x 0.05) = 162

The sample size calculated was 162 but 200 patients were recruited for the study. This sample size calculation was based on the assumption that the syphilis sero-positivity in donors was 12%. The assumed 12% was based on previously reported studies from Ghana (38,39). This sample size was expected to detect the sero-positivity prevalence with a good precision of 0.05. The calculation did not take into consideration the detection of active infection because that was not the focus of the study.

3.3 Study setup in Ghana

Process of initiating study in KATH

Permission to carry out this study was sought from the many levels of authority including the hospital management, the heads of the departments, and units that were to be involved in this study.

Hospital management

A letter was sent to the hospital management asking for permission to use the hospital and its patients for the study. The management required to see all ethical approvals and my study protocol. Once they were satisfied with all documents, the importance of the study in the hospital and for the scientific community as a whole, the Medical Director issued an internal memo to all heads of all concerned departments and units, to officially introduce the study.

Departments involved

The departments of Medicine, Obstetrics and Gynaecology, Paediatrics, Oncology and Diagnostics (including microbiology and haematology) were directly involved and approvals from their heads of department were required. These approvals were important so as to ensure maximum cooperation from personnel. Meetings were organised individually with the heads and when their approval was given, this was followed by clinical meetings in the departments where details of the study were presented to members of staff of each department. These meetings afforded clinical staff the opportunity to ask questions and establish a rapport that was to help facilitate the conduct of the study.

Transfusion Medicine Unit

The unit was central to the success of the study. Frequent meetings were held with the head and staff of the Transfusion Medicine Unit (TMU) and my study team to ensure that any issues that came up were quickly settled. These meetings also

discussed the benefits from the collaboration for the unit including research capacity building and the use of findings from the study to improve practices. Coordination and strategies were agreed, including allowing study staff members to be stationed in TMU and be responsible for alerting other study team members whenever a transfusion request had been made and blood was being prepared to be issued out for transfusion. The unit was also to allow study team members access to their donor records and all other information required for this study.

Figure 3.1: The accident and emergency building which houses the blood bank

Figure 3.2: The blood bank of Komfo Anokye Teaching Hospital

Figure 3.3: A biomedical scientist in the grouping and cross matching room and a blood fridge containing blood that has been cross matched

Maintaining normal work routine

Care was taken not to disrupt work schedules in the unit or to alter standard operating procedures for all activities in the unit. This study was an observational study and did not intend staff members to modify any activity.

Blood screening algorithm in unit

The process for donation and screening of donated blood is as shown in figure 3.4. All blood which was collected into the blood bags had been screened for the major viruses already.

Figure 3.4: The blood donation and screening process at KATH

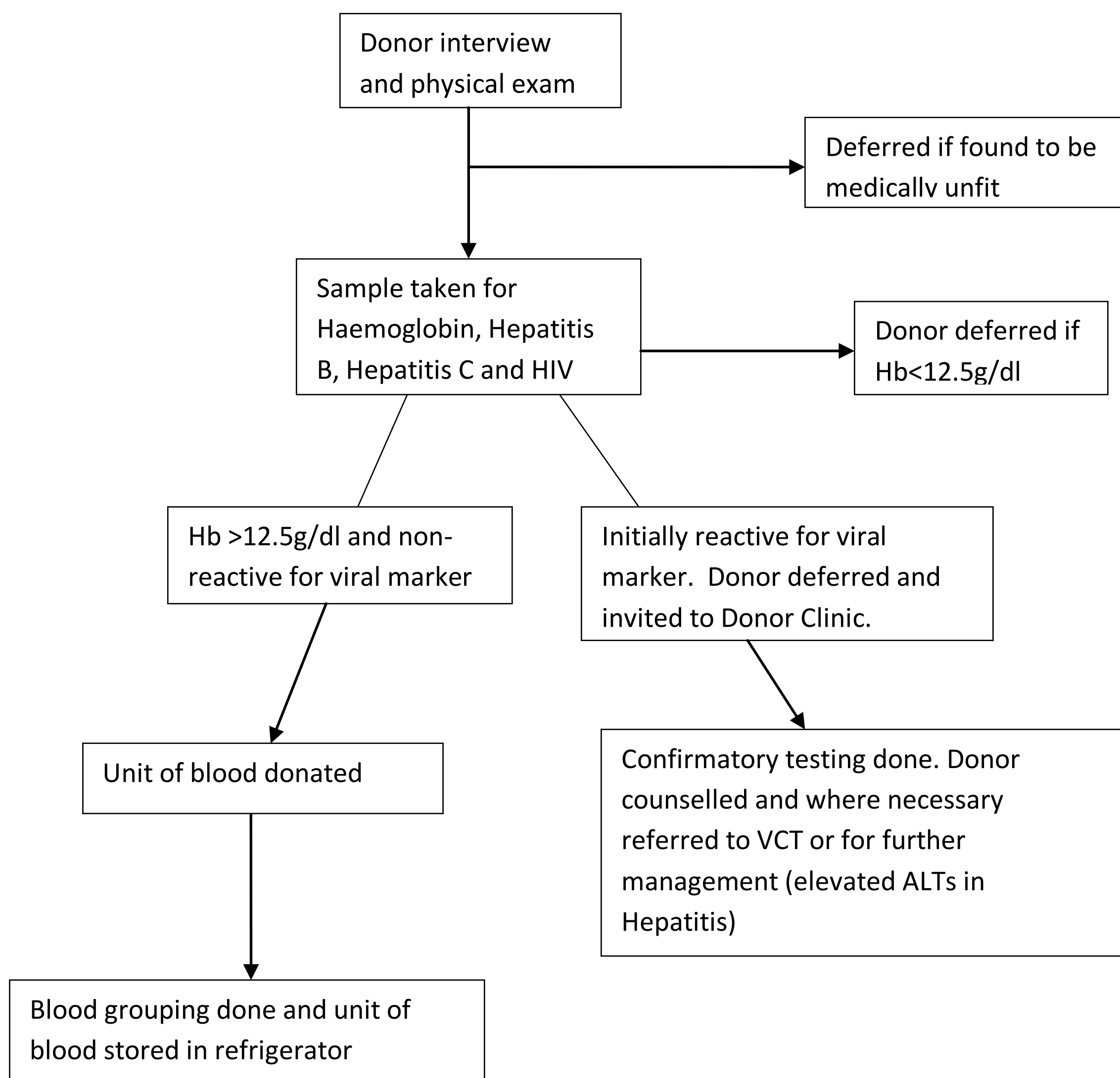
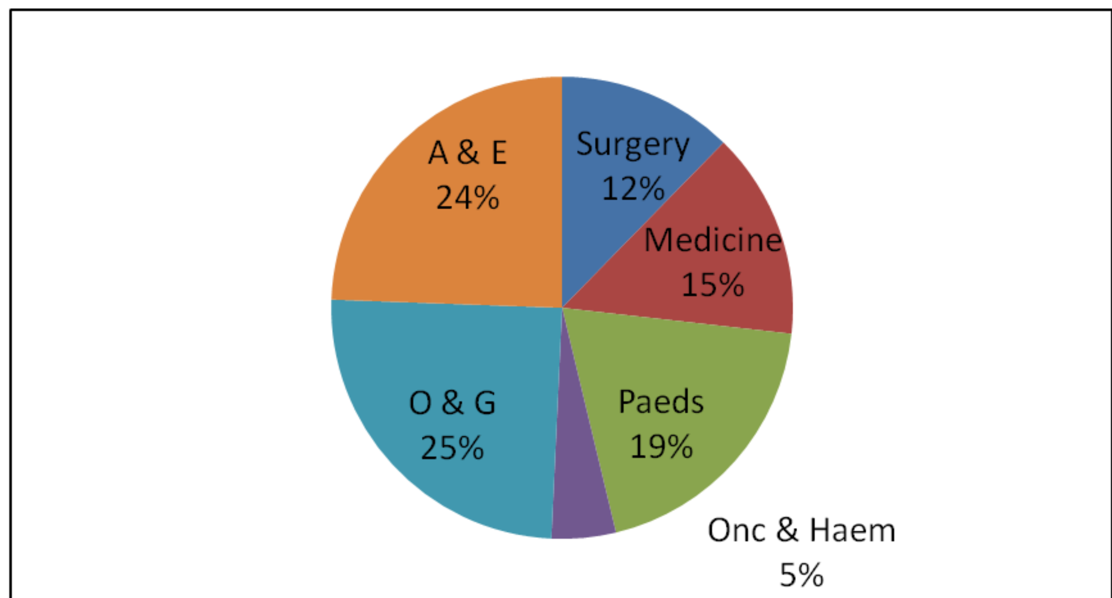


Figure 3.5: A blood donor undergoing a health check prior to blood donation

Figure 3.6: Percentage distribution of blood units in KATH by departments



(A&E-Accident and Emergency, Paeds-Paediatrics, O&G- Obstetrics and Gynaecology, Onc & Haem- Oncology and Haematology).

Maintaining the integrity of the unit of blood

To prevent iatrogenic contamination, the blood bag was only breached at the bedside of the patient at the start of the blood transfusion. Sampling for grouping and cross matching was taken from the accessory tubing. The principle was maintained for this study and no samples were obtained from the blood bag prior to starting transfusion on the patient. The required volume of blood sample was taken from the blood bag at the tail end of the transfusion process after the blood bag had been clipped off. For paediatric transfusions which required volumes less than a unit of blood, the staff in the blood bank did breach blood units according to their protocols to take the amount needed.

Blood use in KATH

Out of the 15,685 blood units collected in 2010, 64% were used by the Departments of Paediatrics, Obstetrics and Gynaecology, Medicine and Oncology, where the study took place. The distribution of blood use in the hospital is shown in figure 3.6.

3.4 Study components

The phase 2 of this project, where the studies were performed in Ghana, was divided into four components and conducted in the following order:

Component I - Conduct of a survey to determine transfusion practices in the different departments

Component II - Conduct of the syphilis aspect of the study to determine syphilis prevalence and sero-conversion.

Component III – Conduct of bacterial contamination portion of the study to determine prevalence of bacterial contamination and transfusion-transmitted bacterial sepsis

Component IV – Conduct of the transfusion-transmitted malaria portion of the study to determine the prevalence of *P. falciparum* parasitaemia in donors and the incidence of transfusion-transmitted malaria in recipients.

The project was separated into these four distinct components for two main reasons;

1. To allow the study team to focus only on one study at a time to ensure efficient conduct of the study and of the follow up of patients. Each of the studies had different follow up days and therefore there was a potential for mix-up. For example, follow up in the bacterial contamination study was up to one week and that for the syphilis study was 30 days post transfusion for only some recipients.
2. To ensure that the same patients were not used for all studies thereby reducing the amount of blood to be taken from each patient. If one patient were to be enrolled in two or more sub-studies, there would have been more blood samples taken

Study team

Experienced study staff were used as much as possible so that not much training was required. This enabled the study to start quickly and smoothly. 'Dry runs' were practised prior to each of the study components to evaluate how efficiently the studies were to be conducted.

Clinical study team: Three clinical research assistants were involved in this study, each one responsible for Medicine and Oncology, Paediatrics and Obstetrics and Gynaecology respectively. The research assistants were responsible for all activities in their assigned department including obtaining informed consent and monitoring transfusions. The research assistants had worked on previous clinical trials in the hospital and were trained in principles of Good Clinical Practice.

Laboratory work: For microscopy and blood cultures, experienced biomedical scientists and technicians from KATH were used for this study.

3.5 Set up and conduct of studies.

General conduct of the study

The principles of Good Clinical Practice were applied throughout the study. All patient records and files were kept under lock and key and were only accessible to the relevant study team member. The laboratory staff were not given access to patient clinical history. This served a dual purpose of maintaining confidentiality and avoiding bias.

Timing of studies

The first component of the study, which involved an observational survey of the routine transfusion practices of clinicians on the wards, was conducted immediately after permission was obtained from hospital management but before meeting the staff of the individual departments. This was to avoid the Hawthorne effect whereby the clinicians would change or modify their practice once they knew their behaviour was being watched. Once the observational study was completed, clinical meetings were held where the study was introduced to raise awareness and the design of the other components of the study was presented to staff.

In addition to observing practices, this survey was to help determine if there were some specific practices that would impede the implementation of the other components of the study. It was observed from the survey that the use of anti-malarials with or after blood transfusion was high and that recruiting children into the study run the risk of a high dropout rate due to anti-malarial use.

Participants

The following groups of patients were to be recruited into the study:

1. Pregnant women
2. Children
3. Immune suppressed patients including those with HIV

4. Malignancies and Chronic diseases (including sickle cell, liver and kidney diseases).

These patients are known to be highly susceptible to malaria in malaria endemic countries. They were chosen as the study population because if transfusion-transmitted malaria exists, it will be clearly seen in these individuals. All these patients were from the departments of Medicine, Oncology, Paediatrics and Obstetrics and Gynaecology. The study was therefore limited to these departments in the hospital.

Patient care

The study team provided supplementary care (laboratory results and follow ups) for patients enrolled into the study but clinical care provided by the regular hospital clinical team was maintained. This was because the study was mainly observational. Results of the patients were shared with their respective doctors, allowing the obstetricians, gynaecologists, paediatricians, physicians and oncologists to continue to give specialised care to their patients. They were at liberty to decide the treatment without seeking approval from the study team. During the study on transfusion-transmitted malaria, the patients were reviewed daily and their vital signs measured 8 hourly.

Follow up clinic

A follow up clinic was set up specifically for the study. Prior to discharge, all study participants were shown where the follow up clinic was located and where the malaria laboratory was. They were then given a card with the dates for each visit and the contact details for the study team. All follow up information was stored electronically. At the end of each day, a list of patients who were due to attend the clinic for follow up the next day was printed and patients were called on phone to remind them of the visit. Patients were also encouraged to come for unscheduled visits whenever they felt unwell or had a fever.

Follow up strategies

On recruitment into the study, a detailed home address and telephone contact was obtained. This helped the study team to locate patients when they didn't return for follow up. Patients would be called and then followed up at home if required.

The advantages of follow up were explained in detail to patients; emphasising that they would not pay anything, would not join any long queues and they would obtain their results quickly. They were provided with a telephone number of the study team to contact if they encountered any difficulties or if they did not feel well.

The participants/relations were given money to cover their transportation costs. Initially patients were given the money when they came for the follow up visit. However it was noticed that some were unable to come to the hospital on their own so they were given these transportation monies on discharge to facilitate their return for follow up.

Laboratory work in KATH

The malaria laboratory was the focal point of all laboratory work for this study. This laboratory was chosen because they have been involved in several clinical trials and are familiar with Good Clinical and Laboratory Practices (GCLP). Room, fridge and freezer temperatures are measured and recorded daily and an excellent audit trail for samples are maintained. Reagents and kit for the study were kept in this lab and sample separation, labelling and storage were performed here. The malaria laboratory is not officially accredited.

Blood cultures were performed in the bacteriology laboratory and assays for syphilis and malaria ELISA, TPHA and RPR were performed in the serology and virology laboratories. Full blood count, malaria microscopy and malaria rapid testing were performed in the malaria laboratory.

Test kits and supplies

Diagnostic kits had to be purchased for two aspects of the study; malaria testing and syphilis testing. The logistics of when to buy, and where to ship the kits to, was tricky because of the delay in approving the study. Orders that had been made were suspended until approval for the study was received. All syphilis test kits and reagents (EIA, TPHA and RPR) were ordered from BIOKIT Ltd, UK and kept in a cold room in Liverpool until they were carried in a cold box to Ghana. Malaria EIA ordered from Cellabs, Australia was shipped directly to Ghana. A malaria rapid detection test (First Response, manufactured in India) was purchased from the National Malaria Control programme.

Sample shipment to Liverpool

Packing of the blood spots of filter paper were done according to the UN3373 Biological substance Category B specifications for transport of dangerous goods. The necessary documentation and approvals were obtained in Liverpool prior to shipment of samples. Samples were then shipped by DHL courier to LSTM. Upon reception, samples were logged in to the LSTM database via the Pro-Curo software system and stored in the cold room.

Molecular testing

Initial plans for the study were for PCR and genotyping to be done in a research institution in Ghana. Performing these tests became problematic and compelled the molecular work to be performed in the Liverpool School of Tropical Medicine. Two kinds of PCR for *Plasmodium falciparum* were performed, real-time PCR was the method used to screen all samples from donated blood units and nested PCR was used for genotyping of the parasites.

Data management

Four different case report forms, each specifically designed were used in this study. Double entry of the data was done into electronic databases designed in Microsoft Access. Data cleaning was continuous throughout the study after which analysis of data was performed using Predictive Analytics Software (PASW®) statistics 18 package (SPSS Inc, USA).

Summary of activities

Table 3.1: Summary of my activities performed in the study

Activity	Place
Case report forms	LSTM & KATH
Obtaining ethical clearance	LSTM & KATH
Participant recruitment	KATH
Physical examination	KATH
Medical history	KATH
Medical charts review	KATH
Transfusion monitoring	KATH
Follow up clinic	KATH
Sample separation, labelling, storage	KATH
T. pallidum enzyme immunoassay	KATH
Rapid plasma reagin	KATH
T. pallidum hemagglutination	KATH
Malaria microscopy	KATH
Malaria rapid detection test	KATH
Malaria enzyme immunoassay	KATH
DNA extraction	LSTM
Malaria PCR (real-time and nested)	LSTM
Data entry	KATH
Data analysis	KATH & LSTM

LSTM =Liverpool School of Tropical Medicine, KATH = Komfo Anokye Teaching Hospital

3.6 Ethical issues

Ethical approval for the study was sought and granted from the ethics committees of the Liverpool School of Tropical Medicine in Liverpool, UK and the School of Medical Sciences, Kwame Nkrumah University of Science and Technology in Kumasi, Ghana.

Ethical considerations and approval

In my initial study design for transfusion-transmitted malaria, malaria testing of donor blood was not to be done immediately. Patients were to be followed up blindly for 4 weeks, without knowledge of whether the blood they received was malaria positive or negative. At weekly intervals they were to have a blood smear done for parasitaemia. The end points were to be both clinical malaria and parasitaemia. These end points were to be compared retrospectively between those who received malaria positive and malaria negative blood. My initial estimation was that 5% of donor blood will be *P. falciparum* positive and that if transfusion-transmitted malaria was occurring it will result in 10% of recipients becoming positive. This approach was to simply observe the current practices in KATH, which are similar to those in most sub-Saharan countries where there is no screening of blood for malaria.

The goal of the study to measure incidence of infections transmitted by transfusion had an ethical dilemma of apparently allowing infections to progress without intervention. I felt that the current practice of treating patients presumptively with anti-malarials without evidence was even more unethical.

The ethics committee of the Liverpool School of Tropical Medicine (LSTM) however had concerns and raised some major issues. These included the following:

- The committee felt that it was unethical for study participants to be transfused with blood that contained malaria parasites and not be treated.
- The committee wanted assurance that stringent measures were in place to ensure safety of participants who received infected blood
- The committee wanted a justification for why this approach was to be used

Resolving ethical considerations

Some major and minor modifications were made based on comments and concerns raised by the ethical committee. At various stages, I worked together with a paediatrician and a statistician from the ethics committee. The major changes to the study design included keeping recipients of blood in hospital for 3 days to monitor closely and to perform 8 hourly measurements of vital signs including blood pressure, temperature, respirations and pulse rate. The study design was also modified to a matched cohort study (but this did not happen because of impracticalities; there were so few microscopy positive cases that recipients of positive blood could not be matched immediately by department and gender). I proposed that the benefits of this study outweighed risk and that practice of widespread anti-malarial prophylaxis would help spread parasite resistance and was not affordable.

Ethical approval

Ethical approval from the LSTM ethics committee was difficult to obtain and was given only after 4 rounds of re-submissions to the ethics committee. Interestingly, the LSTM ethics committee failed to reach a consensus on the major ethical implications surrounding untreated iatrogenic parasitaemia. An approval was given 6 months after the first submission the LSTM ethics committee and only after the protocol had been referred for an external review.

During the visit by my supervisor, an issue of how to handle positive blood donors was raised. We agreed that syphilis positive donors had to be offered treatment but not for malaria positive donors who may have donated several days earlier and were not acutely ill. The ethics committee of the Kwame Nkrumah University of Science and Technology was consulted but they referred us to the National Malaria treatment guidelines of Ghana which unfortunately did not have answers. We discussed and agreed that any positive donor be invited for another blood screening before treatment. Treatment was to be given if parasitaemia was found in the repeat sample or if the donor had any symptoms.

Informed consent

Signed or thumbprint informed consent was obtained from all patients or their relatives before being enrolled in the study. This sometimes involved using an interpreter. A detailed explanation of the study was given and patient/relatives given time to think about it. To ensure a low dropout rate, patients who were initially hesitant but later willing to give consent were not enrolled. Also patients who were giving consent because they thought 'the doctor knew best' were not enrolled. This was because from previous experience, such patients were unlikely to return for regular follow up. Patients were allowed to withdraw their consent at any time during the course of the study.

3.7 Summary

A description of the study setup including the ethical challenges that were overcome has been presented. Collaborations between different units and departments and the coordination of activities between different laboratories and different personnel is shown to contribute immensely in the setup and completion of this study. This chapter provides the rationale for sample sizes and participants recruited, and give a background understanding to the studies presented in the next four chapters.

CHAPTER 4

THE IMPACT OF INCONSISTENT POLICIES FOR TRANSFUSION-TRANSMITTED MALARIA ON CLINICAL PRACTICE IN GHANA

4.1 Introduction

There are approximately 500 million cases of malaria and 1 million deaths worldwide each year (381) and 90% of this burden occurs in Africa (382). Current measures to reduce the burden of malaria include insecticide- treated mosquito nets, intermittent preventive treatment, indoor residual spraying and malaria vaccines. An area of malaria control which has been neglected in endemic countries is malaria transmission through blood transfusion.

There are no evidence-based international guidelines for the prevention of TTM in sub-Saharan Africa and there is lack of harmonisation between policies produced by blood safety programmes and those produced by malaria programmes. The World Health Organisation (WHO) recommends that donated blood should be tested for malaria "where appropriate and possible" (1) but there is currently no method for screening blood for low-level parasitaemia which is sensitive, practical and affordable for use by transfusion services in endemic countries (104). Other transfusion guidelines suggest that transfusion recipients should be given prophylaxis with anti-malarials (185). In contrast to blood safety policies, malaria policies recommend that malaria treatment should only be prescribed on the basis of laboratory confirmation of infection (383) in order to reduce the development of drug resistance.

There is very little information in the literature about how African countries have incorporated the WHO recommendations concerning transfusion-transmitted malaria into their national policies and how these policies have been translated into practice. In the absence of clear national policies clinicians may rely on international recommendations which may not be applicable in their setting or they use individualised practices that are not based on evidence. As in many other

countries in sub-Saharan Africa, the blood transfusion policy in Ghana makes no mention of screening donated blood for malaria.

The aim of this study was to document how these conflicting or absent policies relating to malaria transmission and blood transfusion impact on clinical practice in a teaching hospital in Kumasi, Ghana. Analysis of discrepancies between policies and practice should enable identification of the priorities for better harmonisation of policies and to identify gaps in the evidence regarding TTM.

4.2 Methods

This study was conducted in the departments of Obstetrics and Gynaecology (O&G), Medicine and Paediatrics of the Komfo Anokye Teaching Hospital.

Participants

Inclusion criteria: Transfusion recipients from the three departments who had received a blood transfusion within the previous 24 hours were eligible to take part in the study. Within the departments, pregnant women, children, immune compromised patients and patients with malignancies and chronic diseases (including sickle cell disease, chronic renal failure, and liver disease) were enrolled.

Exclusion criteria:

- Any patient whose case notes were not traceable
- Patient being transferred to a department not included in this study
- Patient receiving more than one transfusion within the 24 hour time period.

Enrolment

The study was a retrospective review of the clinical notes of transfusion recipients. Data were collected between October and November of 2009. The recipients were selected by convenience sampling from a list of patients who had received blood in the preceding 24 hours. Each day the blood bank staff provided the names of two

blood transfusion recipients in each of the three departments. On six occasions during the study, a department had less than two transfusion recipients in 24 hours and so the name of a replacement participant from another department was provided by the blood bank staff. On one occasion the case notes of a patient from obstetrics could not be traced and as no other obstetric patient was eligible, a replacement was recruited from paediatrics.

Data collection

At recruitment, a data collection form (appendix 3.1) was used to extract information from the clinical notes of the transfusion recipients. The form was adapted from the KATH transfusion monitoring form, which is based on the national guidelines for the clinical use of blood in Ghana. There are no hospital based guidelines for the clinical use of blood or any written policies or protocols concerning transfusion-transmitted malaria but the blood bank has transfusion monitoring forms which are given to ward staff when the blood is issued and are used for reporting any transfusion reactions.

The form was expanded to include information about the patient's diagnoses, laboratory tests (such as malaria microscopy, pre-transfusion haemoglobin) and treatment type (such as anti-malarials, antibiotics, diuretics). Information was also collected concerning monitoring of vital signs, duration of transfusion and documentation of adverse events of transfusion.

Analysis

Predictive Analytics Software (PASW®) statistics 18 package (SPSS Inc, USA) was used to calculate frequency statistics including measures of central tendency and dispersion and the results were compared between the three departments. The practices relating to the prevention and treatment of malaria associated with transfusion were compared with known policies.

4.3 Results

Participant demographics

151 transfusion recipients were recruited into the study from medicine (41, 27.2%), obstetrics (51, 33.7%) and paediatrics (59, 39.1%). The majority of the patients (64.9%) were female, because of the inclusion of the obstetric department, and the median age of all patients was 22.0 years (IQR: 4.0-36.0) (Table 4.1).

Malaria was the leading cause of admissions in transfusion recipients in the department of paediatrics (69.5%) but did not contribute to any admission in the department of medicine (0%). In O&G, malaria accounted for only 17.6% of admissions (Table4.2).

Transfusion practices

25 patients (16.6%) had received a blood transfusion during a previous admission and the highest rates of previous transfusions were in the department of medicine (45.5%). 66% of transfusion recipients from medicine, 65% from obstetrics and 15% from paediatrics had received a transfusion during the current admission prior to enrolment in the study. All patients received either whole blood or packed red cells. The mean duration of transfusion for all study participants was 2.6 hours and was longest for paediatric transfusions (3.3 hours). Ninety four percent of all transfusions were completed (Table 4.1).

The mean pre-transfusion haemoglobin in all transfusion recipients in the study was 4.5g/dl (SD ± 1.2) (Table4.1). The temperatures charts for all transfusion recipients revealed that 34 (23%) developed fever in the 24 hours following transfusion. Post-transfusion fever was a particular problem in obstetric (24%) and paediatric (32%) transfusion recipients (Table 4.1). There were 10 deaths (7%) among recipients within 24 hours of transfusion but none of these were attributed to the blood transfusions (Table 4.1).

Table 4.1: Characteristics and practices among transfusion recipients within three departments in a Ghanaian teaching hospital

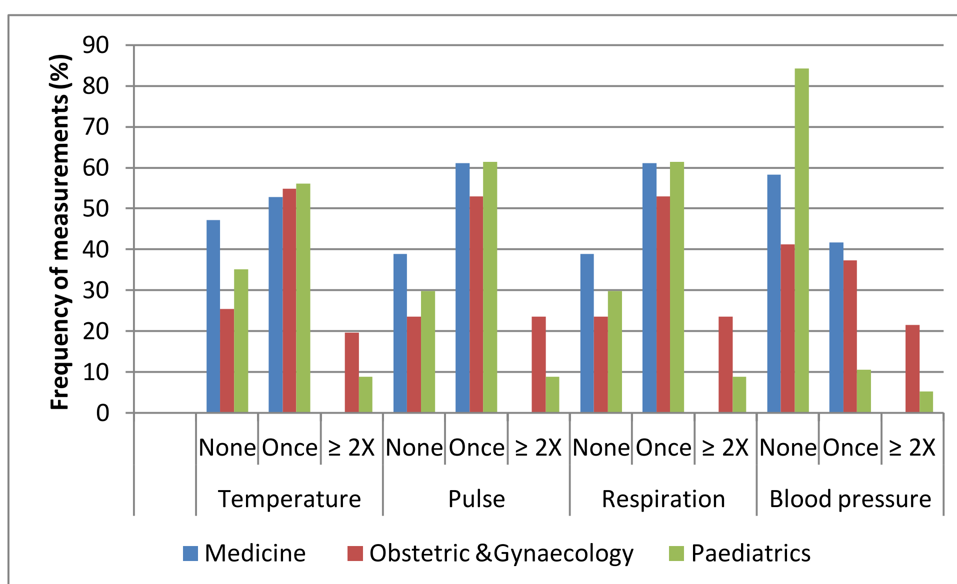
	All (N=151)	Departments		
		Medicine (N=41)	Obstetrics (N=51)	Paediatrics (N=59)
Median age (IQR)yrs	22.0 (4.0-36-0)	41.0 (29.5-56.0)	28.0 (22.0-38.0)	3.0 (1.0-6-0)
Completed transfusion (%)	142 (94.0)	36 (87.8)	51 (100)	55 (93.2)
>1 transfusion received (%)	82 (54.3)	27 (65.9)	33 (64.7)	9 (15.3)
Mean pre-transfusion haemoglobin ±SD (g/dl)	4.5 ± 1.2	4.4 ± 1.7	4.7 ± 1.2	4.3 ± 0.9
Mean duration of transfusion ±SD (hrs)	2.6 ±1.2	2.2 ± 1.1	2.0 ±0.8	3.3 ± 1.0
Documentation of transfusion reactions	13 (8.6)	4 (9.8)	5 (9.8)	4 (6.7)
Post transfusion fever (%)	34 (23)	3 (7)	12 (24)	19 (32)
Death within 24 hrs of transfusion (%)	10 (6.6)	6 (14.6)	2 (3.9)	2 (3.3)

O&G represents Obstetrics and Gynaecology; IQR represents interquartile range; SD represents standard deviation.

Table 4.2: Malaria related clinical diagnoses made in transfusion recipients

Paediatrics	
Severe malarial anaemia	17
Malaria in sickle cell disease	7
Suspected malaria with anaemia	6
Severe malaria	4
Cerebral malaria	4
Malnutrition with malaria	3
Total cases	**41/59 (69.5%)**
Obstetrics and Gynaecology	
Suspected malaria with jaundice/haemolysis in pregnancy	7
Malaria in pregnancy	1
Sickle cell and malaria in pregnancy	1
Total cases	**9/51 (17.6%)**
Medicine	
Malaria (any form)	0
Total cases	**0%**

Figure 4.1: The frequency of vital signs measurements recorded in patient charts



Vital signs

Measurement of temperature, respiration, blood pressure and pulse rate was not done in 33.1%, 28.5%, 59.6% and 28.5% of patients respectively during the transfusion. For all departments the temperature, pulse rate and respiratory rate were frequently measured only once during the transfusion (Figure 4.1).

Anti-malarial drug use

Sixty six (44%) patients received malaria treatment within 24 hours of their blood transfusion. There was marked variation in the use of anti-malarial drugs between departments with 51 (87%) children receiving malaria treatment compared to 12 (24%) obstetric patients and 3 (7%) adult medical patients (Table 4.3). In 51 of these patients (78%) the anti-malarials were prescribed at the same time as the blood transfusion. 84% (43/51) of those who received anti-malarials with the transfusion were children and only one had parasitaemia confirmed by microscopy. In 4 patients an anti-malarial was prescribed in the 24 hours following the blood transfusion, 8 patients had received anti-malarials at least 24 hours prior to transfusion and in 3 patients the timing of the anti-malarials was unknown. Four transfusion recipients with post-transfusion fever received anti-malarials but only one had malaria confirmed on a blood film (Table 4.3).

Information from patients' case notes indicated that clinically-suspected malaria was a reason for hospitalisation in 50 (30%) of the transfusion recipients; 70% of these were children. Overall 20 transfusion recipients (13%), 10/59 (17%) children, 3/51 (6%) obstetric patients and 7/41 (17%) medical patients, received malaria treatment with their transfusion or within 24 hours post-transfusion despite having no underlying clinical diagnosis of malaria, and 64 (42%) transfusion recipients were treated for malaria without any laboratory confirmation of parasitaemia. Quinine (39% of all anti-malarial prescriptions) and artesunate-amodiaquine (33%) were the most commonly prescribed anti-malarial drugs and these were used exclusively by the department of paediatrics.

Table 4.3: Practices related to transfusion-transmitted malaria in three departments of KATH

	All (N=151) n (%)	Medicine(N=41) n (%)	O&G(N=51) n (%)	Paediatrics(N=59) n (%)
Clinical diagnosis of malaria at admission (%)	50 (30)	0 (0)	9 (18)	41* (70)
Anti-malarial use (%)	66 (44)	3 (7)	12 (24)	51 (87)
Type of anti-malarial given				
Quinine	26 (40)	0 (0)	0 (0)	26 (51)
Artemether-Lumefantrine	18 (27)	3 (100)	12 (100)	3 (6)
Artesunate-Amodiaquine	22 (33)	0 (0)	0 (0)	22 (43)
Time of prescribing anti-malarial				
With transfusion	51 (77)	1 (33)	7 (59)	43 (84)*
At least 24 hours before transfusion	8 (12)	1 (33)	1 (8)	6 (12)
Within 24 hours post- transfusion	4 (6)	0 (0)	3 (25) *	1 (2)
Unknown	3 (5)	1 (33)	1 (8)	1 (2)
Other drugs				
Furosemide (%)	60 (40)	2 (5)	6 (12)	52 (88)
Antibiotics use (%)	118 (78)	25 (61)	49 (96)	44 (75)
Post transfusion fever (%)	34 (23)	3 (7)	12 (24)	19 (32)

*confirmed by positive malaria microscopy in one case

Artemether-Lumefantrine (28%) was prescribed in the departments of medicine and obstetrics but not in paediatrics. No chloroquine or sulphadoxine-pyrimethamine was prescribed for any transfusion recipient.

Other treatments

Overall 118 transfusion recipients (78%) received antibiotics, with the highest usage in obstetrics (96%)(Table 4.3). 81% of antibiotics were prescribed prior to the blood transfusion. 40% of all transfusion recipients, predominantly paediatric patients (88%), had furosemide prescribed at the same time as the blood transfusion (Table 4.3). From the clinical notes the reason for prescribing a diuretic was only documented for two patients both of whom had heart failure. In 96% of patients there was no indication of why furosemide was given.

4.4 Discussion

In Ghana, as in many other malaria endemic countries in sub-Saharan Africa, blood for transfusion is not screened for malaria and there are no clear policies about whether or not anti-malarials should be prescribed presumptively with blood transfusions. 44% of all transfusion recipients in our study received malaria treatment with their blood transfusion (77%) or in the 24 hours following the transfusion (6%) but only two (3%) had parasitaemia confirmed on microscopy. This practice of routine prescription of anti-malarials with blood transfusions without laboratory confirmation of infection occurred predominantly in paediatrics. Many of the children had a clinical diagnosis of malaria so it is possible that the anti-malarials were prescribed presumptively for their suspected infection. However, in children, 98% of malaria drugs for which the timing was known, were prescribed simultaneously with the blood transfusion and the number of children prescribed anti-malarials exceeded the number with suspected malaria. This indicates that in paediatric practice anti-malarial drugs are prescribed routinely with blood transfusions.

Prescribing malaria treatment routinely for transfusion recipients in endemic areas reflects WHO guidelines (185) and the advice in several published papers (194-197). In contrast, other papers recommend restricting malaria treatment to selected 'at risk' transfusion recipients such as neonates (34,179,198). These recommendations for neonates were without evidence from the published studies. Evidence of transfusion-transmitted malaria being a significant clinical problem in endemic areas is almost non-existent. One study has documented malaria parasitaemia occurring in recipients of malaria-positive transfusions in Sudan (101).In contrast, the majority of transfusion recipients in Ghana who developed parasitaemia post transfusion did not acquire it from the blood transfusion as parasite genotyping indicated that malaria transmission only occurred in 2% of those who received a malaria-positive blood transfusion (as shown in my results which are presented in chapter 5).

There have been no studies from endemic areas comparing presumptive versus targeted malaria treatment for transfusion recipients and there is very scanty evidence underpinning recommendations for presumptive malaria treatment of transfusion recipients. In contrast, current malaria treatment guidelines recommending that treatment should only be given to those with proven infections (383) are well supported by evidence and are designed to slow down the emergence of *P. falciparum* resistance to artemisinin (384,385). Worldwide, there has been a rise in the proportion of patients with a febrile illness with laboratory confirmation of malaria from 5% in 2000 to 35% in 2009 (76). Although patients with negative malaria tests are still being treated for malaria (386) a Ugandan study has demonstrated that it is possible to improve the rates of diagnostic malaria testing and to restrict malaria treatment to those with positive results (387).

My study found that the restrictive malaria treatment policy is not implemented for blood transfusions in a malaria endemic area. International guidelines do allow for presumptive treatment of malaria if laboratory confirmation is not available (383) but any facility that is able to offer blood transfusion will almost certainly have a laboratory capable of providing a definitive diagnosis of malaria. The practice of treating every fever presumptively for malaria is entrenched (388) and my study

demonstrated that in practice, and particularly in paediatric practice, clinicians choose presumptive treatment for transfusion recipients rather than laboratory confirmation of malaria diagnosis prior to initiation of treatment. Implementation of the WHO recommendations to screen donated blood for malaria is hampered by a lack of suitable screening methods (104) which may partly explain the emphasis on presumptive treatment as an alternative strategy.

The mean duration of transfusion in children was longer than in adults and diuretics were prescribed with the blood transfusion in 88% of children. These practices do not appear to be consistent with any policies or guidelines but are based on assumptions that the symptoms of severe malarial anaemia, particularly respiratory distress, result from biventricular failure (389) and that these children are not hypovolaemic (390). Recent evidence has suggested that children with severe febrile illness and impaired perfusion do not benefit from fluid resuscitation (391). Clinical guidelines (185) indicate that diuretics should only be used in transfusion recipients likely to develop or who develop cardiac failure. 88% of children in our study were given furosemide with their blood transfusion and in almost all cases there was no indication of why it was given.

The prescription of an antibiotic in transfusion recipients ranged from 61-96% across the three departments and is likely to represent treatment for underlying conditions. In clinical practice in Africa it can be difficult to differentiate between severe malaria and sepsis, and both conditions may co-exist (392). Ideally blood cultures should be taken before empirical antibiotics are started (393) but it is common for antibiotics and anti-malarials to be prescribed simultaneously (383,394). There is evidence that this approach may reduce mortality in severe malaria (159).

Monitoring of vital signs was poorly performed in all departments. The national guidelines (395) recommend that vital signs should be measured 15 minutes after the start of transfusion and then hourly until the transfusion is complete. With a 2.6 hr mean duration of transfusion, at least 3 records of vital signs measurement were expected but it they were mostly done once or not at all.

Table 4.4: Summary of policies and practices concerning transfusion-transmitted malaria

WHO recommendations	Policies in Ghana	Practice in KATH	Research knowledge gaps
Donated blood should be tested for malaria (59)	All units to be tested for HIV I & II, Hepatitis B and C and syphilis and any other transfusion transmissible disease (379)	No malaria screening of donated blood	Screening methods for malaria that are practical and sensitive enough for use by transfusion services
In endemic areas, there is a high risk of transmitting malaria by transfusion. All transfusion recipients should receive routine treatment for malaria (185)	No policy on routine treatment for malaria	33% of transfusion recipients received anti-malarials with their transfusion or within 24 hours post-transfusion	Cost-effectiveness of malaria screening in different transmission zones
Anti-malarials should only be prescribed for proven malaria infections (383)	Confirm cases of malaria before initiating treatment (treatment can be started in severe cases but a confirmation from the laboratory is needed)(396)	3% (2/66) had a laboratory confirmation of malaria before treatment	Rates of malaria transmission by transfusion with genotyping to confirm that malaria was acquired through transfusion
In patients at risk of circulatory overload, red cells are preferable to whole blood. Treat volume overload and cardiac failure with diuretics(185)	Diuretics should only be given in recipients with heart failure. For patients with heart failure lower the rate of transfusion(395)	88% of children were given diuretics	Effectiveness of presumptive malaria treatment of transfusion recipients compared to treatment restricted to proven infections Effective approaches for ensuring that transfusion guidelines are evidence-based, regularly reviewed and implemented

4.4.1 Limitations of study

The study was conducted within a two month period, and that may not be representative of what happens throughout the year. Considering the fact that October and November represents the end of the rainy season and the incidence of malaria is not high, the use of anti-malarials may have reduced. A survey all year long or a survey that compares practices during the dry and wet seasons may be appropriate.

The study was unable to establish the rationale for anti-malarial use. It may have been helpful through qualitative methods to directly ask doctors the rationale for initiating treatment with anti-malarials. In addition, the period for which clinical staff has been in a particular department could influence the practice there. For this survey, the duration of employment in the various departments was not ascertained. Some factors such as pre-transfusion fever could have influenced clinicians practice but this was not evaluated.

The study was also limited with its restriction to few departments rather than all departments in the hospital. Such a survey would have presented a more general overview of hospital transfusion practices.

4.5 Summary

This study has shown that where international malaria and transfusion policies are in conflict, such as restricting anti-malarials to proven infections versus presumptive treatment of transfusion recipients, clinicians choose to adhere to transfusion policies rather than malaria policies (Table 4.4). This is despite the fact that malaria policies have a much stronger evidence base than transfusion policies. There is an urgent need for harmonised policy-making by malaria and blood transfusion programmes so that clinicians, and particularly paediatricians, receive a clear message about how to reduce malaria transmission through blood transfusions. This harmonisation needs to be preceded by research to generate better knowledge about the prevalence and clinical impact of transfusion-transmitted malaria, and about the cost-effectiveness of various blood donor screening

strategies taking account of the need to avoid exacerbating blood shortages by unnecessarily excluding donors. My study has demonstrated that until these critical gaps in the evidence about transfusion-transmitted malaria are addressed and the relevant policies are harmonised, these mixed messages will continue to be translated into inconsistent clinical management to the detriment of patient care and public health.

CHAPTER 5

TRANSFUSION-TRANSMITTED MALARIA

5.1 Introduction

Blood shortages and unsafe blood, are the two crucial issues relating to blood transfusion in the developing world, particularly Africa. These issues can lead to serious health consequences such as death from post partum haemorrhage or the transmission of life threatening infections (397). As major progress is made in the prevention of the major transfusion-transmitted infections namely HIV, hepatitis B, hepatitis C and syphilis, one disease that requires attention and which can impact on blood safety and a shortage is malaria.

Malaria is seen as the most devastating parasitic infection in the world (398). *P. falciparum* is the most dangerous species of plasmodium and has the highest rate of complications and mortality. In 2007 it caused an estimated 451 million cases of clinical malaria in malaria endemic countries (399). When transmitted through blood transfusions in non-endemic countries, malaria can have serious consequences including being rapidly fatal. The effects of transfusion-transmitted malaria (TTM) on recipients living in the endemic regions are yet to be defined. It has been suggested that TTM is a problem experienced by non-immune individuals but not the semi-immunes who when transfused with blood infected with malaria parasites, acquire infections but may not necessarily exhibit clinical symptoms (400).

In SSA, blood donors are adults, who by virtue of living in a malaria endemic area have acquired immunity, which can be associated with low grade parasitaemia (401). In screening such donors, the blood may be declared negative only because parasitaemia is below the detection threshold of the method used. Such a person may still have malaria parasitaemia and will be able to induce TTM if his/her blood is transfused. This poses a diagnostic challenge in screening for malaria in endemic areas (402). Microscopy, rapid detection tests (RDT), enzyme immunoassays (EIA)

and PCR methods are tests used for diagnosis of malaria but their efficacy as screening tests has not been demonstrated.

As discussed in chapter 4, there are policies that recommend screening for malaria and that recommend presumptive treatment for transfusion recipients in endemic areas but these are not based on evidence. The aim of this study therefore was to establish the incidence of TTM in transfusion recipients and to determine which test was most appropriate for screening donated blood.

5.2 Materials and Methods

Study design and sample size modification

The sample size calculated for this study was not used because of the difficulty in enrolling the required 600 subjects within the one year time frame of the fieldwork. The study design of matching recipients of parasitaemia positive transfusion recipients by age and gender would also have enrolled very few patients so it was not pursued. For example, 21 cases of microscopy positive donor blood were found for the period of the study, implying that with two controls for each case only 63 cases would have been recruited for the study. It was decided that enrolment was to go on continuously until the end of study period.

Participants and recruitment

Participants were recruited from the Departments of Obstetrics and Gynaecology, Paediatrics, Medicine and Oncology at Komfo Anokye Teaching Hospital (details of the study site have been discussed in chapter 3). The following transfusion recipients were eligible for enrolment; pregnant women, children (aged between 1 and 15 years), immune compromised (including HIV and malnourished) patients and patients with malignancies and chronic diseases (including sickle cell disease, chronic renal failure, liver disease).

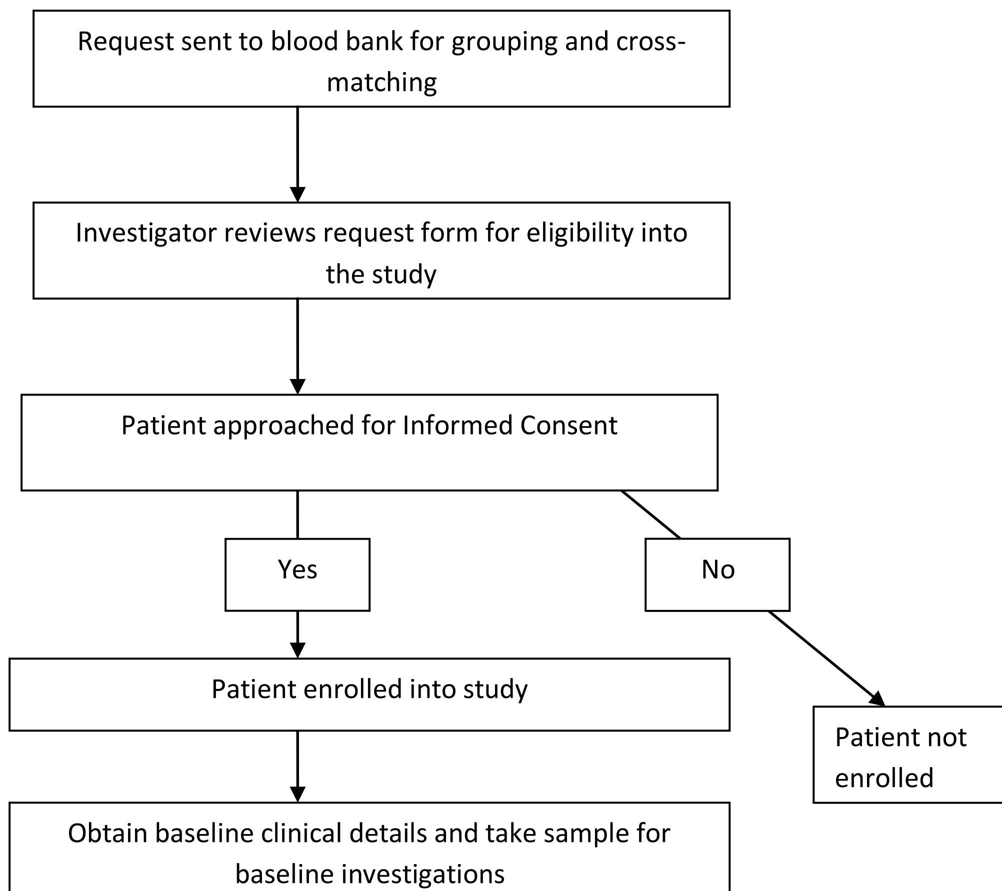
Exclusion criteria: Patients were excluded from the study if they were found to be parasitaemic by microscopy at pre-transfusion or if they had taken anti-malarials 5

days prior to the transfusion. Patients were also excluded if they were transfused in the operating room or considered too ill (unconscious, shocked or delirious) such that signs and symptoms could not be elicited. Patients with a clinical diagnosis of malaria were not excluded if they did not fulfil any exclusion criteria.

Study enrolment

Patients for whom a transfusion had been requested were identified from the blood bank records. Eligible patients were evaluated by scrutinising their clinical notes and interviews before obtaining a written informed consent. An algorithm for the process of enrolment is shown in figure 5.1. Patients who consented to participate were assigned unique study numbers. Each blood unit received by the patient was uniquely identified.

Figure 5.1: Process of enrolment into the study



Patient monitoring and follow-up

Patients were followed up for 14 days post-transfusion. They were not excluded from the study if they received more than one unit of blood. The duration of storage of the transfused blood units was retrieved from the blood bank records. Patients' vital signs, blood pressure, temperature, pulse and respiratory rate, were measured at the start of the transfusion, 15 and 30 minutes after the start of the transfusion and then hourly until the transfusion had been completed. After the transfusion, and for the next 3 days when patients were in hospital, patients were examined daily and their vital signs were measured every 8 hours. Subsequently, scheduled follow up of patients was performed on day 7 and 14. The activities performed on each day is presented in table 5.1.

Table 5.1: Study procedures performed for each patient enrolled in the study

Time point	Activities
Pre-transfusion	History and clinical examination Blood sample
During transfusion	Vital signs measurement at 15 min, 30 min and hrly until transfusion is complete
Immediate post transfusion	Vital signs measurement
Day 1	Clinical examination Vital signs measurement (3X) Blood sample taken
Day 2	Clinical examination Vital signs measurement (3X)
Day 3	Clinical examination Vital signs measurement (3X) Blood sample taken
Day 7	History and clinical examination Blood sample taken
Day 14	History and clinical examination Blood sample taken

Patient management

After transfusion, five follow up visits were planned; the first 3 while in hospital and the next 2 were as outpatients. At each of these follow up visits a blood sample was examined for parasitaemia by microscopy. In addition, haemoglobin was measured. Besides the scheduled visits, patients were encouraged to come back to the study

team anytime they did not feel well (unscheduled visit). The regular physicians of the patients were maintained throughout the study. They were provided with all results for the patient and for the blood they received and were responsible for patients' treatment. Any patient who was found to be parasitaemic by microscopy was treated with anti-malarials according to local guidelines. Their physicians were also free to give patients any other appropriate medications they deemed necessary.

Figure 5. 2: Blood transfusion in a paediatric ward

Table 5.2: Definitions used in the study

Serious adverse event An event that resulted in stopping an ongoing transfusion. An event that occurred in relation to the transfusion, which resulted in death, life threatening or incapacitating conditions for patients or which prolonged hospitalisation or morbidity.
Post-transfusion malaria Parasitaemia occurring after a patient received a transfusion, irrespective of the source of the parasitaemia.
Transfusion-transmitted malaria (TTM) Parasitaemia occurring in a patient post transfusion, where the parasite was genotypically the same as that in the transfused unit of blood.
Clinical TTM TTM in a patient with a temperature >37.5 °C.

Sample processing and testing

Transfused blood:

Two millilitres of blood were obtained from each unit of transfused blood. Blood samples obtained from the transfused unit of blood were screened for *P. falciparum* by means of microscopy, rapid diagnostic test (RDT), enzyme immunoassay (EIA) and polymerase chain reaction (PCR). The parasite detection focussed on *P. falciparum* because falciparum is responsible most of severe malaria and causes significant mortality and morbidity (403).

RDT was performed immediately and microscopy was done within 24 hours of obtaining sample. One millilitre of blood was placed in an Eppendorf tube and stored immediately in a freezer at -20°C for malaria EIA testing. Four concentric blood spots each about 50µl (2 drops) were placed on 3M filter paper; air dried and sealed in a plastic envelope together with a desiccant. The filter paper was stored at room temperature until it was transported to Liverpool for PCR and genotyping.

Recipient blood:

One millilitre of blood was taken from patients at pre-transfusion and on all the follow up days for haemoglobin measurement and detection of *P. falciparum*.

Haemoglobin measurement was by means of a Sysmex KX21N (Sysmex Corporation, Kobe, Japan). Microscopy was performed immediately for *P. falciparum* detection. All samples were in addition stored as spots on filter paper, stored at room temperature and for PCR and genotyping. Only patients who received *P. falciparum* positive blood (determined by PCR on transfused blood) had their follow up samples tested by PCR. This is because when the donated blood had no parasitaemia then there was no basis for comparison. In situations where the follow up sample was also positive, genotyping was done on parasites from the transfused unit and the patient to determine if they were the same parasite.

Figure 5.3: The staining area in the malaria laboratory

Figure 5.4: The microscopy bench in the malaria laboratory

Test methods

<u>Microscopy</u>

Thick and thin blood smears for microscopy were prepared from venous blood that had been collected in EDTA bottles. The detailed preparations for thick and thin film are shown in appendix 6.3. The smears were stained with 10% Giemsa and examined for the presence of parasites with X100 oil immersion lens of a microscope (Olympus, Japan). Slides were only declared negative after 100 high power fields were examined. Slides were examined by two microscopists and reviewed by an expert microscopist when there was disagreement. Ten percent of slides were also reviewed by the expert microscopist.

<u>Rapid detection test</u>

First Response® Malaria Antigen test kit (Premier Med Ltd, India), which detects Histidine rich protein 2 (HRP2) was used for rapid detection of *P. falciparum*. A drop of blood (25µl) was placed into a sample well. Two drops of an assay buffer was added through a buffer well below it. The results were read in 20 minutes, a positive test being the presence of two bands on the control and test lines.

Enzyme immunoassay

The Malaria Antigen CELISA (Cellabs, Sydney, Australia) test kit used for this study is based on the detection of HRP2 and was performed according to the manufacturer's instructions. Briefly, 100 µl of each blood sample which had been frozen and thawed twice (to ensure complete haemolysis) was pipetted into individual micro-wells of the CELISA plate. These wells had been pre-coated with an anti-*P. falciparum* monoclonal capture antibody. The plate was incubated in a humid chamber at room temperature for one hour. After washing five times with a wash buffer, 100 µl of the conjugate was added into each well and incubated under similar conditions for an hour. The plate was washed again five times and 100 µl of freshly prepared substrate pipetted into each well. After incubation at room temperature in the dark for 15 minutes, 50µl of a stop solution were added and the results read in a spectrophotometer at an absorbance of 450nm. For each batch of samples run, two positive and negative controls were included. The cut-off level was calculated as the sum of the optical density of the negative control plus 0.1. Any sample with an absorbance value above the determined cut-off level was considered reactive for *P. falciparum* antigen. No confirmatory tests were required.

DNA extraction from blood spots on filter paper

DNA was extracted using the chelex/saponin method (404). A 6 mm punch out of the blood soaked filter paper was fully immersed in a 1.5ml Eppendorf tube containing 1 ml of freshly prepared 0.5% saponin in 1X PBS and left overnight at room temperature. The saponin solution was removed and 150 µl of 6 % chelex suspension made up in nuclease-free water added to fully cover the filter paper. The tubes were then placed in dry heating blocks that had been set to 100˚C and allowed to boil for between 20 and 25 minutes. Cooling was allowed for two minutes on the bench and to allow the chelex to settle. Finally, the Eppendorf was centrifuged for 2 minutes and 100µl of the DNA supernatant eluted. This was stored at -20°C until PCR was to be performed.

Quantitative PCR

A SYBR-Green real-time PCR assay, specific for *falciparum* species, was employed to detect *P. falciparum* DNA extracted from the blood spots. Brilliant III SYBR Green PCR mix (Agilent Technologies, Santa Clara, CA, USA), forward (rtPfa1F) and reverse primers (rtPfa1R)((Eurofins MWG Operon, Ebersberg, Germany) and 1 µl of template DNA was used in a total reaction volume of 20 µl. The amplification was performed on a Stratagene MX 3005P real-time PCR system (Agilent Technologies) with the following cycling program: 95°C for 3 min; 40 cycles of 95°C for 10 sec, 55°C for 10 sec and 60°C for 1 min. The PCR product identity was confirmed using a melt curve analysis as previously described (405). Dissociation and amplification curves are presented in appendices 6.5 to 6.7. Serial dilutions of the International Standard ranging from 1 X 10^6 to 10^3 IU/ µl were included in duplicate in each real time PCR run, allowing an approximate quantification of the samples to be carried out. The quantification was derived from standard curves which were produced from the dilutions of the standard.

Table 5.3: Primers used for *P. falciparum* real time PCR

Name	Sequence (5' ->3')
rtPfa1F (forward primer)	GCCGAAAGGCGTAGGTAATC
rtPfa1R (reverse primer)	GTACAAAGGGCAGGGACGTA

P. falciparum strain comparison

Genetic analysis of *P. falciparum* in patients and the corresponding transfused unit of blood were carried out according to published protocol, using nested PCR for the detection of allelic diversity at the merozoite surface protein 2 (msp2) loci (172).

Nested PCR

Nested PCR involves a primary reaction, where the primers span the entire genetic segment of block 3 (Table 5.4) while for the nested reaction primers were specific for the 3D7 or FC27 allele (Table 5.5). Both primary and nested amplifications were carried out in 25 µl reaction volumes. For the primary PCR, the final concentration

of the master mix contained 0.04 U/µl of Taq polymerase 1, 1x PCR buffer, 1.5mM $MgCl_2$, 200 µM of dNTPs, 300 nM each of forward and reverse primers (Table 5.4) and 5 µl of the extracted DNA.

Table 5.4: Primers used for primary PCR for *msp*2

Name	Sequence (5' ->3')
M2-OF	ATGAAGGTAATTAAAACATTGTCTATTATA
M2-OR	CTTTGTTACCATCGGTACATTCTT

A Techne TC-4000 thermocycler (Bibby Scientific, Staffordshire, UK) was used for amplification under the following cycling conditions: initial denaturation 94°C for 2 min; 30 cycles of 94°C for 30 sec, 54°C for 1 min and 72°C for 1min, and 72°C for 5 min. One µl of the primary PCR product was re-amplified in a separate nested reaction for the 3D7 or FC27 family. Each reaction consisted of 1x buffer, 300nM of forward primer S1fw, 1.5mM $MgCl_2$, 200 µM of dNTPs, 0.04 U/ µl of Taq polymerase and 300nM of a specific 3D7-type or FC27-type allele reverse primer (N5 for 3D7 and M5 for FC27) (Table 5.5). The conditions for the nested amplification were as follows: initial denaturation 94°C for 2 min; 30 cycles of 94°C for 30 sec, 50°C for 45 sec and 70°C for 1min 30 sec, and 70°C for 5 min.

Table 5.5: Primers used for 3D7 and FC27 allele-specific PCR

Name	Sequence (5' ->3')
S1fw (forward primer)	GCT TAT AAT ATG AGT ATA AGG AGA A
M5rev (reverse primer for 3D7)	GCA TTG CCA GAA CTT GAA
N5rev (reverse primer for FC27)	CTG AAG AGG TAC TGG TAG A

Electrophoresis and band detection

Following the nested PCR reactions, electrophoresis of products was carried out on 2% agarose gel.

Figure 5.5: PCR product detection on agarose gel

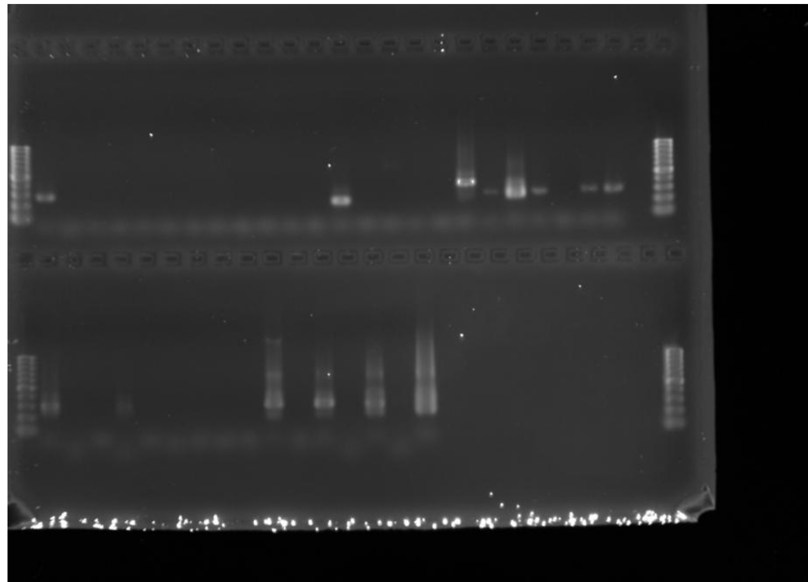
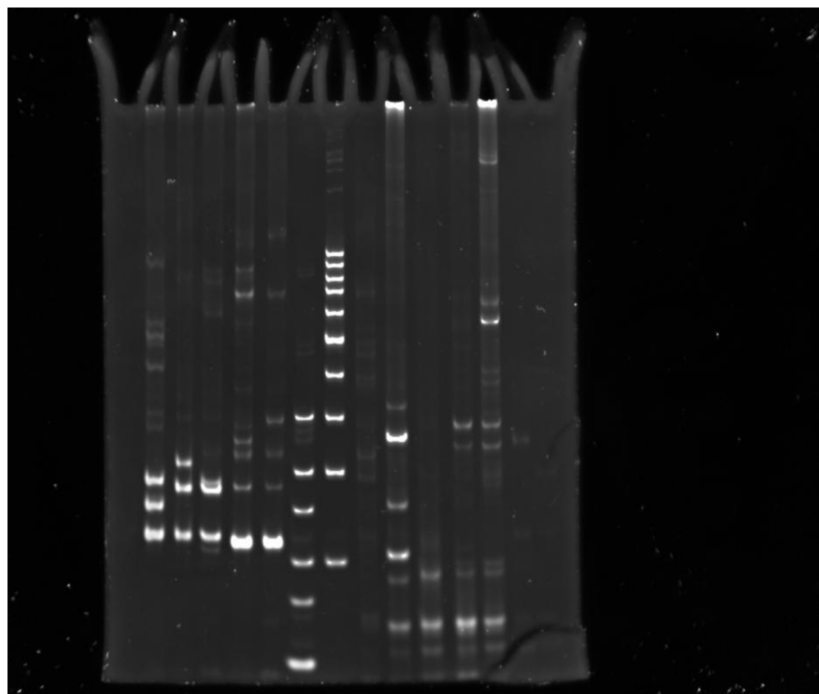


Figure 5.6: PCR product detection on polyacrylamide gel



Figures 5.5 and 5.6 shows the 2-step electrophoresis performed after the nested PCR. DNA products were initially run on 2% agarose gel (Figure 5.5). When products were identified to be present, they were then run on 10% polyacrylamide gel which gives a better resolution (Figure 5.6). Automated band sizing was then performed on the polyacrylamide gel.

Where DNA products were detected in both the transfused blood and in the patient's follow up sample, the paired samples were then run on 10% polyacrylamide gel, stained with ethidium bromide and visualised using a Gel Doc™

EZ imaging system (Biorad, Hercules, CA, USA). Automatic band detection and fragment sizing was carried out using Image Lab software (Biorad). For both the 3D7 and FC27 families, alleles were considered to be the same if molecular weights were within 10 base pairs of each other. If alleles from the blood bag and patient were the same, transfusion-transmitted malaria was said to have occurred.

Restriction digest

To increase the discrimination power of the msp2 genotyping, the products of nested PCR amplification for the 3D7 family were subjected to digestion with the ScrF I restriction enzyme (Fermentas Inc, Glen Burnie, MD, USA). A reaction mix with a 20 µl total volume, containing the nested PCR product and the enzyme is incubated in a water bath at 37°C for 30 minutes. PCR fragments were visualised and sized as described above.

5.2.1 Quality assurance and quality control

The microscopists were qualified biomedical scientists who have worked as microscopists in GCLP compliant malaria drug and vaccine trials. To prevent artefacts on slides, only new slides were used and the Giemsa stain for microscopy were freshly prepared daily. An experienced microscopist resolved any differences and also independently reviewed 10% of slides.

The RDT was stored in an air-conditioned room to preserve its sensitivity as it is known that extremes of temperature can reduce sensitivity.

Negative and positive controls were included for each batch of samples tested by the HRP2 EIA. The negative control was to have an optical density value of less than 0.1. For both PCR and EIA runs, some wells were randomly left empty to check if spill over was taking place.

Positive and negative controls were used for all PCR reactions. For all plates, the last two rows were used for negative controls and standards. Two types of negative controls, negative human sample and water and positive controls including international standards in different dilutions were used in duplicates. These are

used for the detection of contamination, quality of reagents, normalisation and quantification as mentioned earlier.

In all laboratories, specific standard operating procedures for specific test methods were strictly followed to ensure consistency.

Data capture and statistical analyses

Case report forms designed to capture all relevant information were used to record data (see appendix 6.1). Information on the blood including the type of donors and the duration of storage of the refrigerated blood was obtained from the donor records at the blood bank. All signs and symptoms elicited, diagnoses, investigations and treatment given was recorded in these case report forms. All statistical analyses were performed with the Predictive Analytics Software (PASW®) statistical package 18 (SPSS Inc, USA). Descriptive statistics including measures of central tendency and dispersion were used where appropriate. Parasite densities of *P. falciparum* identified by microscopy and DNA copies determined by qPCR were converted into logarithm values and correlation assessed by Pearson's coefficient r.

To compare the accuracy of the four different test methods used to detect *falciparum* parasitaemia, the composite reference standard method was used (406). A reference 'gold standard' test was constructed by combining the results of PCR and microscopy such that a positive result by either test in combination or singly (i.e. +PCR/-microscopy, -PCR/+microscopy and +PCR/+microscopy) was considered a reference positive. The reference test was negative when both PCR and microscopy were negative. Using the constructed gold standard, the sensitivity, specificity, negative predictive and positive predictive value of PCR, microscopy, EIA and RDT was calculated. This method was used because though microscopy which is the gold standard for parasite detection, it is not as sensitive as other methods such as PCR. It is therefore be inappropriate for use when assessing the performance of malaria screening tests.

5.3 Results

Recipient characteristics

Three hundred and seventy two patients were enrolled and the majority (43.6%) were from the department of medicine (Table 5.6). The department of paediatrics had the least number of patients, 6.7% (25/372). There were more females than males (64.5%), the reason being all patients from O&G were females. Chronic diseases accounted for 58.2% of patients in medicine and 37.9% of patients in paediatrics and were the most common diagnoses in both departments. Pregnancy related bleeding /anaemia (65.3%) was the most common diagnosis in O&G. Anti-malarials had been given to 23.4% of the patients (87/372), either more than 5 days before recruitment (50.6%; 44/87) or post-transfusion (49.4%; 43/87). The most frequently used anti-malarial (74.7%) was artemether-lumefantrine. Thirteen percent (11/87) of anti-malarials prescriptions were for a single agent artesunate, contrary to current WHO recommendations.

Table 5.6: Characteristics of 372 transfusion recipients

Characteristics		
Department N (%)	Medicine	162 (43.6)
	O & G	141 (37.9)
	Oncology	44 (11.8)
	Paediatrics	25 (6.7)
Sex N (%)	Male	132 (35.5)
	Female	240 (64.5)
Median age (years)(IQR)		35.0 (25.0 -49)
Mean transfusions ± SD		2.6 ± 1.6
Anti-malarials use N (%)	> 5 days before transfusion	44 (50.6)
	After transfusion	43 (49.4)
Antibiotics use N (%)		228 (61.3)

Characteristics of donated blood

Voluntary blood donors provided 65.8% of the units of blood transfused (Table 5.7). The median duration of storage of units of blood transfused in this study was 2 days, however the maximum duration observed was 30 days. There was no significant difference for detecting parasitaemia in refrigerated blood stored for more than 14 days (2/23) compared to blood stored for 14 days or less (OR 0.418, 95% CI 0.096-1.829).

Table 5.7: Characteristics of 372 donors and donated blood

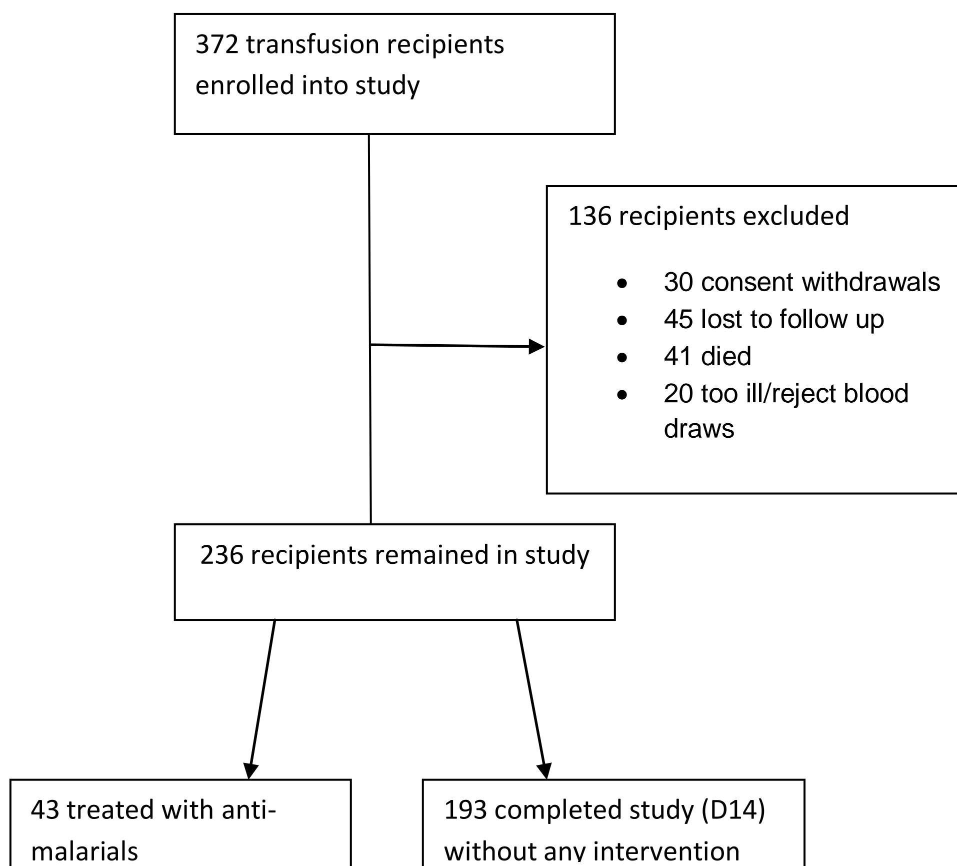
Donor characteristic		
Median age	Median age (inter-quartile range) years	21.0 (18.0 – 30.0)
Type of donor	Voluntary	245 (65.8)
	Replacement	127 (34.1)
Sex of donor	Male (%)	275 (73.9)
	Female (%)	97 (26.1)
Duration of storage	Median duration (inter-quartile range) days	2 (1-6)

Study dropouts

Although 372 transfusion recipients were enrolled in the study, 136 (36%) did not complete the required 14 days for evaluation (figure 5.2). There were 30 patients who withdrew their initial consent and 45 who did not come for a follow up visit. There were 20 patients who wanted to remain in the study but did not want their blood taken; they were excluded because they could not be evaluated for parasitaemia. The majority (76%) of deaths (n= 41) in this study were from the department of medicine with only one death (2%) from paediatrics, four (10%) from O&G and five (12%) from oncology. Malignancies together with chronic liver and kidney disease accounted for 70% of the deaths in this study group.

Of the 236 patients who could be evaluated, 43 (18%) received anti-malarials from their physicians. However only 11 (26%) of these 43 were treated based on microscopic parasitaemia.

Figure 5.7: Study flow chart

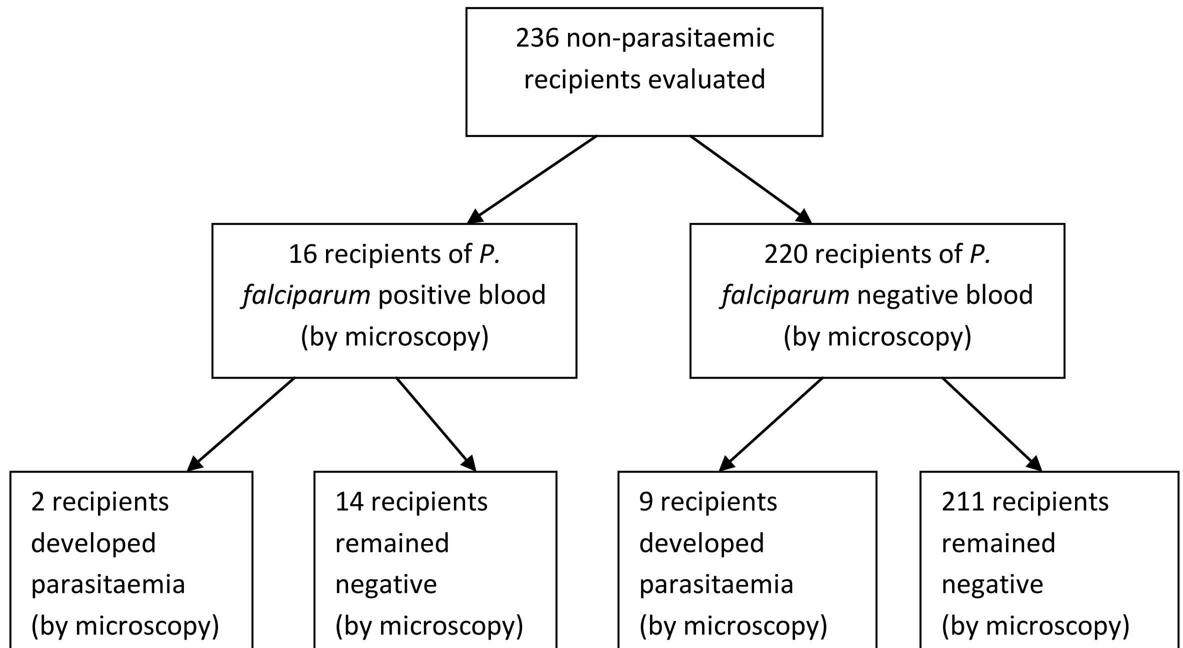


Malaria in transfusion recipients: evaluation by microscopy

There were 236 recipients who could be evaluated. Sixteen of these transfusion recipients received blood that was determined to contain *P. falciparum* and two of the 16 recipients (12.5%) subsequently developed parasitaemia at follow up (Figure 5.8). Of 220 recipients who received blood that did not contain *P. falciparum*, nine (4.1%) developed parasitaemia during the 14 day follow up period (Figure 5.8).

In all, *P .falciparum* was detectable by microscopy in eleven transfusion recipients during the 14 day follow-up period. The incidence of post transfusion malaria was therefore 4.6% (95% CI 2.0-7.2%). Only two (18.2%) of these recipients had received a microscopy confirmed *P. falciparum* positive blood. There were 32 recipients who were treated with anti-malarials despite having a negative blood film for parasites.

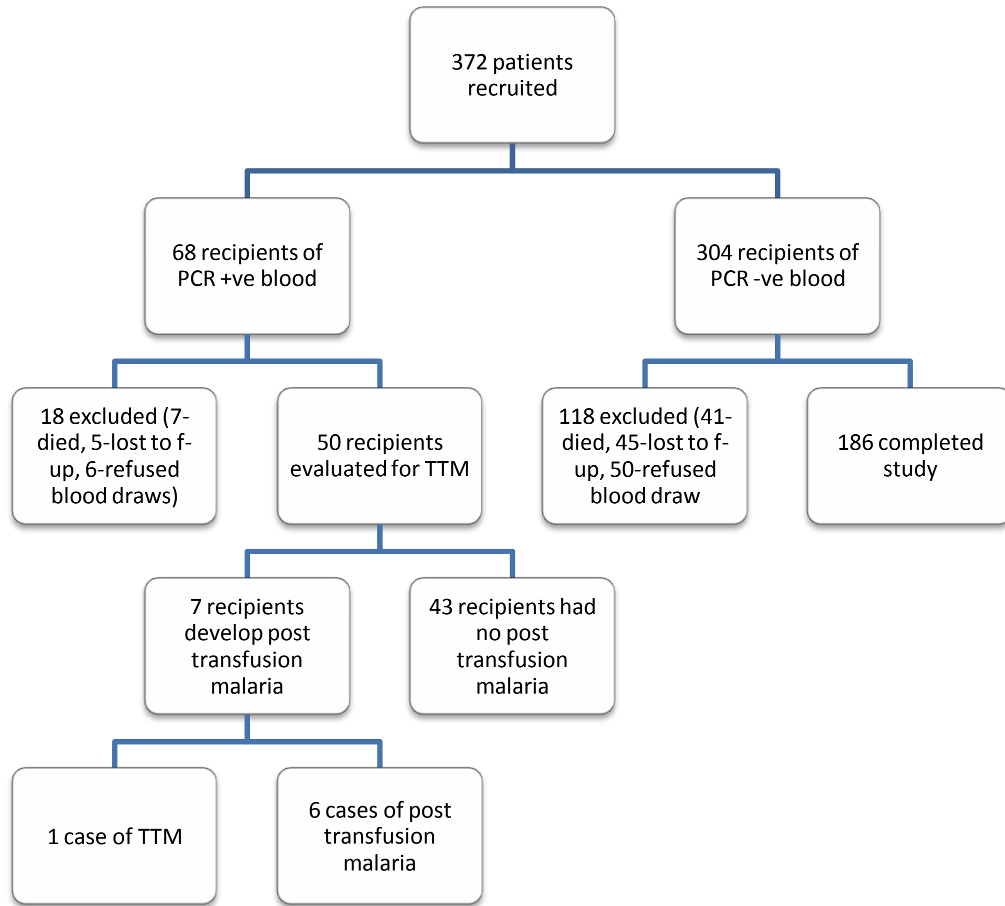
Figure 5.8: Outcomes in transfusion recipients when parasitaemia detection was by the microscopy method.



Malaria in transfusion recipients: evaluated by PCR

There were 68 recipients of PCR positive *P. falciparum* blood but only 50 could be evaluated (Figure 5.9). Among those not evaluated, seven died, five were lost to follow up and six refused blood draws. Of the 50 that were evaluated, 16 received anti-malarials during the study for different reasons. Five were treated for being microscopy positive at follow up and the other 11 were treated presumptively, based on clinical symptoms but without parasitological confirmation by microscopy.

Figure 5.9: Outcomes of patients who received PCR positive P. falciparum blood



Genotyping demonstrated that *P. falciparum* in recipient, ID 223, and in the blood unit transfused was the same (Table 5.8). This recipient was afebrile and asymptomatic but was treated with anti-malarials because *P. falciparum* had been detected by microscopy. Genotyping of parasites from patient ID 356 were found to be of the same family (3D7) as those in their transfused blood, but the parasites were distinguishable on digestion with Scr1F. Results from patients ID 184, 301, and 369 showed that the *P. falciparum* in the recipient and the transfused blood were from the same 3D7 family of msp2 allele but were different clones. For patients 9 and 319 the *P. falciparum* parasites in the patient samples and the corresponding blood bag were from different families of the *msp2* alleles, demonstrating that these were post-transfusion infections and not TTM.

Thirteen transfusion recipients received blood that was positive for malaria by both microscopy and PCR. Three of these recipients developed post transfusion malaria detected by PCR alone and two had post transfusion malaria which was detected by microscopy alone.

Genotypically confirmed TTM therefore occurred in one (2.0%, 95% CI -2 – 6%) of fifty patients who received a blood transfusion positive for *P. falciparum* by PCR. There was no case of clinical TTM.

Table 5.8: Genotyping of positive paired samples of patients and transfused blood unit

	Screening results of blood bag				Patient results		MSP2 Genotyping results					TTM confirmed?
							Presence in sample		Comparison of fragments			
Pt/blood ID	**Micro**	**RDT**	**EIA**	**PCR**	**day of +ve ff-up**	**PCR**	**3D7**	**FC27**	**3D7 family**	**FC27**	**3D7 digest**	**Yes/No**
9					D3	+	-	+	ND	ND	ND	No
BU-9	-	-	+	+			+	-				
184					D1	+	-	+	ND	ND	ND	No
BU-184	+	+	-	+			+	-				
223					D1	+	+	+	same	same	same	Yes
BU-223	+	+	+	+			+	+				
301					D7	+	+	-	Different	ND	ND	No
BU-301	-	-	+	+			+	-				
319					D1	+	+	-	ND	ND	ND	No
BU-319	+	+	+	+			-	-				
356					D1	+	+	-	Same	ND	different	No
BU-356	-	+	+	+			+	-				
369					D3	+	+	-	Different	ND	ND	No
BU-369	+	+	+	+			+	-				

TTM=transfusion-transmitted malaria; Micro=microscopy; RDT=rapid detection test; EIA=enzyme immunoassay; PCR=polymerase chain reaction; ND=not done; ff-up= follow up; Pt ID=patient identification, BU=Blood unit

Table 5.9: Sensitivity and specificity of malaria screening tests using a composite gold standard as the reference test

Screening test		Gold standard result		Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
		+ve	-ve				
PCR	+ve	71	0	93.4	100	100	98.6
	-ve	5	355	(84.7 - 97.6)	(98.7 - 100)	(93.6 - 100)	(96.6 - 99.5)
Microscopy	+ve	18	0	23.7	100	100	85.5
	-ve	58	355	(14.9 - 35.1)	(98.7 - 100)	(78.1 - 100)	(82.1 - 89.1)
RDT	+ve	32	25	42.1	92.9	56.1	88.2
	-ve	44	330	(31.0 - 53.9)	(89.6 - 95.3)	(42.4 - 69.0)	(84.4 - 91.2)
EIA	+ve	47	48	61.8	86.5	49.5	91.4
	-ve	29	307	(49.9 - 72.5)	(82.4 - 89.8)	(39.1 - 59.8)	(87.7 - 94.0)

PPV=positive predictive value; NPV=negative predictive value; RDT=rapid detection test; EIA=enzyme immunoassay; PCR=polymerase chain reaction; +ve=positive; -ve=negative.

Malaria screening tests

In the study period, 445 units of blood were transfused in 372 patients. The prevalence of *P. falciparum* in the transfused blood units was 4.7% (N=445) by microscopy, 13.7% (N=440) by RDT, 18% (N=436) by PCR and 22.2% (N=442) by EIA. Using the composite reference standard method to construct a gold standard, table 5.9 shows the accuracy of each of the four test methods used to detect parasitaemia. PCR has the highest sensitivity (93.4%; 95% CI 84.77 - 97.6%) while microscopy had the lowest sensitivity (23.7%; 14.9 – 35.1%). Specificity for all tests were high, the lowest being 86.5% (95%CI 82.4 – 89.8%) for the EIA method and the highest being 100% for PCR and microscopy (Table 5.9). Of the 431 transfused units which had been tested by all four malaria screening methods, 67.9% (293) were negative by all 4 tests and 12 were positive by all 4 tests (Table 5.10).

Table 5.10: Comparison of malaria tests on 431 transfused units by four different methods

Number of units that tested positive	Malaria test
by 4 tests (12)	
12	PCR + RDT + EIA + Microscopy
by 3 tests (15)	
13	PCR + RDT + EIA
1	PCR + RDT + Microscopy
1	RDT + EIA + Microscopy
by 2 tests (40)	
20	PCR + EIA
4	PCR + RDT
12	RDT + EIA
2	RDT + Microscopy
2	EIA + microscopy
by 1 test (71)	
35	EIA
13	RDT
2	Microscopy
21	PCR

In the 13 samples that were positive by both PCR and microscopy the parasite densities of transfused blood determined by microscopy were positively

correlated with the *P. falciparum* DNA concentration as determined by real-time PCR (Pearson's correlation coefficient r = 0.528, p = 0.043).

Transfusion reactions

Four hundred and thirty two transfusions were monitored during the period of this study. Signs and symptoms that patients experienced are summarised in table 5.11. Severe adverse reactions occurred in 4.6% (20/432) of the transfusions given and 17 (3.9%) of these transfusions were stopped before its completion. Acute transfusion reactions were the commonest serious adverse event, making up 16 of the 20 cases recorded. Death, a transfusion associated circulatory overload (TACO) and an acute haemolytic transfusion reaction were the other SAEs observed. One patient who was very anxious and hyper-ventilating but without any pathology on examination could not be categorised.

Table 5.11: Signs and symptoms experienced post transfusion

Symptom present	Yes N (%)
Fever	70 (16.2)
Restlessness	26 (6)
Rigors/chills	52 (12)
Cyanosis	1 (0.2)
Chest pain	2 (0.5)
Loin/Back pain	7 (1.6)
Palpitations	39 (9)
Unexplained bleeding	2 (0.5)
Wheezes/stridor	3 (0.7)
Urticaria	6 (1.4)
Anxiety	35 (8.1)
Headache	24 (5.6)
Pruritus	50 (11.6)
Dyspnoea	11 (2.5)
Rash	9 (2.1)
Dark urine	10 (2.3)
Respiratory distress	4 (0.9)

5.4 Discussion

My study has found that recipients of blood transfusions who live in malaria endemic regions are at risk of TTM but that the incidence of transmission, even among particularly vulnerable patients, was not high. Only one out of fifty

patients (2%) who received blood which was positive for malaria by PCR contracted malaria from the blood transfusion. The other 49 patients who received a PCR malaria-positive transfusion had either no molecular evidence of infection (43 patients) or the malaria parasite detected post-transfusion was not the same as in the transfused blood (6 patients).

There have been almost no studies documenting the incidence of post-transfusion malaria in endemic areas and none that have used genotyping to confirm parasite transmission. In Sudan, all 12 patients who received malaria infected blood developed microscopically confirmed malaria (101) compared to only 12.5% (2/16) in this study. Malaria parasitaemia has been documented post transfusion in recipients who received microscopy-negative blood although the incidence in the 14 days following transfusion was higher in this study (4%) compared with the Sudanese study (0.52%). A study investigating TTM has recently been carried out in KATH, where this study was also performed. A follow up of transfusion recipients 3 months after their transfusion showed a 14-28% incidence of TTM (personal communication with Prof Allain). The differences between the incidences detected by the two studies may be due to the comparatively large sample volume (0.5mls of cellular fraction) from which DNA was extracted compared to the blood spots used in this study.

Refrigeration of blood at 4°C results in a 7.1 fold reduction in *P. falciparum* parasitaemia after 14 days storage (107). In many countries in Africa blood shortages are so acute that donated blood is used very rapidly. In our study blood was stored for a median duration of two days before being transfused. This is insufficient time to destroy the malaria parasites and so refrigeration is unlikely to account for the low incidence of TTM observed.

All transfusion recipients were carefully monitored for signs of malaria after their transfusion. For ethical reasons they were treated promptly if malaria parasites were detected and this may explain why no recipient developed symptoms of malaria. Clinicians who were responsible for the patients in our study treated 19% of transfusion recipients for malaria post-transfusion although only a quarter of these patients had proven parasitaemia. WHO

recommends that malaria treatment should only be prescribed for confirmed infections (383) whereas some national blood transfusion policies indicate that transfusion recipients in endemic regions should receive malaria treatment routinely (59). Although we specifically selected recipients who would be at high risk of TTM, malaria transmission occurred infrequently suggesting that routine prophylaxis for all recipients of blood transfusion is probably unnecessary in this group of patients.

Depending on the number of parasites inoculated, the symptoms of malaria may develop days to weeks after transfusion (183). The prompt treatment initiated after parasitaemia was found during the active follow up of patients did not allow parasitaemia to multiply to show any effects and may be the reason for the low number of clinical post transfusion malaria and no clinical TTM cases.

Screening for parasitaemia in blood donors has inherent difficulties. Healthy donors who are resident in malaria endemic regions may generally harbour low levels of parasitaemia, which will not be detected by tests such as microscopy and RDT but will still be a high enough in a unit of blood. The limits of sensitivity of microscopy and RDT are 10-100 parasites per microlitre (139,162,407) which would be equivalent to 5-50 x 10^6 parasites per unit of transfused blood. For a test to be useful at ruling out a disease, it must have a high sensitivity and for it to be useful in confirming a disease, it must have a high specificity (408). In this study, specificity of microscopy and PCR was very high (100%) indicating that these two methods may be good confirmatory tests. PCR was also the most sensitive test. Further, the sensitivity of PCR can be increased by using larger volumes of blood for testing (409). PCR is not a practical method for routine use where resources are scarce and the relationship between PCR positivity and infectivity is not clear. It is capital intensive, requires well trained staff and may not be affordable in many blood banks or hospital centres where transfusion is done. PCR also requires electricity and this may not be available in many rural areas. Even in cities, electricity supply is irregular. PCR as a screening test in malaria endemic countries comes with another major challenge of seriously

compromising blood supply. Furthermore, a test which is unnecessarily sensitive may result in inappropriately high donor rejection rates which will exacerbate blood shortages and increase mortality. For a similar reason of being unable to reject all positive blood donors, blood centres in Francophone African countries, were all not screening blood for *Plasmodium* (410). Based on results from this study, 18% of blood would have been discarded for being parasitaemic. Previously published evidence suggest that up to 50% of donors harbour parasitaemia and could be rejected (104).

The absence of an ideal gold standard in diagnostic or screening studies is not peculiar to malaria but occurs in most clinical conditions in medicine (411). Various ways have been suggested to overcome this deficiency including the composite reference method which was used in this study to assess the accuracy of the test methods for malaria parasitaemia. A major difficulty in the composite reference standard approach is whether the combination of reference test results (in this case PCR and microscopy) results in a 'true positive'. It however continues to be one of the most used ways of constructing gold standard tests in different fields (412,413).

There is presently no standardized approach to genotyping as this depends on the transmission patterns and multiplicity of infections but by increasing the resolution of the genotyping method and the number of loci assessed, discriminatory power may be increased adequately (414). In an effort to harmonise processes, there is recommended genotyping procedures initiated by two organisations Medicines for Malaria (MMV) Venture and the WHO (172). This recommended genotyping is what was used in this study. *Msp1, msp2 and* glutamate-rich protein (*glurp)* are the most commonly used molecular markers for *P. falciparum* (415) and we used the *msp2* locus as it is the marker with the most discriminatory power (416-418). The most commonly used method of genotyping involves amplification of specific alleles of msp1 and msp2genes using nested PCR and gel electrophoresis (414,415). When used on agarose, the interpretation of results may vary but for my study this was averted by using 10% polyacrylamide gel and digitalised automation which gives better

resolution. Other more sensitive methods for genotyping such as the microsatellitism, isoenzyme electrophoresis, and single nucleotide polymorphism exist but they are more expensive.

5.4.1 Study limitation

A limitation of this study may be the inability to detect low level *P. falciparum* clones in the transfused blood. Semi-immune adults living in malaria endemic sub-Saharan Africa can have low-level infections with up to 10 genetically distinct *P. falciparum* variants or clones (419) simultaneously and nested PCR methods may not be sensitive enough to detect low abundance variants, especially if mixed infections are present(416,420).

The study was unable to follow the original study design and enrol the proposed sample size of 600. Age and sex matched exposures may have given a more robust outcome with few confounders compared to the current study design. Again the bigger sample size may have given better results and eliminated the negative confidence interval obtained for the incidence of TTM.

There was a high dropout rate for a variety of reasons and this contributed to reduce the number of recipients who were evaluated. A further limitation of this study is the small number of children recruited which related to the high number of children already receiving malaria treatment. The study was therefore unable to evaluate TTM in relation to children, who are an important high risk group.

5.5 Summary

TTM occurred in only 2% of recipients of *P. falciparum* PCR positive blood. TTM also did not occur in majority of recipients of both *P. falciparum* PCR positive and microscopy positive blood. The widespread practice of treating transfusion recipients presumptively for malaria, and the assumption that transfused blood is the source of malaria occurring post-transfusion, are based on misperceptions

about the true burden of TTM. There is also insufficient evidence to support policies that advocate screening donated blood for malaria. Major gaps exist in knowledge concerning the risks of TTM and its clinical impact in semi-immune populations, and there are no screening tools for malaria which are practical, affordable and suitably sensitive for use by blood banks in Africa. The prevalence of malaria in blood donors is variable. By EIA, prevalence was 22.2% but can reach 50% in some parts of West Africa. Implementation of any policy which advocates rejection of all such donors will have a major impact on the availability of blood for transfusion and undoubtedly increase mortality particularly among pregnant women and children.

CHAPTER 6

BACTERIAL CONTAMINATION AND TRANSFUSION RELATED SEPSIS

6.1 Introduction

Transfusion of a bacterially contaminated blood product can have serious consequences including death and serious morbidity in transfusion recipients (421). The greatest risk of transfusion- transmitted infection in the UK currently is bacterial contamination. Between 1996 and 2009, 40 (60%) of 66 reported incidents of transfusion-transmitted infections were due to bacteria compared to 17 (25%) that were due to viruses including HBV, HCV, HEV, HIV and human T-cell lymphotrophic virus (HTLV). During the same period, transfusion-transmitted bacteria caused 73.3% (11/15) of deaths due to transfusion-transmitted infections (SHOT report). Despite this, blood safety has focussed mostly on viral infections resulting in a remarkable reduction of transmission of these viruses (215).

There is little information concerning bacterial contamination in sub-Saharan Africa. Evidence has only become available in recent years that bacterial contamination occurs in the region, with estimates ranging from 8.8% to 17.5% (36,37,205). Hassall et al showed that the frequency of bacterial contamination of whole blood transfused to children in Kenya is 2500 times greater than that found in red blood cells in industrialised countries (205). The effects of such bacterial contamination on the recipients of transfusion are unclear as there have been no published studies in sub-Saharan Africa. Bacterial contamination of whole blood may be a significant but unrecognised hazard of transfusion in Africa.

This chapter describes a study that investigated the prevalence of bacterial contamination of donated blood in Kumasi and the clinical effects it may have on recipients of these units of blood.

6.2 Materials and methods

Study site

This was a prospective study conducted at the Komfo Anokye Teaching Hospital (KATH) in Kumasi Ghana. The background information for KATH has been provided in Chapter 1.

Eligibility Criteria

All transfusion recipients in the hospital were eligible for enrolment into the study if they fulfilled the following inclusion and exclusion criteria.

Inclusion criteria: Patients from the departments of Paediatrics, Obstetrics and Gynaecology, Medicine and Oncology; who were to receive blood transfusions were eligible for recruitment into the study.

Exclusion criteria

- Any patients with an altered state of consciousness such that they would be unable to identify and report any symptoms that develop during the transfusion. This included:
    1. Patients in shock
    2. Patients who were confused,
    3. Comatose patients
- Any patients that received a transfusion in theatre or who was being sent to the theatre. Such patients will not have the chance to be monitored regularly by the study team or have been able to give an informed consent.

Study enrolment process

Informed consent: Patients due to be transfused were identified by their request forms which had been sent to the blood bank and were evaluated by the eligibility criteria. If they qualified for enrolment, the details of the study

were explained to either the patient or his/her relative before a signed or thumb printed informed consent was obtained.

Enrolment: Recruitment into the study was by convenience sampling. Enrolled patients were given unique sequential study numbers until the proposed sample size of 200 patients had been attained. The study number was subsequently used as the identifier for all study purposes. To ensure reliable follow ups, the patient's home address and telephone numbers were obtained so they could be called back for follow up in case the blood they received yielded a positive culture result.

The medical history and clinical examination findings were recorded on a study pro-forma prior to the patient receiving the blood transfusion. The patient's vital signs including blood pressure, temperature, pulse rate and respiratory rate measurement were monitored and documented 30 minutes after the start of the transfusion and then hourly until the transfusion was complete. Patients were reviewed immediately after the completion of the transfusion, 24 hours after the transfusion and each day until they were discharged from hospital. A temperature greater than 37.5°C was classified as fever and when it occurred within 24 hours after transfusion, it was called post transfusion fever.

Patient follow-up

Patients who received blood that was found to be contaminated with bacteria were followed up. Those who had been discharged were recalled and those still in hospital were seen in their ward. They were clinically examined and blood samples were taken for bacterial culture, irrespective of whether they looked well or unwell, or had been treated with antimicrobials.

The follow up schedule involved a mandatory follow up when the culture results of the donated blood unit was ready. A second follow up was planned for patients who were unwell or who had a positive culture result from their follow up sample.

Classification and diagnosis

A unit of blood was said to be contaminated when culture of a sample from that blood unit yielded a positive isolate. At the end of the study all recipients of contaminated blood were classified into one of four case definitions.

The classification for transfusion-transmitted bacterial infections that I used for this study was adapted from the standard definitions from the Canadian Public Health Agency's Guidelines for Investigation of Suspected Transfusion-Transmitted Bacterial Contamination (210).

These guidelines were proposed for investigating transfusion reactions and therefore made provision in the classification for not performing a blood culture. However because our study was prospective and all blood cultures were to be done, the criteria was modified to eliminate those uncertainties. A summary of the classification of case definitions are shown in table 6.1

Treatment

This was an observational study so the decision concerning whether and when initiate treatment was entirely the prerogative of the patient's primary physician. The study team provided the physicians with all preliminary and final laboratory results, in addition to any other findings made at follow up as soon as it became available. A patient who was unwell at review was immediately referred to their physician for evaluation and management after blood culture was taken.

Table 6.1: Classification of transfusion-transmitted bacterial infection cases used in the study

Case description	Criterion used
Definite transfusion-transmitted bacterial infection	• Same bacteria found in the transfused blood and the recipient • Contamination of blood sample or laboratory contamination is not suspected.
Probable transfusion-transmitted bacterial infection	• Positive culture from transfused blood • Contamination of blood sample or laboratory contamination is not suspected. • Developed signs/symptoms during or within 24 hrs of transfusion • Recipients blood culture is negative (recipient taken/taking antibiotics)
Possible transfusion-transmitted bacterial infection	• Positive culture from transfused blood • Contamination of blood sample or laboratory contamination is not suspected. • Presence of signs/symptoms prior to transfusion • Worsening or persisting symptoms • Recipients blood culture is negative (recipient taken/taking antibiotics)
Unlikely transfusion-transmitted bacterial infection	• Positive culture from transfused blood • No signs/symptoms during or after transfusion • Contamination of blood sample or laboratory contamination suspected

Sample collection and processing

Two types of blood samples were obtained for bacterial culture; blood from the donated unit of blood that was transfused and blood from patients who had received a 'contaminated' blood transfusion.

Sample collection from donated blood

To obtain samples from the donated unit of blood that was being transfused, the transfusion was stopped almost at the end, when at least five mls of blood remained in the bag. The blood was collected aseptically by using a sterile syringe to draw five mls of blood through the port that had been cleaned with 70% isopropyl alcohol. The blood was immediately inoculated into 20 mls of brain heart infusion (BHI) broth.

Sample collection from patients

The antecubital fossa of the patients was vigorously cleaned with 70% isopropyl alcohol starting from the centre and cleaning in wider circles. This was done for 30 seconds and the alcohol allowed to dry before 5 mls of venous blood was drawn, using a sterile needle and syringe. The blood was immediately inoculated into 20 mls of BHI and sent to the microbiology laboratory. The needle used for phlebotomy was changed before inoculation into the blood culture broth.

Sample processing

The BHI without sodium polyanetholsulfonate (Oxoid Ltd, Basingstoke, Hampshire, UK) when sent to the laboratory was immediately incubated aerobically for up to 7 days at 37°C. Anaerobic cultures were not performed.

The blood culture broth was inspected daily for turbidity as a sign of bacterial growth. Sub-cultures of the broth were performed onto 5% human blood agar and MacConkey agar (Oxoid Ltd, Basingstoke, Hampshire, UK) plates if the broth was turbid, and blindly on days 2 and 7. The plates were incubated aerobically at 37 °C for between 18-24 hrs. Colonies that grew on the plates were identified by colonial morphology, Gram stain reaction and standard biochemical and sugar fermentation tests. Gram negative organisms were further identified by Mini API rapid ID 32E (BioMeriuex) test strips. Serology was not performed to confirm the *Shigella* spp.

Figure 6.1: The microbiology laboratory of KATH where the study was conducted

Figure 6.2: Samples being processed in the microbiology laboratory

Susceptibility testing

The antimicrobial susceptibility patterns of *Pseudomonas* spp, *P. aeruginosa*, *Alcaligenes* spp, *Shigella* spp and *Citrobacter freundii,* were tested using the disc diffusion method [CLSI method used]. Single antibiotic impregnated discs from Oxoid were tested on Muller-Hinton agar including the following antimicrobials: gentamicin (10 µg), amikacin (30 µg), ceftazidime (30 µg), co-trimoxazole (25 µg), co-amoxyclav (30 µg), chloramphenicol (30 µg), cefuroxime (30 µg), ceftriaxone (30 µg), and ciprofloxacin (50 µg).

6.2.1 Quality assurance and control

Sterility testing was done on the batches of BHI broths, and agar plates used for culture to ensure that no contamination had taken place. For the quality control in antimicrobial susceptibility testing, control strain *E. coli* ATCC strains -25922 was tested against the antimicrobials. The bacteriology laboratory participates in the World Health Organisation's External Quality Assurance System for Antimicrobial Susceptibility Testing (EQAS-AST). This proficiency testing has been ongoing for nearly a decade and the laboratory has been recording very good results.

As a further part of quality control, sterility testing was also performed on blood bags that had not been used to collect blood. The citrate was aspirated and cultured to ensure that there was no prior contamination of the blood bags. These cultures did not isolate any bacteria.

Data management

A Microsoft Access based electronic database was used to record data generated in this study. The data was cleaned on an ongoing basis and exported into PASW Statistics v18.0 package (IBM, Chicago, IL, USA) for statistical analysis. Descriptive statistics, including measures of central tendency, dispersion, cross tabulation and histogram were used where appropriate. Odds ratios and the 95% confidence intervals were calculated as a

measure of association between the donor and recipients characteristics and risk of giving or receiving contaminated blood units.

6.3 Results

Recipient characteristics

Two hundred recipients of blood transfusion were enrolled in the study. The majority (42%) were from the Department of Medicine and Oncology. There were slightly more females (51.5%) in the study because all patients from the department of O&G were females (Table 6.2).

Table 6.2: Characteristics of transfusion recipients

Recipient characteristic	
Total number of recipients	200
Female recipients (%)	103 (51.5)
Median age (IQR) years	25.0 (4.2 – 39.0)
Department of recipient (%)	
Medicine and Oncology	84 (42)
Paediatrics	74 (37)
Obstetrics and Gynaecology	42 (21)
Median length of stay in hospital (IQR) days	6.5 (4.0- 13.0)
Antimicrobial use during admission (%)	129 (64.5)
Mortality in hospital (%)	21 (10.5)

64.5% of recipients received antimicrobials during their admission. A total of 21 recipients died giving a mortality rate of 10.5%. None of the deaths were considered to result directly from the blood transfusion (Table 6.2). The patients who died included six from paediatrics, all acute infections (3 cases of severe malaria, 2 cases of sepsis *E. coli* and *Klebsiella* spp and a case of meningitis), one death from O&G due to cervical cancer and 14 from Medicine and Oncology (8 malignancies, 4 chronic kidney and liver diseases, and 1 case each of atypical pneumonia and hypertensive heart disease).

Donated blood units

The characteristics of the donated units are in Table 6.3. Voluntary donors accounted for 71.5% of blood used for transfusion in the study. The mean duration of transfusion was 2.3 hours. A bacterium was isolated from 23 of the 200 transfused units of blood, a contamination rate of 11.5% (95% CI 7.0-16.0%).

Table 6.3: Characteristics of blood from 200 donors

Donor and blood unit characteristics	
Voluntary donors (%)	143 (71.5)
Male donors (%)	132 (66)
Median duration of storage of blood (IQR) days	3.0 (1.0-5.0)
Mean duration of blood transfusion ± SD (hours)	2.30 ± 1.5
Positive culture from blood (%)	23 (11.5)

Contamination rates in blood units sent to different departments

Almost half of the contaminated blood units were sent to the Paediatrics department (Figure 6.3). 30% and 22% of isolates were from blood units sent to Medicine and Oncology, and O&G departments respectively. The departments of Medicine and Oncology received the lowest number of contaminated units while Paediatrics had the highest number.

Bacterial isolates

The organisms isolated are shown in figure 6.4. Gram positive organisms including *Bacillus* species and coagulase negative Staphylococcus (CNS) accounted for the majority (70%) of organisms and CNS was the most common isolate. Gram negative organisms made up 30% and included *Pseudomonas aeruginosa*, *Pseudomonas* spp, *Shigella* spp, *Alcaligenes* spp. and *Citrobacter freundii*.

Figure 6.3: Bacterial contamination rates within departments

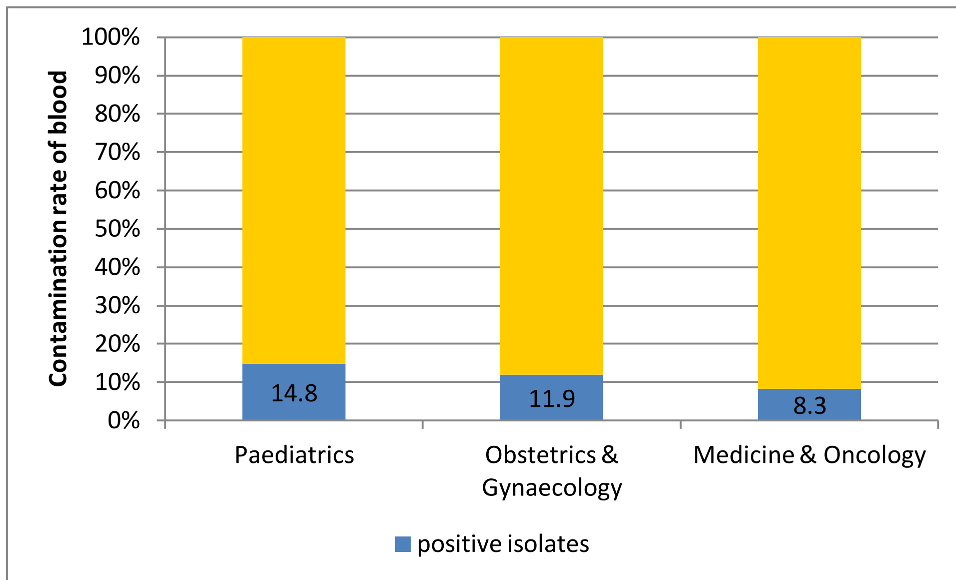
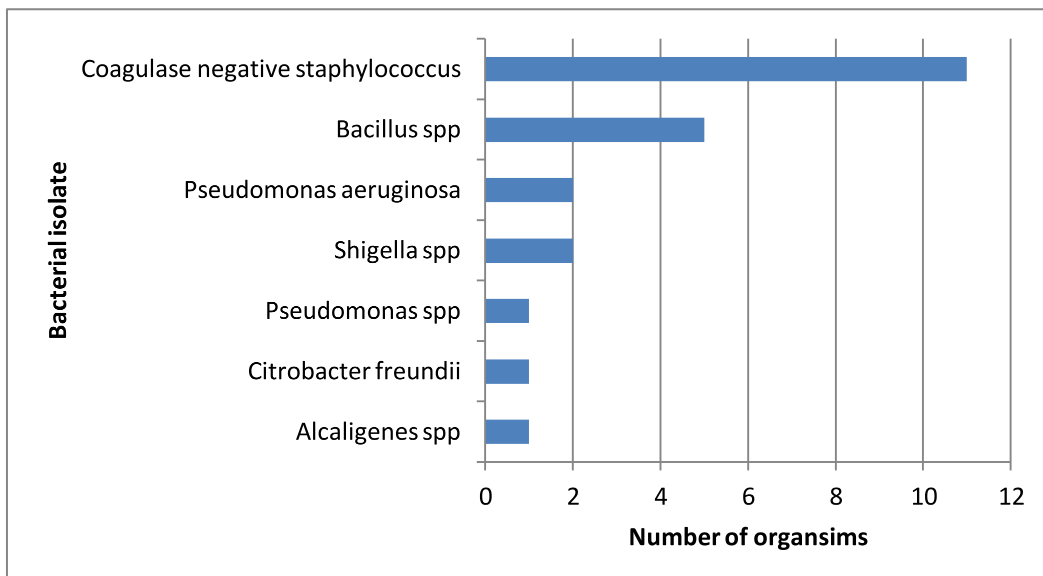


Figure 6.4: Bacterial isolates cultured from donated blood



Susceptibility of bacteria isolates

The susceptibility patterns of the Gram negative organisms are shown in table 6.4. All of the three *Pseudomonas* spp. identified were susceptible to amikacin and ceftazidime but two of out of the three were resistant to gentamicin. *Citrobacter* was sensitive to all antimicrobials tested. *Alcaligenes* was sensitive

to gentamicin, ceftriaxone and ciprofloxacin but resistant to chloramphenicol and amikacin. Both *Shigella* identified were sensitive to co-amoxyclav, and ceftriaxone (Table 6.4).

Table 6.4: Sensitivity pattern of Gram negative bacterial contaminants

Antimicrobial	Susceptibility n (%)			
	Pseudomonas (N=3)	*Shigella* spp (N=2)	*Citrobacter* (N=1)	*Alcaligenes* spp (N=1)
Co-amoxyclav	-	2/2 (100%)	1/1 (100%)	-
Co-trimoxazole	-	1/2 (50%)	1/1 (100%)	-
Chloramphenicol	-	1/2 (50%)	-	0/1
Gentamicin	1/3 (33.3%)	2/2 (100%)	1/1 (100%)	1/1 (100%)
Amikacin	3/3 (100%)	-	-	0/1
Ceftazidime	3/3 (100%)	-	1/1 (100%)	-
Ceftriaxone	-	2/2 (100%)	-	1/1 (100%)
Cefuroxime	-	1/2 (50%)	1/1 (100%)	-
Ciprofloxacin	-	1/2 (50%)	1/1 (100%)	1/1 (100%)

Associations of the donor with contaminated units

Donor characteristics: Blood stored for up to 10 days was less likely to be contaminated than that stored for a greater number of days (OR 0.30, 95%CI 0.10 -0.87). The gender of donor was not significantly associated with a contaminated unit (Table 6.5). Similarly there was not a statistically significant difference in contamination rates for the type of donor but the difference was large (6.5% in replacement donors compared to 14% in voluntary donors; p =0.21).

Associations of the recipient with receiving a contaminated unit

Fever in recipients: There were 50 (25%) recipients of transfusion who were febrile at pre-transfusion assessment and 22 (11%) of recipients who were still febrile 24 hours post-transfusion (Figure 6.5). The number of patients who were not febrile at pre-transfusion but became febrile during or after the

transfusion ranged from 30 (15%) at 30 minutes post transfusion to 9 (4.5%) at 24 hours post transfusion.

There were 6/23 (26%) recipients who received a contaminated unit and became febrile after the start of transfusion compared with 24/177 (13%) patients who received an uncontaminated unit and became febrile after the start of the transfusion (OR 2.250, 95% CI 0.6921-3.9754) (Table 6.6). There was no association between receiving a contaminated unit of blood and developing fever even after adjusting for antimicrobial use (OR 1.635, 95% CI 0.623 – 4.291).

Figure 6.5: Post transfusion fever in transfusion recipients

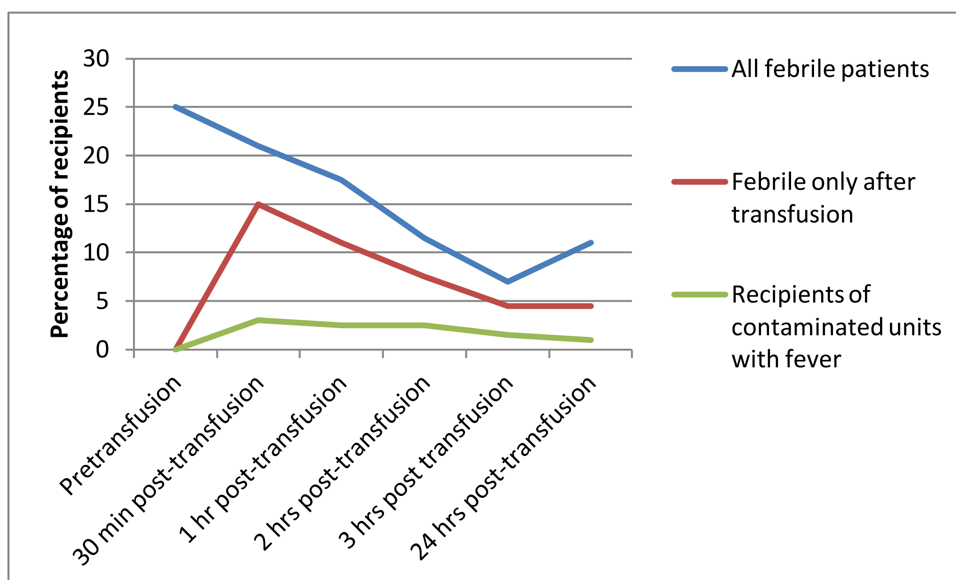


Table 6.5: Risk determination in the blood donors for contaminated blood units

Donor characteristic		Contaminated units N (%)	Non-contaminated units N (%)	P Value	Odds ratio (95% CI)
Sex of Donors	Male	15 (65.2)	117 (70.5)	0.606	0.785 (0.289 – 2.178)
	Female	8 (34.8)	49 (29.5)		
Type of Donor	Replacement	3 (13.0)	43 (25.9)	0.207*	0.429 (0.096 - 1.633)
	Voluntary	20 (87.0)	123 (74.1)		
Storage duration	0-10 days	17 (73.9)	150 (90.4)	0.021	0.302 (0.094 - 1.000)
	>10 days	6 (26.1)	16 (9.6)		

*P value using Fisher's Exact Test; CI = Confidence Interval

Table 6.6: Risk association for contaminated blood units in the transfusion recipients

Recipient characteristics		Contaminated units (n=23)	Non-contaminated units (n=177)	P value	Odds ratio (95% CI)
Department	Paediatrics	11 (48.0)	63 (35.6)	0.260	1.659 (0.637 – 4.310)
	other	12 (52.0)	114 (64.4)		
Fever	Yes	6 (26.1)	24 (13.6)	0.113	2.250 (0.710 - 6.883)
	No	17 (73.9)	153 (86.4)		
Chills	Yes	3 (13.1)	13 (7.3)	0.343	1.892 (0.390 – 8.037)
	No	20 (86.9)	164 (92.7)		
Antibiotic use	Yes	17 (73.9)	118 (66.7)	0.485	1.417 (0.491 – 4.265)
	No	6 (26.1)	59 (33.3)		
Prolonged admission	Yes	9 (39.1)	48 (27.1)	0.230	1.728 (0.640 – 4.608)
	No	14 (60.9)	129 (72.9)		
Outcome	Died	2 (8.7)	19 (10.7)	1.000*	0.792 (0.118 – 3.927)
	Alive	21 (91.3)	158 (89.3)		

*P value using Fisher's Exact Test; CI = Confidence Interval

Other signs and symptoms

Recipients of contaminated blood did not exhibit many signs and symptoms besides fever. None of the patients developed post transfusion, hypotension or bradycardia. One recipient experienced post transfusion tachycardia. Two patients complained of restlessness and three developed chills post transfusion.

Antimicrobial use

Among the recipients who received contaminated blood, 17/23 (74%) were treated with antimicrobials compared with 118/177 (66.7%) who received uncontaminated blood (Table 6.6). There was no association between being given antimicrobials and receiving a contaminated blood unit. The commonly given empirical antimicrobials were gentamicin, chloramphenicol, flucloxacillin and cefuroxime in paediatrics and metronidazole in combination with cefuroxime or co-amoxyclav in O&G. In the Department of Medicine, ceftriaxone and metronidazole were commonly used but a wide range of other antimicrobials were also prescribed, depending on the condition being treated. The presence of fever at pre-transfusion or the development of fever post transfusion was not significantly influenced by the use of antimicrobials (Table 6.7).

Table 6.7: Determination of the effects of antibiotic usage on the presence of fever in transfusion recipients

	Antibiotic use		
FEVER	Yes (%)	No (%)	P-value
Pre-transfusion fever (yes)	42 (31.1)	20 (30.8)	0.961
Fever at 30 min post transfusion (yes)	31 (26.5)	11 (19.3)	0.297
Immediate post-transfusion fever (yes)	32 (24.1)	11 (17.2)	0.274
Fever 24 hours after transfusion (yes)	20 (15.4)	5 (8.9)	0.236

Other recipient characteristics

There were no association between risk of receiving a contaminated unit of blood and death as a study outcome, prolonged admission and receiving blood in the paediatrics department (Table 6.6).

Patient follow- up [Table 6.8, 6.9, 6.10]

Twenty one of the 23 patients who received a contaminated blood unit were evaluated within seven days. Two could not be followed up; one child had died from *Klebsiella* spp. sepsis, which was diagnosed pre-transfusion and an adult died from liver cirrhosis.

There were nine patients still in hospital at the time of follow up. Seven of them had received or were still receiving an appropriate antimicrobial. For two patients their physicians decided on additional antimicrobials because of the contaminated blood they had received. One patient was being treated for puerperal sepsis and anaemia and the obstetricians added ciprofloxacin to cefuroxime and metronidazole when *Alcaligenes* spp was isolated from the transfused unit of blood. A patient with HIV/TB received additional co-trimoxazole when *Shigella* spp was isolated from the transfused blood.

Twelve patients had already been discharged home but were recalled. All patients were clinically well with no signs of ongoing sepsis. However, one patient, who received blood contaminated with *Pseudomonas aeruginosa*, required readmission for anaemia and was transfused again. He did not show any signs of sepsis and the paediatricians decided against treatment with antimicrobials.

Transfusion-transmitted bacterial infections

The blood cultures taken at follow-up visits did not yield bacteria including those identified from the transfused unit of blood. None of the recipients was therefore classified as a case of definite transfusion-transmitted bacterial infection.

There were 26 % (6/23) probable (Table 6.8) and 21% (5/23) possible cases (Table 6.9) of transfusion-transmitted bacteria resulting from 23 donated blood units contaminated with bacteria. The remaining 53% (12/23) of patients were considered unlikely to have had a transfusion-transmitted bacterial infection (Table 6.10).

Table 6.8: Cases of probable transfusion-transmitted bacterial infection cases identified in the study

Age	Diagnosis	Pre-transfusion fever	Post-transfusion fever	Post-transfusion chills	Post-transfusion restlessness	Antimicrobials received in hospital	Isolate from donated blood	Outcome at Discharge	Transfusion-transmitted bacterial infection (TTI)
25	Antepartum haemorrhage	No	No	Yes	No	Co-amoxyclav, Metronidazole	*Bacillus* spp	Alive	Probable TTI
48	Hepatic failure	No	Yes	No	No	Ceftriaxone, Metronidazole	*Citrobacter freundii*	Alive	Probable TTI
3	Severe malaria	No	Yes	No	No	Co-amoxyclav	CNS	Alive	Probable TTI
1	Klebsiella sepsis	No	Yes	No	No	Gentamicin, Cefuroxime	CNS	Dead	Probable TTI
2	Severe malarial anaemia	No	Yes	No	No	Gentamicin, Chloramphenicol	*Pseudomonas aeruginosa*	Alive	Probable TTI
34	Post Caesarean section	No	Yes	No	No	Co-amoxyclav, Metronidazole	*Pseudomonas aeruginosa*	Alive	Probable TTI

Table 6.9: Cases of possible transfusion-transmitted bacterial infections identified in the study

Age	Diagnosis	Pre-transfusion fever	Post-transfusion fever	Post-transfusion chills	Post-transfusion restlessness	Antimicrobials received in hospital	Isolate from donated blood	Outcome at Discharge	Transfusion-transmitted bacterial infection (TTI)
30	Puerperal sepsis	Yes	Yes	Yes	Yes	Cefuroxime. Metronidazole Ciprofloxacin[1]	*Alcaligenes* spp	Alive	Possible TTI
2	Severe malarial anaemia	Yes	Yes	Yes	Yes	Flucloxacillin Chloramphenicol	*Bacillus* spp	Alive	Possible TTI
2	Staph aureus sepsis	Yes	Yes	No	No	Flucloxacillin Chloramphenicol Gentamicin	CNS	Alive	Possible TTI
30	Liver cirrhosis with sepsis	Yes	Yes	No	No	Ceftriaxone	CNS	Dead	Possible TTI
25	G6PD full defect with pancytopenia	Yes	Yes	No	No	Metronidazole Flucloxacillin Co-amoxyclav	CNS	Alive	Possible TTI

[1] Antimicrobial added following receipt of the blood unit bacterial culture result

Table 6.10: Cases of unlikely transfusion-transmitted bacterial infections

Age	Diagnosis	Pre-transfusion fever	Post-transfusion fever	Post-transfusion chills	Antimicrobials received in hospital	Isolate from donated blood	Outcome at Discharge	Transfusion-transmitted bacterial infection (TTI)
43	HIV with TB	Yes	Yes	No	Anti-TB medications, Co-trimoxazole[1]	*Shigella* spp	Alive	Unlikely TTI
43	Liver cirrhosis	No	No	No	Metronidazole	*Bacillus* spp	Alive	Unlikely TTI
34	Chronic anaemia	No	No	No	-	*Bacillus* spp	Alive	Unlikely TTI
24	Severe anaemia 2IVH	No	No	No	Cefuroxime, Co-amoxyclav, Metronidazole	*Bacillus* spp	Alive	Unlikely TTI
12	Sickle Cell anaemia	No	No	No	Cefuroxime	CNS	Alive	Unlikely TTI
3	severe malarial anaemia	No	No	No	-	CNS	Alive	Unlikely TTI
27	HIV with *E.coli* sepsis	No	No	No	Co-trimoxazole, Metronidazole, Ciprofloxacin	CNS	Alive	Unlikely TTI
3	Sickle cell Dx with S. aureus sepsis	No	No	No	Cefuroxime	CNS	Alive	Unlikely TTI
42	Ca Cervix	No	No	No	-	*Pseudomonas* spp	Alive	Unlikely TTI
1	Severe malaria	No	No	No	-	*Shigella* spp	Alive	Unlikely TTI
1	Severe anaemia	No	Yes	No	-	CNS	Alive	Unlikely TTI
2	Severe malaria	Yes	No	No	-	CNS	Alive	Unlikely TTI

All patients did not have post-transfusion restlessness. [1] Antimicrobial added following receipt of the blood unit bacterial culture result

6.4 Discussion

This study has demonstrated a prevalence of 11.5% of bacterial contamination in donated blood. This high prevalence is comparable to earlier studies with estimates of 8.8% and 9% in Kenya and Southern Ghana respectively (36,37). An even higher level of contamination (17.5%) was found in Northern Ghana (36). It has been suggested that prospective bacterial cultures of whole blood or red cell units have a higher incidence of bacterial contamination than there actually is (246) because of the difficulties in maintaining an aseptic environment during sample transfer, leading to false positive test results (223). These consistently high rates of bacterial contamination however cannot be ignored and should be a cause for concern as we seek to improve blood safety in Africa. The study presented in this chapter is unique because in previously reported studies, samples of blood were taken while the blood was in the blood bank. Whereas in this study samples were taken right at the end of transfusion and this may be a truer reflection of what was transfused to the recipient. It is possible that during or after sampling in the blood bank, even more bacteria may be introduced into the blood thereby increasing the patient risk.

This study has demonstrated some clinical effects on recipients. Although no definite case of transfusion-transmitted bacterial infection was identified, 5.5% (11/200) of all transfusion recipients had probable or possible transfusion-transmitted bacterial infection. No comparable studies investigating the clinical effects of bacterial contamination on recipients has been identified in Africa. Even in developed countries with good haemovigilance systems in place, establishing a definite case of transfusion-transmitted bacterial infection has not been easy. In Canada, out of 39 reported septic transfusion reactions over a 4-year period, 20% were classified as definite, 64% as probable and 15% as possible bacterial contamination incidents (422). In the US, Benjamin et al reported that 12/20 (60%) of septic transfusion reactions were judged to be probable because the incriminating organism could not be isolated from the patient's blood; seven of them because antimicrobial therapy had already been instituted (423).

The prescription of antimicrobials was high in the study population. Although there was no statistical difference between antimicrobial usage in recipients of contaminated blood and recipients of non-contaminated blood, more (74%) antimicrobials were prescribed for recipients of contaminated blood. This may be a reason for why bacteria could not be cultured from the recipients. Apart from resulting in a negative blood culture, antimicrobial use can mask overt signs and symptoms of septic transfusion reaction (241). The clinical severity of a transfusion- associated septic reaction can vary considerably (213), depending on a number of factors. The bacterial species implicated, its virulence and the total number of bacteria infused are as important as recipient characteristics such as underlying disease, the status of the immune system, and whether the recipient is receiving concomitant antibiotic therapy (241). None of the patients in our study showed signs of overwhelming sepsis. In those who were not treated with antibiotics and did not show any signs of sepsis, pathogens recovered from their transfused units may have been as a result of contamination during sample collection and transport or contamination during processing of samples.

Fever is one of the signs that were evaluated as a possible indicator of sepsis but there was no significant association established in this study. This is not surprising as there are many other causes of fever. It was seen that fever at all times, including at pre-transfusion, 30 minutes after the start of transfusion, immediate post transfusion and 24 hours after transfusion, was more common in recipients who received antimicrobials than those who did not. This finding may not support the suggestion that antibiotics mask signs of sepsis. However, because most recipients were febrile pre-transfusion and most antimicrobials were also started pre-transfusion, it suggests recipients were prescribed antimicrobials for other co-morbid conditions that were present before the transfusion started.

Some organisms are less virulent than others and therefore may cause less overt sepsis. For example, CNS may not be considered a serious threat in healthy adults with strong immune system. CNS has however been implicated in

a number of deaths and severe transfusion reactions (215,246). In this study the majority (70%) of the organisms were Gram positive bacteria. CNS and *Bacillus* spp are skin commensals that when found in blood may indicate poor skin cleansing techniques before donor blood is obtained. CNS was similarly the major cause of contamination in both previous studies conducted in Ghana (36,37) but not in the study from Kenya (205).

Gram negative bacteria may generally be more virulent and can quickly produce septic shock through the effects of endotoxin. Gram negative bacteria such as *Yersinia spp* and *Pseudomonas spp* can also survive in temperatures of 2-8°C and cause bacterial contamination and sepsis even when blood is stored under refrigerated conditions (239). *Pseudomonas* may be transiently present on skin and inadequate skin cleansing of donors can result in the presence of *Pseudomonas* in the donated blood. *Pseudomonas* was the commonest Gram negative bacteria in this study and has been reported in previous studies in Africa (37,205).

Shigella spp was an unexpected finding as a blood contaminant as it is not a usual pathogen isolated from blood. It also is able to survive under refrigeration conditions. The clinical details from the 2 donated units and the 2 recipients were reviewed but their transfusions were 2 weeks apart and they were from different departments. The antimicrobial susceptibility patterns were also not the same and therefore it was unlikely to have been the same *Shigella* from both samples. Unfortunately it was not possible to confirm the *Shigella* species because of lack of antisera for the required agglutination tests. *Shigella* is commonly spread by the feacal-oral route so breaches in aseptic technique while collecting the blood could have resulted in the contamination by *Shigella.*

The contamination of red cell products may be directly related to its storage time. For example, most cases of *Yersinia* contamination occur in blood bags older than 25 days (239) and the majority of septic transfusion reactions associated with contaminated RBCs usually occur with units that have been stored for more than 21 days (424). In this study, blood stored for more than 10 days was significantly more likely to be contaminated. Other published studies

from Africa do not establish an association of contamination with duration of storage. The 10 day risk of contamination compared to the 21 days found in developed countries may be due to unreliable refrigeration conditions which have been suggested as a factor contributing to the hazard of bacterial contamination (205). Monitoring of the fridges was not performed during the study.

The observation of higher contamination rates in donor blood from voluntary donors compared to replacement donors may be due to different skin cleansing practices under different conditions. Blood from replacement donors are obtained in the hospital while voluntary blood is donated at mobile sessions including school outreach programmes and FM radio station blood drives. For these mobile sessions which are outside the hospital, there is the likelihood that skin cleansing procedures may not be done appropriately. This may be due to staff not spending the adequate time to clean the skin because of the many donors waiting to give blood or the absence of clean running water for proper hand washing. Such conditions may increase the risk of contamination compared to conditions which may exist in a hospital setting which has been created for blood collection. It may be helpful if larger studies are conducted to compare contamination rates.

Bacterial contamination may result from exogenous sources such as from the skin of donors, and manipulation of blood units into smaller volumes. Blood units sent to the Department of Paediatrics had the highest contamination rates although the likelihood of a paediatric unit being contaminated was not significantly higher than blood sent to the other departments. Blood prepared for the paediatrics department was usually less than 500 mls and therefore the original donated unit had to be breached to take out the required volume. This process of manipulation, especially if done without full aseptic conditions, may account for the increased pathogen isolation. Leaky seals, damaged tubing, or micro-punctures in collection bags have been linked to episodes of bacterial sepsis (237). Contamination of blood transfusion bags has also been reported (228). Further, heavy contamination of the exterior of blood packs with *Serratia*

marcescens has lead to septicaemia in recipients following transfusions with red cells or platelets (425). Inadequate skin cleansing is one of the commonest causes of bacterial contamination. 70% povidine iodine has been recommended worldwide but at KATH alcohol is still being used for the skin preparation. Sterilization of skin surfaces has limits when the phlebotomy needle cores out a skin plug as it passes through the skin. This inherently non-sterile skin plug can be carried into and seed the collected blood. The majority of organisms present in both culture studies and case reports of platelet-associated sepsis is usually normal skin flora as it may be virtually impossible to decontaminate human skin. Blood culture results show an incidence of positive cultures, ranging from 2% to 6% after the cleansing of the skin (227).

Contamination can also be from an endogenous source in which a donor may be having a transient bacteraemia or a subclinical infection. Donor bacteraemia is not easily identified but it may be reduced by an efficient donor screening programme. Screening will however not be able to identify donors with transient bacteraemia. Individuals infected with *Yersinia enterocolitica* are typically asymptomatic at the time of blood donation (259,426,427).

Risk reduction strategies include careful selection of blood donors, appropriate attention to skin preparation and to the detection of bacteria in samples of blood prior to transfusion (241). Sample diversion, where the first 20 -40 mls of blood collected are directed away from the blood bag is another method that reduces bacterial contamination. Studies have shown that either alone or in combination, improved donor arm disinfection and sample diversion leads to a 40-88% reduction of bacterial contamination of donated blood (231,264,265).

6.4.1 Study limitations

Follow up in this study was limited to patients who received blood that yielded bacterial isolates from the laboratory. However, a look back investigation of patients who developed signs and symptoms suggestive of transfusion associated bacterial infection may possibly have identified some false negative culture results. Such an investigation simulates bedside scenarios where

patients who develop transfusion associated bacterial infections are retrospectively diagnosed by culturing the donated blood.

Agar prepared with human blood is associated with poor bacterial isolation rates. Human blood agar has been shown to be suboptimal for the growth and susceptibility testing of some organisms including *Streptococcus pneumoniae, Streptococcus pyogenes and Staphylococcus aureus* (428). The use of human blood agar may have resulted in the recovery of fewer organisms than there actually was.

The inability to recover organisms from the recipients of contaminated blood raises questions of whether all organisms isolated from the culture of transfused blood was actually transfused into the patient.

Anaerobic cultures were not done. It is therefore possible that anaerobes, such as *Clostridium perfringes* which has been implicated in transfusion sepsis was missed in this study.

6.5 Summary

Bacterial contamination occurred in 11.5% of donated blood units. Not all recipients of contaminated blood units developed clinical sepsis or bacteraemia. 'Probable transfusion-transmitted bacterial infection' occurred in 26% of recipients of contaminated units and 21.7% of recipients had 'possible transfusion-transmitted bacterial infection'. The lack of 'definite transfusion-transmitted bacterial infection' or the apparent lack of clinical effects of septicaemia in recipients of contaminated blood may be due to the high rate of antimicrobial usage among recipients of blood transfusion or false positive cultures.

Bacterial contamination can occur at different points including collection of blood from donor, processing and storage of blood, sample collection and laboratory processing. To reduce the risk of transfusion recipients receiving blood contaminated by bacterial we recommend a multi-step approach to eliminate possible sources of contamination. A carefully directed donor

questionnaire should include a recent history of tooth extraction, sigmoidoscopy or minor medical procedures which can cause transient bacteraemia. This can be used to identify and defer such donors to eliminate endogenous sources of bacterial contamination. Strict adherence to proven skin cleansing procedures before phlebotomy, and a sample diversion strategy in which the first 20 mls of drawn blood is discarded, can eliminate exogenous causes of bacterial contamination. Furthermore, the use of paediatric packs if available, although expensive, will remove the need for manipulations that breech the integrity of donated blood units to produce the smaller volumes required for paediatrics.

CHAPTER 7

TRANSFUSION-TRANSMITTED SYPHILIS

7.1 Introduction

Syphilis is a public health problem with serious consequences if not prevented or treated (279). Worldwide 12 million people are infected with syphilis each year and more than 90% of infections occur in low and middle-income countries (429). An estimated 2 million pregnant women are infected with syphilis, resulting in the birth of at least 500,000 babies with congenital syphilis (430). Congenital infection may cause a further half a million annual abortions, still births and other cases of peri-natal deaths each year (431). The burden of congenital syphilis is concentrated in Africa where it has not received serious attention, hence its description as the 'forgotten priority' (432).

To prevent the transmission of syphilis by transfusion, screening of all donated blood donations is recommended (59). Though a legal requirement in most countries (378), screening remains a challenge in developing countries. Twenty percent of donated blood in sub-Saharan Africa is transfused without screening for any of the major transfusion transmissible infections (433) and in Ghana, only 20% of donated blood is screened for syphilis (23).

The burden of syphilis attributable to transfusion-transmitted syphilis is not known but pregnant women and children, who are the major recipients of blood transfusion in sub-Saharan Africa, are particularly at risk of contracting transfusion-transmitted syphilis. Routine screening of blood donors, a decrease in the prevalence of syphilis, and the refrigeration of the donated blood prior to its use (66,297) may be the reasons why no transfusion-transmitted syphilis has been reported over the past few decades. There has recently been a resurgence of syphilis cases across Europe and North America (286,434,435). This increase indicates that risky sexual behaviours are increasing in the blood donor population and therefore a continuous vigilance is required in blood services (295).

This chapter deals with transfusion-transmitted syphilis and describes a study carried out to establish the prevalence of syphilis in the donor population in Kumasi, and to determine if sero-conversion had taken place in transfusion recipients.

7.2 Materials and methods

Participants and recruitment

Participants were recruited between November and December 2009 from the Departments of Paediatrics, Obstetrics and Gynaecology, Medicine and Oncology of the Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana. The blood bank in KATH provides blood and blood products for all transfusions in the hospital. Blood donors are currently screened for HIV, hepatitis B and hepatitis C infections but not for syphilis. Only donors who are negative by these screening tests are allowed to donate blood.

Eligibility

Inclusion criteria: All recipients of blood transfusion in the three departments (Paediatrics, Obstetrics and Gynaecology, Medicine and Oncology) were eligible for recruitment into the study if they gave their written informed consent.

Exclusion criteria:

i) Patients who were to be transfused in the operating theatres, where the transfusion could not be monitored.

ii) Any patient in shock, unconscious or with an altered mental status which prevented them from being able to identify any symptoms experienced during transfusion.

iii) All patients receiving their transfusion before 8am and after 6pm. This was because they could not be monitored by the study team.

Recruitment into study

Participants were given consecutive and unique study identification numbers upon recruitment into the study. Prior to transfusion, a complete medical history was obtained and a clinical examination was performed and documented. The patient's diagnoses was also retrieved from their case notes and recorded. Plasma from paired blood samples, namely a pre-transfusion sample from the patient and a sample from the unit of transfused blood were obtained and stored in a -20°C freezer for later testing in batches. Socio-demographic data of the blood donors and the duration of storage of the transfused blood units were retrieved from the blood bank and recorded in the patients case report form.

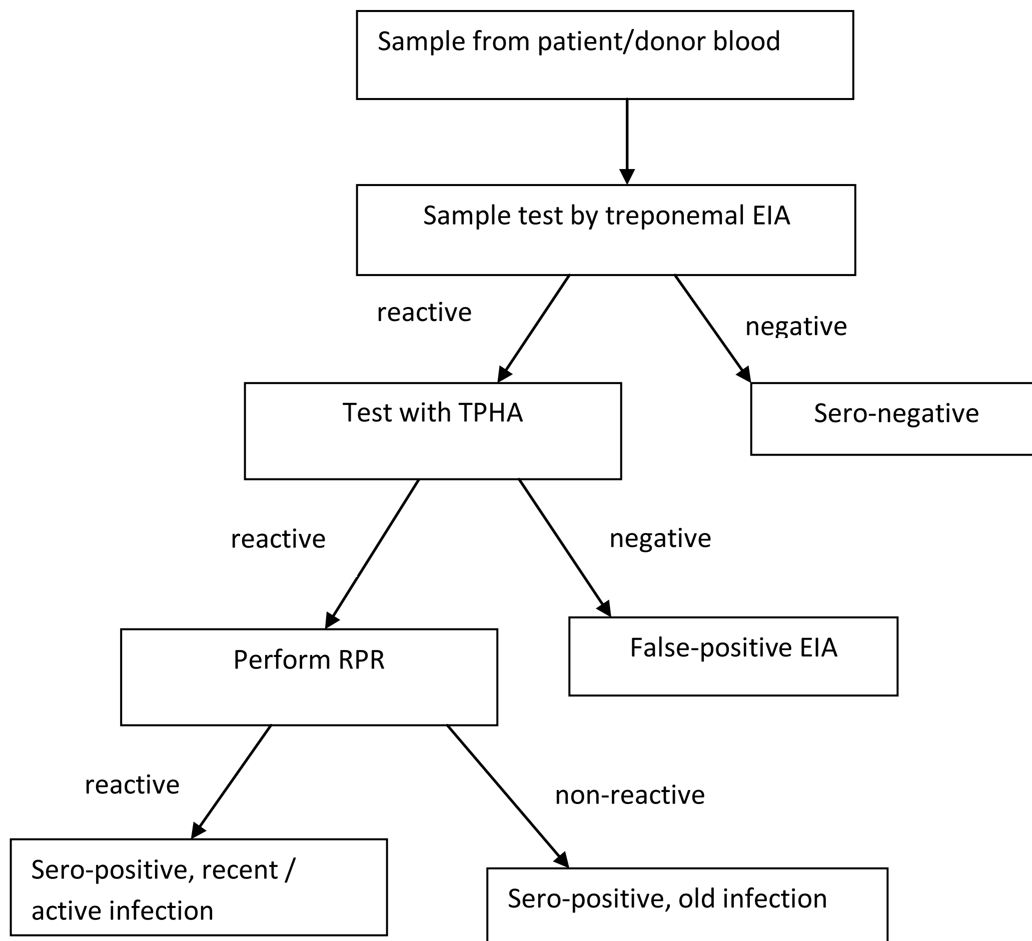
Patient monitoring and follow up

Patients were monitored while receiving their transfusion by hourly measurement and documentation of their vital signs, including the temperature, the pulse rate, respiratory rate and blood pressure measurements. Immediately after the transfusion patients were asked for any symptoms they experienced during the transfusion. When testing had been carried out on donor blood and patient samples, sero-negative recipients of sero-positive blood were recalled for a clinical re-evaluation and re-testing to determine if sero conversion had occurred. The recalled patients were also questioned for fever or skin lesions on symptoms to determine if they had developed any symptoms after the transfusion. Recipients, whose pre-transfusion samples were sero-positive, were not evaluated for sero-conversion. All blood donors and transfusion recipients who were found to have recent/active syphilis were recalled and if they attended were offered counselling and treatment of syphilis in accordance with national guidelines.

Classification of donated blood and recipients

Based on the EIA and TPHA results, blood donors and recipients were retrospectively classified as sero-negative or sero-positive. A sero-positive result was defined as a reactive result in both the EIA and TPHA assays and sero-negativity was defined as a negative result in the EIA assay or a reactive EIA result but negative TPHA result. Reactivity to the RPR test in sero-positive patients/donors was consistent with recent/active infection and those with non-reactive plasma were classified as old syphilis infections (Figure 7.1).

Figure 7.1: Screening algorithm used for testing for syphilis



Sample collection and processing

Two mls of the donor blood was obtained from the blood bag at the end of the transfusion when the blood bag was clipped off. The sample was taken at the end of the transfusion to ensure that the integrity of the unit of blood was maintained and avoid potential iatrogenic contamination during sampling. At pre-transfusion and at follow up, a minimum of one ml of blood each was drawn from the patients. Samples were collected in clearly labelled EDTA tubes and centrifuged. Plasma was aliquoted from both patient and donor blood into labelled corresponding Eppendorf tubes, and stored in a -20°C freezer.

Sample testing

Three tests for syphilis were performed in the following order; enzyme immunoassay (EIA), treponemal hemagglutination assay (TPHA) and carbon agglutination. Bioelisa Syphilis 3.0, (Biokit, S.A. Barcelona-Spain), a third generation immunoenzymatic assay was the test kit used for the EIA. Syphagen TPHA (Biokit, S.A. Barcelona-Spain) and Biokit RPR Reditest (Biokit, S.A. Barcelona, Spain) were the test kits used for TPHA and RPR assays respectively. These tests have been used in previously reported studies (299,436-438). All assays were performed according to the manufacturer's instructions. Sample testing was done as indicated in figure 7.1. All samples reactive to the EIA assay were confirmed with TPHA. If reactive to TPHA, sample testing was repeated with both EIA and TPHA. Samples that were not reactive to EIA did not have a TPHA assay or RPR performed on it. EIA/TPHA positive samples were tested with RPR and the RPR was repeated the sample was if reactive.

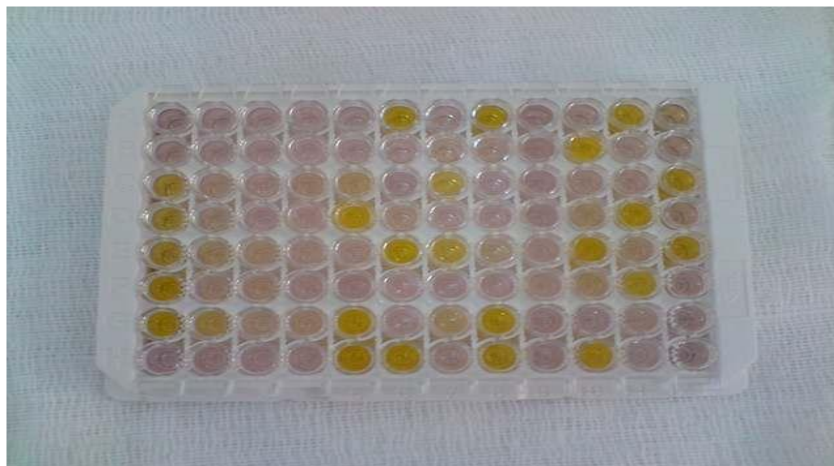
Enzyme linked immunosorbent assay

<u>Principle</u>: Bioelisa Syphilis 3.0, used for the initial screening of all samples, detects IgG and IgM antibodies to *T. pallidum* by a sandwich ELISA capture process. The wells of the microplate provided are coated with p15, p17 and p47 *T pallidum* recombinant antigens which capture the corresponding antibodies. The recombinants antigens which have been conjugated to enzyme peroxidise

are added to bind the captured antibodies. This complex turns blue when an enzyme substrate and chromogen is added, indicating the presence of *T. Pallidum* antibodies.

Figure 7.2: The ELISA reader and washer used for the study

Figure 7.3: An ELISA plate with the yellow wells indicative of a positive test result



Procedure: After bringing all materials to room temperature, 50 µl of each patient sample was added to 50 µl of sample diluent in individual microplate wells. Two negative controls, 3 low positive controls and 2 high positive controls were included in each batch of samples tested. The microplate was covered,

mixed gently and incubated for an hour at 37°C. The contents of the wells were aspirated and plates washed four times using an ELISA washer (BIORAD PW 40 Microplate Washer) (Figure 7.2) after which 100 µl of diluted conjugate was added to individual wells except that for the blank. Washing was repeated after incubating the plate at room temperature for 30 minutes.

A 100 µl freshly prepared substrate-chromogen was added into each well with the solution turning blue if the sample contained anti-*T. pallidum* antibodies. This was incubated at room temperature for 30 minutes and 100 µl of stop solution was added, turning the blue solution into yellow. The absorbance was read at 450 nm by means of a microplate photometer (Thermo Scientific Multiskan EX).

A mean absorbance calculated from the 3 low positive controls was used as the cut-off value in each ELISA assay. A reactive sample was one with an absorbance higher than the determined cut-off value.

Treponema Pallidum Hemagglutination Assay

Principle: The *Treponema pallidum* hemagglutination assay (TPHA, Biokit Syphagen TPHA), used in this study is an indirect hemagglutination test for the detection of specific antibodies to *T pallidum*. Chicken erythrocytes sensitised with an antigenic extract of *T. Pallidum* (Nichols strain) will agglutinate with specific antibodies present in the serum or plasma of syphilitic patients

Procedure: Test samples, round bottom microtitre plates and reagents (antigen, control, diluents, positive and negative controls) were brought to room temperature by allowing them to sit on the work bench. Each sample used four wells, the first two of which were for diluting samples and the other two wells were the control and test wells. Using the diluents, the samples in wells three and four were made up to a 1:20 dilution. These wells had 75 µl of control and antigen reagents added separately to the wells, to bring the final dilution to 1:80 (Table 7.1).

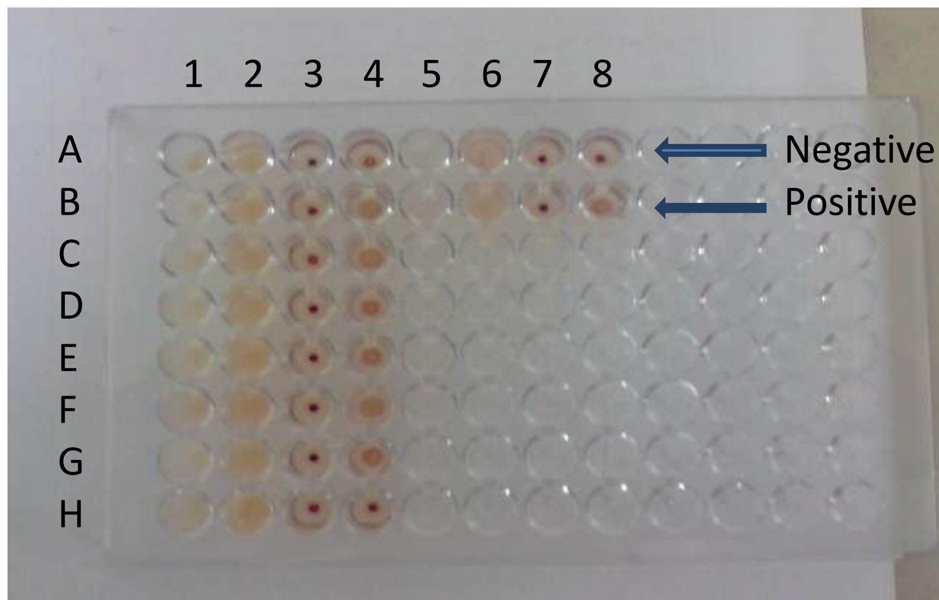
Table 7.1: Summary of steps used for confirming syphilis by the test method of Treponema pallidum hemagglutination assay

Well number	1	2	3 Control	4 Test
Diluent solution µl	25	100	25	25
Sample µl	25			
Min and transfer µl	25	25	25	25 25
Sample dilution	1:2	1:10	1:20	1:20
Control reagent µl	-	-	75	-
Antigen reagent µl	-	-	-	75
Final dilution			1:80	1:80
Incubate for 60 minutes at room temperature				

The contents were mixed by slightly tapping on the sides of the plate. The plate was then covered and incubated for an hour at room temperature. Positive and negative controls were included for each batch of samples tested. The results were then read qualitatively.

A positive result was declared when a smooth mat of agglutinated cells covered the entire bottom of the well (4+), or a smooth mat partially covered the bottom of the well (3+), or a smooth mat of agglutinated cells surrounded by a ring of cells (2+), or a smooth mat of agglutinated cells surrounded by a heavy ring of cells (1+). A button of cells with or without a very small hole in the centre implies a negative result (figure 7.4).

Figure 7.4: Picture of a TPHA test performed on EIA positive samples



In this picture, plate A represents a partially used micro titre plate. Different samples are tested in rows A to H but column 1 to 4 have the same sample. Columns 5 to 8 are negative (row A) and positive controls (row B).

Rapid Plasma Reagin test

Principle: Biokit RPR Reditest is a macroscopic non-treponemal test. The RPR antigen is a cardiolipin suspension containing charcoal micro particles which detects IgG and IgM antilipid antibodies traditionally called 'reagin' that are present in the plasma of syphilitic patients and also in some acute or chronic diseases.

Procedure: The antigen containing vial, the samples to be tested and the controls were left on the bench to allow them to attain room temperature. A drop of the sample was placed and spread in each circle on the test card provided. The antigen vial was gently shaken and a drop added without mixing. The card was placed on a mechanical rotator for 8 minutes at 100rpm after which the test reaction is read macroscopically as the card is tilted from side to side. Positive and negative controls were included with each run. A sample was said to be reactive to RPR when the characteristic black clumping of charcoal particles was observed.

7.3 Results

Recipient characteristics

The 200 recipients of blood transfusions recruited into the study had a median age of 28 years. The majority of recipients (45.5%) were from the Departments of Medicine and Oncology (Table 7.2). The mortality in this group of transfusion recipients was 10.5%.

Causes of admissions

The diagnoses of transfusion recipients recorded in their case notes are summarized in table 7.3. The majority (45.5%) of the transfusions in paediatrics were for malarial anaemia but in medicine/oncology chronic organ (kidney and liver) diseases and gastrointestinal bleeding was more common. HIV and tuberculosis was more frequently seen in recipients from medicine/oncology than in O&G and paediatrics where no HIV was diagnosed. The most frequent reason (62.7%) for transfusions in the department of O&G was haemorrhage (Table 7.3).

Table 7.2: Characteristics of transfusion recipients

Total number of recipients	200
Number of male recipients (%)	98 (49.0)
Median age (IQR, range) years	28 (8–39, 1-86)
Department of recipient (%)	
Medicine and Oncology	91 (45.5)
Paediatrics	63 (31.5)
Obstetrics and Gynaecology	46 (23)
Antimicrobial use during admission (%)	131 (65.5)
Mortality in hospital (%)	23 (10.5)
EIA reactive	38 (19.0)
EIA and TPHA reactive prior to transfusion (%)	26 (13.0)
RPR reactive (% RPR reactive/TPHA positive)	9/26 (34.6)

IQR = inter quartile range, EIA = enzyme immunoassay, TPHA = *Treponema pallidum* hemagglutination assay and RPR = rapid plasma reagin.

Sero-prevalence in recipients

Twenty six transfusion recipients were confirmed sero-positive for syphilis by EIA and TPHA prior to transfusion, giving a syphilis infection sero-prevalence of 13.0% (95% CI 8.3-17.7%) (Table 7.2). Nine of these (34.6%) were RPR reactive, giving a prevalence of recent/active infections of 4.5% (95% CI 1.7- 7.3%). Most of the EIA/TPHA positive patients were in the Department of Medicine and Oncology (84.6%), with 11.5% in Obstetrics and Gynaecology and 3.8% in Paediatrics.

While in hospital, 65.5% of recipients received concomitant antimicrobial therapy in hospital for their underlying conditions.

Table 7.3: Diagnoses of recipients of blood transfusion in the study

Medicine/oncology	Number (%)	Paediatrics	Number (%)	O & G	Number (%)
Chronic organ disease	20 (21.9)	All malaria	30 (45.5)	Haemorrhage	32 (62.7)
GI bleeding	19 (20.9)	Sickle Cell disease	10 (15.1)	All malignancies	11 (21.6)
Other anaemia's	15 (16.5)	Sepsis/pneumonia/ meningitis	8 (12.1)	Sepsis/pyelonephritis	3 (5.9)
HIV/Tuberculosis	13 (14.3)	All malignancies	7 (10.6)	HIV/Tuberculosis	3 (5.9)
All malignancies	11 (12.1)	Malnutrition	4 (6.1)	Other anaemia's	2 (3.9)
Sickle cell disease	5 (5.5)	Other anaemia's	4 (6.1)		
Septicaemia	4 (4.4)	Chronic organ disease	2 (3.0)		
***HMS / Hepatosplenomegaly**	4 (4.4)	GI Bleeding	1 (1.5)		
		HIV/Tuberculosis	0		
Total	**91 (100)**	**Total**	**66 (100)**	**Total**	**51 (100)**

*HMS represents Hyper-reactive Malarial Splenomegaly; O & G represents Obstetrics and Gynaecology

Donor blood characteristics

Voluntary donors contributed 68% of the 200 blood units transfused to recipients in this study. The majority (72.5%) of donors were males (Table 7.4). All the 200 donated units of blood were kept refrigerated until they were issued for use but 56.8% of the transfused blood was kept refrigerated for less than 4 days.

Table 7.4: Characteristics of units of donated blood

Total number of transfusion units	200
Donated blood from male donors (%)	145 (72.5)
Donated blood from voluntary donors (%)	136 (68.0)
Median duration of storage (IQR, range) days	3.0 (1-5, 1-21)
Number of blood units kept for ≤3 days (%)	109 (56.8)
EIA reactive	24 (12.0)
EIA and TPHA reactive (%)	16 (8.0)
RPR reactive (% RPR reactive/TPHA positive)	7/16 (43.8)

IQR = inter quartile range, EIA = enzyme immunoassay, TPHA = *Treponema pallidum* hemagglutination assay and RPR = rapid plasma reagin.

Syphilis sero-prevalence in donor blood

Sixteen of the 200 donated units of blood were sero-positive by the EIA and TPHA assays, giving a syphilis sero-positivity of 8% (95% CI 4.3-11.7%) in donated blood units (Table 7.4). In seven (43.8%) of the sero-positive samples the RPR test was positive, giving a 3.5% (95% CI 1.0-6.0%) prevalence of recent/active syphilis infection in the donors. There was no significant difference in the proportion of samples positive by EIA/TPHA between the recipients and donors (p=0.14) or the proportion of samples positive by EI/TPHA and RPR between the two groups (p=0.80)

Follow-up findings

There were 16 recipients of sero-positive blood (Table 7.5). Eight were not followed up for the following reasons: four of them had died during the hospital admission; two were sero-positive at pre-transfusion; and two were lost to follow up. Eight recipients of sero-positive blood were followed up and had a repeat syphilis screen at 30 days post-transfusion. Six of the eight were EIA negative and 2 were reactive. One of the 2 reactive recipients (recipient ID 13) was a false positive as sample was not confirmed by TPHA testing (Table 4). The other positive sample (patient ID10) was confirmed by TPHA and also reactive in the RPR test.

Nine out of the 16 recipients (56.3%) were prescribed antibiotics during hospitalisation. In various combinations, metronidazole and cefuroxime were given to four patients, amoxycillin-clavulanic acid given to three patients and ciprofloxacin, gentamicin and ceftriaxone were given to 2 recipients. All nine of them received an antibiotic closely related to the penicillin's, which is the recommended treatment for syphilis. These were amoxicillin, cefuroxime and ceftriaxone.

Recipient with sero-conversion

The recipient (ID 10) identified as having sero-converted was an 8 year old girl who had been admitted for suspected severe malarial anaemia. She was transfused because her initial packed cell volume was 12% and she had persistent intra-vascular haemolysis with 'coca-cola urine'. She developed post transfusion fever but both blood culture and peripheral blood film for malaria parasites were negative. She was treated with cefuroxime and gentamicin and discharged after a week. At her follow up visit on day 36, there was no fever or relevant sexual history and she had felt completely well for the period since her discharge from hospital. Clinical examination did not reveal any muco-cutaneous lesion or lymphadenopathy.

Table 7.5: Characteristics of 16 recipients of syphilis sero-positive blood transfusions

	RPR results for transfused blood	Duration of storage	Results for pre-transfusion testing			30 day follow up visit?	Recipient post –transfusion testing			Sero conversion	Comment
	RPR	Days	EIA	TPHA	RPR	Yes/No	EIA	TPHA	RPR	Yes/No	
1	Reactive	12	Neg	ND	ND	No					Died
2	Non-reactive	2	Neg	ND	ND	No					Died
3	Non-reactive	2	Neg	ND	ND	No					Died
4	Non-reactive	1	Neg	ND	ND	No					Died
5	React	4	Neg	ND	ND	No					LFU
6	Non-reactive	1	Neg	ND	ND	No					LFU
7	Non-reactive	2	Pos	Pos	Non-reactive	No					PPT
8	Non-reactive	6	Pos	Pos	Reactive	No					PPT
9	Non-reactive	3	Neg	ND	ND	Yes	Neg	ND	ND	No	
10	Reactive	1	Neg	ND	ND	Yes	Pos	Pos	Reactive	Yes	
11	Non-reactive	2	Neg	ND	ND	Yes	Neg	ND	ND	No	
12	Reactive	1	Neg	ND	ND	Yes	Neg	ND	ND	No	
13	Reactive	3	Neg	ND	ND	Yes	Pos	Neg	Non-reactive	No	
14	Non-reactive	2	Neg	ND	ND	Yes	Neg	ND	ND	No	
15	Reactive	1	Neg	ND	ND	Yes	Neg	ND	ND	No	
16	Reactive	4	Neg	ND	ND	Yes	Neg	ND	ND	No	

EIA = Enzyme immunoassay; TPHA = *Treponema pallidum* hemagglutination assay; RPR = rapid plasma regain; LFU = Lost to follow up; PPT = Positive at Pre-transfusion; ND= Not done, Pos = positive; Neg = Negative; + = positive seroprevalence; - = negative seroprevalence

The child was referred to the paediatricians after confirmation of her sero-positive status and was treated with a course of penicillin. The source of the positive blood was a male voluntary donor and the blood was kept refrigerated for only one day before being issued for use.

7.4 Discussion

The positive syphilis sero-conversion in an initially sero-negative child is most probably due to transfusion-transmitted syphilis and will be the first report in over three decades (439). The last case reported in medical literature was in 1983 (309). Other treponemal infections such as yaws cannot be differentiated serologically from syphilis. Since yaws is not endemic in Kumasi and because there was no clinical evidence, yaws is unlikely to be the cause of the sero-conversion. Furthermore, the 8 year old girl was not sexually active and therefore not likely to have contacted the infection sexually. The most likely cause of the sero-conversion in this patient was the serologically positive blood she received by transfusion.

The 8% prevalence of sero-positivity to syphilis in donated blood found in this study is consistent with previously published rates of 7.5% to 13.5% among donors in Ghana (38,39), although the type of donors were different. More than two-thirds of the donors in this study were voluntary donors (68%) compared to the study by Ampofo et al where 96.3% were replacement donors (39).

The sero-reactivity prevalence rates of 7.5-13.5% in Ghana published in the peer-reviewed literature are at variance with sero-reactivity rates in donor blood reported by the national transfusion service of Ghana of 0.75% - 2.14% (23). This disparity may be related to the different methods of testing used. The tests used for screening in the blood service are the non treponemal tests such as RPR and Venereal Diseases Research Laboratory (VDRL) which detect only recent infections. For the published studies *Treponema pallidum* EIA or particle agglutination assays which were used have a better sensitivity (346) and in addition to recent infections can detect old infections,

The difference in sero-positivity prevalence among donors (8%; 95% CI 4.3-11.7%) and recipients (13%; 95% CI 8.3-17.7%) was not significant. The HIV positive donors and donors with other high risk factors have been eliminated by the screening process prior to the syphilis testing and may account for the low prevalence seen in donors compared to the recipients in this study that include HIV positive patients who may have a higher risk for syphilis. Also pregnancy and malignancies are known to be associated with false positive results (344). These may account for the difference in prevalence between the donors (that did not include pregnant women, people with malignancies or HIV) and the recipients.

The risk of acquiring transfusion-transmitted syphilis in Ghana is not known. A high syphilis sero-prevalence among donors may increase the risk posed to the recipients. However, factors that may reduce the risk include refrigeration of blood, antimicrobial use, passive surveillance and an efficient donor deferral system (356).

More than half (56.8%) of the donated blood used in this study was stored for 3 or less days, and this is insufficient time to kill *T. pallidum* (366). The current average blood storage duration in Ghana does not provide an adequate margin of safety against transfusion-transmitted syphilis. This is the situation that prevails across many blood banks in sub-Saharan Africa where replacement donation is the major source of blood and where, because of inadequate supply, blood is used as soon as it becomes available (440). In Kumasi, refrigeration is not one of the factors that reduce the risk of transfusion-transmitted syphilis.

The use of antimicrobials has been one of the reasons proposed for the decline of TTS (321) because *T pallidum* remains very susceptible to penicillin. Many recipients of blood transfusion in Africa are sick enough to require antimicrobials. This is likely to kill any bacteria that may have been transfused thereby preventing syphilis in recipients. In my study, 56.3% of the recipients who received EIA/TPHA sero-positive blood were prescribed antibiotics. At least one of the antibiotics received by each recipient is closely related to the recommended drug (penicillin) for *T. pallidum*. These were cefuroxime, ceftriaxone and amoxicillin-clavulanic acid. Amoxicillin and cefuroxime are not routinely used to treat syphilis but ceftriaxone is a recommended alternative for penicillin. Besides the recipient who sero-

converted, there were two other recipients who received blood from donors who were sero-positive with a recent infection. All three donations were stored for only one day and the three recipients had concomitant antibiotics but one recipient sero-converted (Table 7.5). The recipient who sero-converted was treated with cefuroxime and gentamicin while the other 2 received cefuroxime only and amoxycillin-clavulanic acid and metronidazole respectively. Since the true presence of *T. pallidum* could not be ascertained, the efficacy of the antimicrobials used cannot be established. It is possible that *T. pallidum* would be killed if it were present in the transfused blood. All the antimicrobials given were for treatment of other co-existing conditions and not to treat suspected syphilis.

It has been shown that relatively few blood donors with infectious syphilis report relevant risk factors for syphilis and donor questionnaires did not promptly defer these donors (278). This may be particularly relevant for replacement donors as such donors may not volunteer a history of risky behaviour because of their concern that their relative may not get the required blood. Under these circumstances, screening for syphilis cannot be compromised.

There is a shortfall of blood supply in sub-Saharan Africa with an average annual blood collection rate of 5.14 units per 100 populations (17). The consequences of such shortages can be severe as seen in a systematic review which identified that 26% of maternal deaths was due to lack of blood (12) Such consequences could be further worsened if 8% of blood is to be discarded due to syphilis sero-positivity. What is needed is a way of identifying the true infectious units of blood thereby saving and using blood from treated donors who may be sero-positive but not infectious.

7.4.1 Study limitations

1. It would have been ideal to titrate the positive TPHA and RPR results. A rising titre would indicate the presence of an acute or ongoing infection. There was however insufficient plasma to carry out these added tests.

2. The serological tests used for the diagnosis of syphilis are indirect methods and they do not tell us whether the positive blood is infectious or not. The existence of cross reactivity to other treponemes suggests that there is the possibility that not all serologically positive samples are cases of syphilis.

7.5 Summary

There is a sero-positivity prevalence of 8.0% in blood donors in Kumasi, Ghana. This high syphilis prevalence and the identification of syphilis sero-conversion in a child indicate that transfusion-transmitted syphilis is still a problem in Kumasi, Ghana. Transfusion recipients are therefore at risk in centres where donated blood is not screened for syphilis. Because blood is not stored for prolonged refrigeration periods, there is an added risk of transfusion-transmitted syphilis. Urgent steps should be initiated to establish syphilis screening in Kumasi and other centres where screening is not done and to achieve a 100% syphilis screening of all donated blood at all blood centres. A follow up study to determine cost effectiveness of a screening algorithm is recommended.

CHAPTER 8

GENERAL DISCUSSION

Introduction

In this thesis, I have presented a series of studies that investigated the prevalence, and effects of *P. falciparum* malaria, bacterial contamination and syphilis on transfusion recipients. In chapters four, five, six and seven, results and a detailed discussion of the results of the specific disease areas were presented. In this chapter, the results are discussed in the broad context of transfusion medicine and blood safety.

8.1 Donor screening and blood safety

Screening constitutes the mainstay of ensuring the safety of our blood supplies. In developed countries, improved screening methods have drastically reduced transfusion-transmitted viral infections (441). Screening with microscopy or highly sensitive EIA is recommended by WHO to prevent TTM (59). My study has shown that of four methods used for *P. falciparum* detection in donated blood, only PCR had a high sensitivity and can be used as a screening test. The sensitivity of microscopy and EIA was low. The four indices of test validity (sensitivity, specificity, positive predictive value and negative predictive value) were all high for PCR. In addition to PCR, microscopy had a specificity of 100%. Tests with high specificity are good confirmatory tests so microscopy may be used as a confirmatory test in screening blood for *P. falciparum*. In assessing a screening test, the disease under consideration and the proposed policy should be considered in addition to validity of the test (442,443). The disease should constitute a significant public health problem, and should be readily treatable. Concerning policy, the screening programme must be cost effective and facilities for diagnosis and treatment must be readily available. The incidence of TTM in this study was low and does not constitute a major public health problem in these patients. The incidence of TTM should however be compared across regions of different malaria transmissions to

establish whether the burden of TTM is different. Cost effective studies will be needed in specific regions before any particular screening intervention is recommended for routine use. Very few cost effective studies exist in Africa. In the only study retrieved, Rajab et al determined that the cost per case prevented of TTM is higher for recipient anti-malarial prophylaxis than pre-transfusion screening with an automated technique (192).

Routine screening for bacterial contamination is not realistic. Blood culture, which is the gold standard, is expensive, requires expertise and cannot be done in many district and lower level centres where regular electricity is not available. A limitation with blood culture is that it can take up to 7 days for a final result to be declared. Delayed results will not be clinically relevant for the physician or the patient who will have received the blood already. My study has also demonstrated the difficulty of interpreting results from blood culture as the laboratory is unable to determine whether all culture isolates were true contaminants of transfusion or if they were due to poor aseptic techniques in collecting the blood culture. The presence of a bacterial isolate at culture does not necessarily imply the organism was in the donated blood and has been transfused into the transfusion recipient. Further, not all recipients of such 'contaminated blood' develop transfusion reactions associated with these bacteria.

Screening for syphilis presents a different challenge. Whereas screening for malaria and bacteria are not routine, routine screening is recommended for syphilis. An argument against screening has been made in some developed countries where the incidence of syphilis is low, donors repeatedly give blood and the blood is kept refrigerated for prolonged periods that inactivate *T. Pallidum*. These factors, which all significantly reduce transmission risk, were not seen in this study. Rather, my study has shown shorter refrigeration storage conditions and high syphilis prevalence. These conditions may increase the risk of syphilis transmission. In my study only one case of probable transfusion-transmitted syphilis was found. These conditions may give cause for concern in especially in centres not screening for syphilis.

In summary, based on my study findings, routine screening for *Plasmodium* and routine blood cultures to detect bacterial contamination cannot be recommended but syphilis screening, which is already a worldwide recommendation, should be implemented in KATH and all centres not screening for syphilis.

8.2 Donor screening and blood supply

The adequacy of blood supply is a critical issue that faces transfusion services in Africa (13). Bates et al showed that 26% of maternal haemorrhage deaths were due to lack of blood (12). These shortages could be further compromised if screening tests are introduced without adequate evaluation. Using the prevalence found in my study, about 13% (8% for syphilis and 4.7% for *P. falciparum*) of blood units would have been rejected. In previously reported studies across Africa where incidence of syphilis in donors is over 10% and up to 50% for malaria, the consequences on blood supply of rejecting these blood units will be disastrous.

Screening for syphilis presents a dilemma because testing is by indirect means and does not identify infectious samples. Antibodies to *T. Pallidum* remain positive even after treatment at a time when the blood is no longer infectious. Such treated and non infectious donors should be able to donate blood. The use of reactivity to RPR assays in some centres to identify and reject only donors with recent infections helps reduce the rejection rate by half as seen in my study. There is however no evidence that all recent infections result in infectious donors (444). Making donated blood units available that are rejected unnecessarily could save many lives. A test is urgently needed that will identify infectious samples. Until such a test is available, current algorithms will have to be re-evaluated to establish, not only the most cost effective but also the test that yields the lowest rate of false positivity.

8.3 Transfusion monitoring and blood safety

Blood transfusion monitoring is one of the measures which improve blood safety, especially when transfusion-transmitted bacterial sepsis is involved. The onset of signs and symptoms is very rapid for transfusion related bacterial sepsis, usually

within a few hours of starting the transfusion (241). If the transfusion is being monitored, such reactions can be quickly identified and the transfusion stopped. Efficient monitoring which results in an immediate stoppage of the transfusion, investigation and immediate treatment can be life saving. My observation of transfusion practices in KATH as presented in chapter 4 showed that vital signs were often not measured at all or measured only once. Similarly in Uganda, there was no recording for vital signs in 97% of transfusions that took place in a regional referral hospital (445). It is unclear why monitoring of transfusion is not being done in Africa. Haemovigilance is generally lacking in Africa and only few countries such as South Africa and Zimbabwe have developed some systems (200). Transfusion monitoring is an important part of blood transfusion safety and its implementation in Africa should become a priority.

As was expected, immediate transfusion reactions did not have an association with TTM. Even for the non immune transfusion recipients TTM takes several days to weeks for symptoms to develop. Beyond 24 hours, there are many other causes of fever, including malaria not caused by the transfusion. In my study post transfusion malaria (12%) was more common than TTM (2%). Transfusion monitoring does not have a role to play in TTM and neither can it be used to identify transfusion-transmitted syphilis.

8.4 Anti-malarial and antimicrobial usage in transfusion recipients

The use of anti-malarials was high in transfusion recipients especially in paediatrics. In KATH, 77% of children were given anti-malarials with their transfusion. This high use of anti-malarials in paediatrics prevented many children from being enrolled into my study. It was often not clear whether children were being treated for malaria as a possible cause for anaemia or were being given anti-malarials presumptively for TTM. Therefore in relation to TTM in children, it is difficult to make any conclusions. For the recipients who were enrolled into the study, 25% received anti-malarials, but only 12% (11/93) of those prescriptions were based on microscopic confirmation. This observation is intriguing because physicians knew that patients were being actively followed up for malaria and yet still felt inclined to

give anti-malarials when microscopy was negative. This practice is clearly against the WHO treatment guidelines for malaria (383). Two possible reasons for this behaviour may be the recommendation of presumptive treatment for TTM and the old teaching of 'every fever is malaria unless proven otherwise'. The use of anti-malarials must be rational and evidence based to protect the development and spread of resistant strains.

Antimicrobial use was also observed to be very high among transfusion recipients. In all studies presented in this thesis, the rate of use of antimicrobials was consistently between 60 -66%. The concomitant use of antimicrobials results from two reasons; non surgical recipients being sick enough to warrant blood culture investigations and antimicrobial therapy, and the surgical patients being given prophylactic antimicrobials. The high use of antimicrobials has two potentially beneficial effects in the context of blood transfusions. It may prevent the development of a severe septic reaction to bacterially contaminated blood and it could be treatment for syphilis. Antimicrobial use has been one of the reasons given for the low incidence of transfusion-transmitted syphilis (310). Antimicrobials may have some beneficial effects on transfusion recipients but studies are needed to clearly define these benefits including whether they prevent transfusion transmitted syphilis and bacterial infections. But just like anti-malarials, misuse or abuse of antimicrobials could lead to the development of resistant strains of bacteria (446). Antimicrobials should not be used as prophylaxis in transfusion.

The frequent use of anti-malarials and antibiotics is of interest to paediatricians, physicians and public health specialists. This is because malaria and bacterial infections cannot easily be differentiated clinically and therefore doctors may start both anti-malarials and antibiotics until a firm diagnosis is made (394). There is also concern that a dogma that translates as 'anti-malarials for RDT positive, antibiotic for RDT negative' is in the offing (447). A concerted effort from clinicians, microbiologists, public health and transfusion practitioners and policy makers is required to ensure we do not 'shoot ourselves in the foot ' but rather provide guidance in judicious use of antimicrobials and anti-malarials.

8.5 Blood safety and blood storage duration

The duration of storage of refrigerated blood has different effects on TTM, bacterial contamination and syphilis.

The viability of *T. Pallidum* is lost when blood is stored beyond 5 days at 4°C (448). The start of blood banking and the associated extended storage of blood in fridges has contributed to the low levels of transfusion-transmitted syphilis. In many developing countries where blood donation is by replacement donors and the blood collection is hospital based, blood is not usually kept for long periods. There are however some countries such as Kenya where a National Blood Service exists and blood is collected centrally for distribution. In such systems blood is likely to be refrigerated for longer periods. In KATH, where this study took place in Ghana, the median duration of storage is 2 days. To keep blood for more than 5 days will probably require a well thought out written policy which all staff has to be aware off. This will most likely not be feasible unless blood supply is plentiful. This will not come without operational and ethical challenges. In dire obstetric emergencies, staff will grapple with whether to issue blood not stored for stipulated period in order to save a patient versus keeping the blood to kill *T pallidum*. People who donate blood will not wait for several days before their relative receives a transfusion. There are many scenarios that will challenge such a policy especially during 'lean seasons' in blood banks.

Prolonged refrigeration is unattractive when bacterial contamination of donor blood is considered because my study has shown that storage for more than 10 days is associated with increased chance of contamination. Other published studies suggest that the risk of contamination of blood is highest after storage for 14 to 25 days (215,426). Irrespective of the number of days, the evidence suggests that the longer the period of storage of blood, the more likely the risk of bacterial contamination. In 1991, a recommendation to reduce the storage duration of blood from 42 to 25 days generated controversy and was finally rejected because of the potential impact on blood supply. Additionally it was argued reducing the storage duration would only reduce but not eliminate contamination (215). Prolonged

storage will have challenges in settings where a regular supply of electricity is not assured leading to temperature fluctuations in fridges.

It is uncertain whether *P. falciparum* lose viability after 14 days of refrigeration. The blood of the patient who developed TTM in my study had been stored for 2 days.

Prolonged storage of blood as a way of reducing the risk for transfusion-transmitted syphilis has many potential challenges and may not be the best alternative.

8.6 Pathogen reduction and transfusion safety

Pathogen inactivation technologies for treatment of platelet or plasma components based on methylene blue, psoralen and riboflavin technologies are in routine use. Technologies for treatment of whole blood are in development, making pathogen reduction of all blood products using one system achievable (271). Many bacteria, protozoa and viruses are susceptible to pathogen inactivation. The broad applicability of pathogen inactivation therefore holds promise and is seen as an important safeguard against future and emerging infectious risks. Implementation of pathogen inactivation technology could decrease or eliminate the residual risk of infectious complications (449). There are still safety concerns that must be addressed but sufficient benefit and cost-effectiveness of the technology must be established to warrant implementation.

8.7 Policy making in transfusion

As discussed in chapter four, the recommended policies in blood safety for malaria conflict with each other and do not give a clear direction. The WHO recommends screening for malaria in endemic countries and also recommends presumptive treatment (59). This is confusing because it does not state whether malaria positive blood should be discarded or if only malaria negative blood should be transfused and presumptive treatment given. Combining these two recommendations may be expensive but the end result will probably be the same as giving every recipient presumptive treatment.

Also current recommendation for parasitological confirmation before malaria treatment contradicts with the policy of presumptive treatment (59,383). Policies and guidelines should not be ambiguous so that they can be interpreted the same way by everyone. This ambiguity may account for the different transfusion practices within different departments. For example, in paediatrics, anti-malarials are used frequently with transfusion and across all departments anti-malarials are used without parasitological confirmation.

These policies as they exist now need revision and harmonisation. The revision should be based on evidence and any region or country that takes on board a policy should ensure that the evidence used for those policies is relevant to that particular area. For policies in TTM, malaria control programmes and transfusion services will have to review the evidence available to come out with evidence based practices which do not conflict with each other. Guidelines can be developed along standardised formats such as the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. It offers a transparent and structured process for developing and presenting summaries of evidence and is now used widely (450,451).

Summary

Table 8.1 summarises three criteria used to assess whether a screening test should be introduced for transfusion-transmitted syphilis and transfusion-transmitted malaria. Transfusion-transmitted syphilis has been a well known problem and screening is already taking place. A good reason is needed to discontinue a process that has been so effective in making transfusion safe. My study has provided evidence to the contrary and found that syphilis transmission goes on in the absence of screening. In institutions such as KATH, where syphilis screening is not being performed, the implementation of a screening strategy is recommended.

A policy of routine screening for TTM has not been implemented and the burden of TTM has not yet been established. Although my study suggests the incidence of TTM is low, larger studies are needed to establish the magnitude of the problem. PCR may be a good test but it needs to be validated as a screening test. It is currently not affordable and is impractical to implement on a large scale. In this study PCR will reduce blood supply by 18% but on the continent up to 50% of donors are positive. Such a reduction will compromise blood supplies. Cost effectiveness studies have not shown that the benefits of introducing screening outweigh any harm that results from screening. Under the current circumstances, routine screening for malaria parasites is not recommended.

Table 6: Criteria assessed for implementing a screening test

Criteria	**Transfusion-transmitted syphilis**	**Transfusion-transmitted malaria**
The disease: Is the disease a public health problem?	Transfusion transmitted syphilis is a well known public health problem (but possibly on the decline) .	Transfusion-transmitted malaria may not be a huge public health problem
The test: Is it reliable and valid (i.e. sensitivity, specificity, positive and negative predictive values)?	There are recommended screening algorithms which have been validated	There are no valid tests recommended for screening
The policy: Is screening cost-effective? How implementable?	Screening is cost effective and has been accepted worldwide.	Exact policy is unclear (anti-malarial prophylaxis or screening donor blood or both). Cost effectiveness has not been assessed.

CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS

9.1 Conclusion

The aim of my study was to determine the effects of blood transfusions containing malaria or bacteria (including syphilis) on transfusion recipients living in a malaria endemic area. There were four specific objectives to which this study sought answers in order to achieve the study aim. The conclusions of this study are provided according to each objective in this chapter. In addition the implications of this study and recommendations are presented.

Objective 1: To describe the impact of policies relating to malaria transmission and blood transfusion on clinical practice in endemic countries.

Conclusion

Clinical practice differs among hospital departments. Anti-malarial drugs are commonly prescribed routinely with blood transfusions. This contravenes malaria treatment guidelines of laboratory confirmation before treatment but is in accordance with less well-evidenced blood safety guidelines.

Objective 2: To determine the prevalence of malaria parasitaemia and bacterial contamination in donated blood and blood products for transfusion.

Conclusion

The prevalence of *P. falciparum* in donor blood was 4.7% by microscopy, 13.7% by RDT, 18% by PCR and 22.2% by EIA.

The prevalence of syphilis sero-positivity in blood donors was 8% for all infections and 3.5% for recent infections.

The prevalence of bacterial contamination in donated blood units was 11.5%.

Objective 3: To identify any adverse effects on recipients of donated blood/blood products that had malaria parasites or bacterial contamination.

Conclusion

The incidence of transfusion-transmitted malaria (TTM) was 2.0% (one patient). The patient with TTM did not show any clinical signs and symptoms of malaria.

The incidence of transfusion-transmitted syphilis was 0.5% (one patient).

The incidence of probable or possible transfusion-transmitted bacterial sepsis was 5.5%.

Objective 4: To compare microscopic, serological and molecular methods for screening blood for malaria parasites

Conclusion

The PCR test method showed the highest sensitivity (93.4%), specificity (100%), positive predictive value (100%) and negative predictive value (98.6%). Microscopy had the lowest (24%) sensitivity but had the highest specificity (100%) and positive predictive value. The sensitivity and specificity of RDT and EIA were low.

Summary of the conclusions

Overall, this study showed that malaria and syphilis were transmitted by blood transfusions but at low rates. Transfusion recipients did not show any clinical effects of TTM and transfusion-transmitted syphilis. On the other hand bacterial contamination in donated units was high (11.5%) and half of recipients of these units developed signs of sepsis.

9.2 Dissemination of study findings and implications

Throughout the period of the study, regular formal and informal meetings were held within departments and units in KATH to provide feedback of results. On two

occasions, study results were presented to the hospital's Transfusion Committee. A presentation of my results was also held in the Department of Haematology and Transfusion in Korle-Bu hospital in Accra, the biggest teaching hospital in Ghana. My research topic generated not only local but international interest and I was invited to present papers in International meeting and conferences. The topics of my presentations are listed in table 9.1.

Table 9.1: Presentations at international meetings

Meeting	Title of presentation
7th International Alliance for Biological Standardization (IABS) symposium on Advances in Transfusion Safety, Singapore. July 2011	Malaria and transfusion in endemic areas
21st Regional Congress of the International Society of Blood Transfusion (ISBT), Lisbon, Portugal. June 2011	Malaria in the blood supply in Africa: Is it a problem?
British Society of Haematology, Brighton, UK. April 2011	Feasibility of screening for malaria and syphilis in endemic countries
31st International Congress of the ISBT, Berlin, Germany. June 2010	1. Transfusion practices in a teaching hospital in Ghana. 2. Bacterial Contamination of donated blood 3. Syphilis screening in a teaching hospital in Ghana.
International Plasma Fractionation Association, Zagreb, Croatia. May 2010	Screening or Treatment: The dilemma of malaria safety in hyper endemic area.
Division of Transfusion Medicine, Haematology Department, Cambridge, UK. May 2010	Malaria blood safety; approaches in West Africa

The publications that have resulted from my study are shown in Table 9.2. The published articles and abstract of my presentations are provided in appendices 1.1 to 1.6.

Table 9.2: Publications arising from thesis

Published manuscripts 1. Transfusion transmitted syphilis in teaching hospital, Ghana. Owusu-Ofori AK, Parry CM, Bates I. Emerging Infectious Diseases 2011 Nov; 17(11): 2080-2. Letter 2. Transfusion transmitted malaria in countries where malaria is endemic: a review of the literature. Owusu-Ofori AK, Parry CM, Bates I. Clinical Infectious Diseases 2010 Nov 15; 51(10):1192-8. Epub 2010 Oct 7. Review
Submitted manuscripts 1. Impact of inconsistent policies for transfusion-transmitted malaria on clinical practice in Ghana*. Accepted by PLoS One journal* *2.* Transfusion transmitted malaria in Ghana. *Submitted to The Lancet*
Manuscript in preparation Bacterial contamination of donated blood and its effects on transfusion recipients.

The justification for performing this study arose from the apparent lack of evidence in Africa about these transfusion transmissible diseases, namely malaria, syphilis and other bacterial infections (49). It was therefore not surprising that my presentations generated a lot of interest. One interesting but worrying feedback in Ghana was that despite the evidence that was presented, clinicians were sticking to their previous opinions. For example, physicians thought parasitaemia should be higher than my findings showed. Many physicians thought patients should be given anti-malarials even if no parasitaemia was found in the recipients after transfusion.

Some 'experts' were of the opinion that refrigeration of blood should be able to eliminate *T. pallidum* from blood and therefore, refrigeration for at least 5 days should be mandatory. This was a preferred option for many local physicians and also for the transfusion committee, rather than screen with indirect tests that result in unnecessary rejection of non-infectious blood. The logistics, science and ethics of keeping blood for prolonged periods have been discussed in chapter 8.

Scientists at the international meetings were of the opinion that the findings, especially for malaria and syphilis, were important but larger sample sizes were needed to adequately portray the true incidence of these transfusions transmitted infections.

My findings in this study will provide a basis that will stimulate further research to provide more evidence in transfusion-transmitted malaria and bacterial infections.

9.3 Recommendations

1. Presumptive treatment of transfusion recipients in malaria endemic areas is not warranted and should be discontinued.
2. Routine screening of donated blood for *Plasmodium falciparum* is not recommended for transfusions in adult patients.
3. A study to compare the incidence of TTM in different malaria endemic regions and different transmission seasons is recommended.
4. Komfo Anokye Teaching Hospital and other blood centres that are presently not screening for syphilis should re-institute screening.
5. Training of clinical staff on the need for transfusion monitoring is crucial and should be done regularly in hospitals. Transfusion monitoring should be performed for all blood transfusions taking place in the hospital and transfusion reactions systematically investigated.
6. To reduce bacterial contamination and transfusion related sepsis strict adherence to proven skin cleansing procedures before phlebotomy, particularly for mobile blood drives.
7. Transfusion service and malaria programme managers in each country should collaborate to harmonise policies related to prevention of TTM and develop systems that will encourage adherence to these policies.
8. Pathogen inactivation is a promising intervention in whole blood with the potential to make blood safer. Feasibility of clinical trials in this area should be explored in Africa.

9.4 Research questions arising from my thesis

Transfusion-transmitted malaria

Children and infants were not adequately represented in this study. Therefore a study to determine the incidence of TTM in children is needed to provide evidence that will support or refute the current practice of presumptive treatment in children

Bacterial contamination

Sources of contamination were not established in my study. Blood units sent to department of paediatrics had the highest contamination rate. However contamination also occurred in units sent to other departments. Further studies are required that will identify specific causes and sources of contamination so that targeted solutions can be implemented to avoid contamination.

Syphilis screening

The syphilis screening algorithm that includes enzyme immunoassay, *T. pallidum* hemagglutination (TPHA) and rapid plasma regain (RPR) assay is highly sensitive. The complexity of this three tier screening may result in poor user compliance and is more expensive than the single RPR assay commonly used. Different screening algorithms should be compared to establish the most cost effective screening method.

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APPENDICES

Appendix 1.1: Malaria publication in Clinical Infectious Disease Journal

This text box is where the unabridged thesis included the following third party copyrighted material -

Alex K. Owusu-Ofori,
Christopher Parry,
and Imelda Bates
Transfusion-Transmitted Malaria in Countries Where Malaria Is Endemic: A Review of the Literature from Sub-Saharan Africa Clin Infect Dis. (2010) 51(10): 1192-1198 http://dx.doi.org/10.1086/656806

Appendix 1.2: Syphilis publication in Emerging Infectious Diseases Journal

Olivier Barraud, Edgar Badell, François Denis, Nicole Guiso, and Marie-Cécile Ploy

Author affiliations: Institut National de la Santé et de la Recherche Médicale, Limoges, France (O. Barraud, F. Denis, M.-C. Ploy); University of Limoges, Limoges (O. Barraud, F. Denis, M.-C. Ploy); and Institut Pasteur, Paris, France (E. Badell, N. Guiso)

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Address for correspondence: Marie-Cécile Ploy, Institut National de la Santé et de la Recherche Médicale, Equipe Avenir, 2 Rue du Dr Marcland, 87025 Limoges Cedex, France; email: marie-cecile.ploy@unilim.fr

Transfusion-transmitted Syphilis in Teaching Hospital, Ghana

To the Editor: Transfusion-transmitted syphilis, which is caused by *Treponema pallidum* subspecies *pallidum*, is one of the oldest recognized infectious risks of blood transfusion (*1*). Routine screening of blood donors and refrigeration of donated blood before its use has resulted in only 3 reported cases of transfusion-transmitted syphilis over the past 4 decades (*2*–*6*).

The World Health Organization recommends screening all donated blood for syphilis (*7*), but doing so is challenging for many developing countries. Many blood banks in low-income countries, including Komfo Anokye Teaching Hospital in Kumasi, Ghana, do not screen donated blood for syphilis.

This study was conducted at Komfo Anokye Teaching Hospital. The purpose of this study was to determine the prevalence of syphilis among blood donors and whether seroconversion occurred in transfusion recipients. The study was approved by the ethics committees in Kumasi, Ghana, and Liverpool, UK.

Pretransfusion plasma samples from 200 conscious transfusion recipients in adult, pediatric, and obstetric inpatient departments and samples of their transfused blood were tested for syphilis. A positive initial result by enzyme immunoassay (EIA) (Bioelisa Syphilis 3.0; Biokit, Barcelona, Spain) was confirmed by using a *T. pallidum* hemagglutination assay (TPHA) (Syphagen; Biokit). A rapid plasma reagin (RPR) assay (RPR Reditest; Biokit) was used to determine whether seropositivity was caused by recent infection. Seronegative recipients who had received seropositive blood were retested 30 days posttransfusion to identify seroconversions. All donors and recipients with recent infections were offered counseling and treatment in accordance with national guidelines.

A total of 145 (73%) blood donors were male, and 109 (57%) units of blood had been stored for <4 days. Sixteen units (8%, 95% confidence interval [CI] 4.3%–11.7%) were seropositive for syphilis by EIA and TPHA. Of these units, 7 (44%) were RPR reactive, which indicated a prevalence of recent infections of 3.5% (95% CI 1.0%–6.0%) (Table). Twenty-six transfusion recipients (13%; 95% CI 8.3%–17.7%) were seropositive by EIA and TPHA. Of these recipients, blood samples from 9 (35%) were RPR reactive, indicating a prevalence of recent infection of 4.5%.

One recipient, an 8-year-old girl with severe malarial anemia (recipient 10), showed seroconversion after receiving an RPR-reactive unit of blood that had been refrigerated for only 1 day before being issued for

Table. Characteristics of 16 recipients of syphilis-positive blood transfusions, Kumasi, Ghana*

Recipient ID	RPR results for transfused blood	Duration of blood storage, d	Blood sample test results						Outcome
			Pretransfusion			Posttransfusion			
			EIA	TPHA	RPR	EIA	TPHA	RPR	
1	R	12	–	ND	ND	NA	NA	NA	Died
2	NR	2	–	ND	ND	NA	NA	NA	Died
3	NR	2	–	ND	ND	NA	NA	NA	Died
4	NR	1	–	ND	ND	NA	NA	NA	Died
5	R	4	–	ND	ND	NA	NA	NA	Lost to follow up
6	NR	1	–	ND	ND	NA	NA	NA	Lost to follow up
7	NR	2	+	+	NR	NA	NA	NA	Not followed up
8	NR	6	+	+	R	NA	NA	NA	Not followed up
9	NR	3	–	ND	ND	–	ND	ND	Well
10	R	1	–	ND	ND	+	+	R	Seroconverted
11	NR	2	–	ND	ND	–	ND	ND	Well
12	R	1	–	ND	ND	–	ND	ND	Well
13	R	3	–	ND	ND	+	–	NR	Well
14	NR	2	–	ND	ND	–	ND	ND	Well
15	R	1	–	ND	ND	–	ND	ND	Well
16	R	4	–	ND	ND	–	ND	ND	Well

*ID, identification; RPR, rapid plasma reagin; EIA, enzyme immunoassay; TPHA, *Treponema pallidum* hemagglutination assay; R, reactive; –, negative; ND, not done; NA, not available; NR, not reactive; +, positive. All results for transfused blood tested by EIA and TPHA were positive.

use. Posttransfusion fever developed in this recipient, who responded to treatment with cefuroxime and gentamicin, although results of blood culture for bacteremia and peripheral blood film for malaria parasites were negative. She had no relevant sexual history, had been febrile after the transfusion, and showed no evidence of mucocutaneous lesions or lymphadenopathy at her follow-up visit 1 month after the transfusion. She was referred to pediatricians for treatment of syphilis.

This recipient who showed seroconversion most likely had a case of transfusion-transmitted syphilis. Other treponemal infections such as yaws cannot be differentiated serologically from syphilis, and a diagnosis of yaws is based on clinico-epidemiologic features (*8*); however, yaws is not endemic to Kumasi, and because this child had no clinical evidence of yaws, this disease is unlikely to be the cause of the seroconversion.

Refrigeration of units of blood for ≥5 days kills *T. pallidum*, but 57% of the donated blood in this study was stored for <4 days before use. This situation prevails across many blood banks in sub-Saharan Africa where, because of inadequate supply and high demand, blood is used as soon as it becomes available. Such short periods of blood storage do not provide an adequate margin of safety against transfusion-transmitted syphilis. Findings from this study have been discussed with the hospital transfusion committee, and new syphilis screening guidelines and testing algorithms are being developed.

The high prevalence of syphilis seropositivity in blood donors and seroconversion of a transfusion recipient shows that in centers where screening is not conducted, recipients of blood transfusions are at risk for contracting transfusion-transmitted syphilis. This finding is likely in blood banks that have a high demand for blood and where blood is stored only for a few days. This study highlights transfusion-transmitted syphilis as a serious public health issue in developing countries and demonstrates that screening of donor blood for syphilis should be conducted.

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Alex K. Owusu-Ofori, Christopher M. Parry, and Imelda Bates

Author affiliations: Komfo Anokye Teaching Hospital, Kumasi, Ghana (A.K. Owusu-Ofori); Liverpool School of Tropical Medicine, Liverpool, UK (A.K. Owusu-Ofori, I. Bates); and University of Oxford, Oxford, UK (C.M. Parry)

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Address for correspondence: Alex K. Owusu-Ofori, Department of Microbiology, Komfo Anokye Teaching Hospital, PO Box 1934, Kumasi, Ghana; email: alex.owusu-ofori@liv.ac.uk

Congenital Syphilis, Réunion Island, 2010

To the Editor: Syphilis, caused by the bacterium *Treponema pallidum*, is primarily a sexually transmitted infection, but *T. pallidum* can also be transmitted by infected pregnant women to their children. Every year, at least 500,000 children are born with congenital syphilis (CS); maternal syphilis causes another half million stillbirths and abortions, usually in countries with limited resources (*1*). However, CS has been recently found in industrialized countries such as the United States, where the CS rate increased by 23% during 2005–2008, after a 38% increase in the syphilis rate among US women and girls during an earlier period (2004–2007) (*2*).

Réunion Island, a French overseas territory with 810,000 inhabitants, has a health care system similar to that in continental France. Neither syphilis infection, CS, nor other trepanomatosis (yaws) is notifiable. Since 2006, an increase in early syphilis was documented, first in men who have sex with men infected with HIV and second in the general population.

In 2009, we conducted a retrospective study by using data from 2004–2009 to document the situation of CS on the island. Data from all public (n = 4) and private (n = 2) hospitals on the island with neonatology and obstetrical departments were investigated. Birth deliveries at home were not included. Inclusion criteria were positive specific (*T. pallidum* hemagglutination assay) and nonspecific (Venereal Disease Research Laboratory [VDRL]) test results for *Treponema* spp. among children <2 years of age during 2004–2009. Additionally, hospitalized children coded as having congenital syphilis (International Classification of Diseases [ICD] 10 codes A50.0 to A50.9) in the French national hospital database were included. After reviewing medical files of mothers and their children, cases were classified as confirmed or probable CS according to the case definition of the Centers for Disease Control and Prevention (*2*).

Eighteen children had positive syphilis serologic results by *T. pallidum* hemagglutination assay and VDRL tests, according to the selection criteria. Among these 18 test results, 7 were classified as probable CS (late treatment for mother or symptoms linked to CS), 3 in 2008 and 4 in 2009 (Table). The male:female sex ratio was 0.75. Five case-patients were preterm newborns; 3 of the most premature babies had signs linked to CS, such as hepatosplenomegaly, cutaneous mucosal signs, neurologic signs, radiographic signs of CS in long bones, edema, and biologic anomalies. All were screened for *T. pallidum*–specific IgM by using fluorescent treponemal antibody absorption or IgM capture ELISA from immediately after birth to 15 days old. Two case-patients had positive results; 1 was symptomatic. Six of the 7 children who had probable CS received appropriate penicillin G treatment, except for 1 asymptomatic baby for whom long-term medical supervision was recommended by the pediatrician. Survival rates at 3 months of age reached 100%.

Median age of mothers at delivery was 22 years. All mothers were natives of Réunion Island except 1 who was born in Madagascar and received no antenatal follow-up. Medical history indicated previous genital herpes for 3 women. Social difficulties or alcohol consumption were reported for 3 women. The mean age of gestation at which the first syphilis screening was conducted was 23 weeks (5–33 weeks). Two mothers were symptomatic. Syphilis was diagnosed after delivery for 3 mothers; seroconversion occurred during the pregnancy. Except for missing data on 1 mother, all mothers were HIV negative.

In Réunion Island, in our retrospective review, we found 7 CS cases during 2008–2009 but none during 2004–2007. The incidence rate of probable CS cases was estimated to be 28 cases per 100,000 live births during 2009. However, results may have been underestimated because not all parturients with a positive syphilis test result and fetal deaths were investigated. Meanwhile, a fetal death at 30 weeks was reported during the investigation but not included in the selection criteria. The Centers for Disease Control and Prevention definition of CS based on maternal status can also lead to an overestimation. Late screening of syphilis in mothers, lack of antenatal follow-up, higher VDRL titer, or unknown stage of the disease at time of diagnosis have already been described in other studies (*3*–*5*).

Appendix 1.3: Presentation at the 21st Regional Congress of the ISBT, Lisbon, Portugal. 2011

Abstract 4C-S17-01

MALARIA IN THE BLOOD SUPPLY IN AFRICA: IS IT A PROBLEM?

Although international policies recommend that blood for transfusion should be screened for transfusion-transmitted infections, malaria screening is not carried out in most malaria-endemic countries in sub-Saharan Africa (SSA). There are several reasons why this recommendation may not have been implemented. Serological screening tests are unhelpful in areas where malaria is endemic as most of the population have anti-malaria antibodies. There is a lack of evidence to indicate which malaria screening methods are effective for use by transfusion services in malaria-endemic countries in SSA. This is important because alternative malaria screening tests, such as microscopy, ELISA or rapid tests, are not sensitive enough to detect low levels of parasites in potential blood donors.

Critical shortages of blood are common; for example in SSA 26% of maternal haemorrhage deaths between 1970 and 2007 were due to lack of blood for transfusion. Blood transfusion services are understandably reluctant to implement any policy which is likely to exacerbate blood shortages. A review of the literature indicated that 10% of 33,029 blood donors in SSA were positive for malaria. Rejection of all these malaria-positive donors would undoubtedly have a major impact on the blood supply. A further complication is that the prevalence of malaria varies widely across Africa from 0.7% in Kenya to 55.0% in Nigeria making it difficult to devise a single policy that is appropriate for the whole continent.

Even more fundamental is the question of whether or not the transfusion of low levels of malaria parasites to recipients living in endemic areas has adverse clinical consequences and therefore whether a policy recommending malaria screening is justified at all. Prior to the introduction of artemisinin combination therapy (ACT), pre-emptive treatment of transfusion recipients with chloroquine was cost effective. Given the higher cost of ACT's and the need to prevent the spread and rapid development of resistance, screening policies will become invaluable. Despite the considerable costs associated with implementing a malaria-screening policy for blood transfusion, we have not been able to find any studies from SSA that investigated the clinical outcomes of receiving malaria-infected blood. We urgently need to fill these gaps in our knowledge about the benefits, risks and processes for screening blood for malaria so that appropriate and feasible policies about screening blood for malaria can be developed for the SSA region.

Appendix 1.4: Presentation at the 31st International Congress of the ISBT, Berlin, Germany. 2010

Abstract P-0935 Transfusion practices in a teaching hospital in Kumasi, Ghana

Background: Information concerning transfusion practices in developing countries is scanty. The World Health Organization has published several guidelines and policies to help improve transfusion practices and safety. Ghana published a national guideline for blood transfusion in 2001. The level of adherence to these guidelines is not known.

Aims: This study was undertaken to determine whether current transfusion practices in different departments in a teaching hospital in Ghana followed the published guidelines.

Method: Over a four week period, a retrospective review of the notes of transfusion recipients was performed. A standardized pro-forma was used to extract the required information. The pro-forma was adapted from the hospital's transfusion monitoring forms. It included information on patient demographics, diagnoses, use of antimalarials and premedication and information related to the transfusion including monitoring of vital signs, duration of transfusion and documentation of adverse events. Patients were randomly selected from the Departments of Obstetrics and Gynaecology (OBGY), Paediatrics and Medicine.

Results: 151 patient records were reviewed during the four week study period, representing 10.4% of transfusion recipients. These patients comprised 41 (27.2%) from Medicine, 51 (33.7%) from OBGY and 59 (39.1%) from Paediatrics. The median age was 23.5 yrs (range: 1 – 83 yrs). 64.7 % of patients in OBGY, 61.9% of patients in Medicine and 15% of patients in Paediatrics received multiple transfusions (2 or more transfusions). The mean duration of transfusion was 2.6 hours (range 1-6 hrs) and 6.3% of transfusions exceeded the recommended 4 hr duration. Frusemide was used as premedication in 89.7% of Paediatric, 12.2% of OBGY and 6.3% of Medicine patients. Blood pressure (BP) was not monitored in 59.6% of transfusions, was monitored once in 26.5% of cases, twice in 5.3% of cases and more than twice in 4% of cases. The pulse rate was not measured in 28.5% of transfusions, was measured once in 55.6% of cases, twice in 7.9% of cases and more than twice in 3.4% of cases. The monitoring of respiration and temperature followed a similar pattern. 43.7% of patients had received antimalarials within 24 hrs of transfusion and 75% of the antimalarials given were prescribed at the time the transfusion and were given in the absence of a positive malaria test. Paediatrics had the highest usage of antimalarials at 86.5% and Medicine the lowest at 7.3%. Adverse reactions were documented in 8.6% of the transfusions and treatment of these reactions was documented in 6.6% of patient notes. 7% (10/151) of transfusion recipients died within 24h of the transfusion. It was not possible to determine whether these deaths were directly related to the blood transfusion.

Conclusions: This survey shows that monitoring of vital signs during blood transfusion is not performed according to recommended guidelines. Presumptive malaria treatment is common in the Department of Paediatrics. Transfusion practitioners need regular training about the guidelines for transfusion monitoring to improve practice. Studies are required that provide evidence to guide the rational use of antimalarials in blood transfusion.

Appendix 1.5: Presentation at the 31st International Congress of the ISBT, Berlin, Germany. 2010

Abstract P-0589: Bacterial contamination of donated blood in a teaching hospital in Ghana

Background: Transfusion associated bacterial sepsis from bacterial contamination of blood/blood products is associated with a high mortality. In developed countries bacterial contamination has usually been associated with transfusion of platelets rather than red cells or whole blood. Whereas red cells and whole blood are stored refrigerated, platelets are stored at room temperature and this can provide a suitable environment for bacterial proliferation. In developing countries where fridge temperatures are not monitored and electricity supply is erratic, whole blood may be subject to similar bacterial contamination and proliferation. There is little data about bacterial contamination of donated units of blood and its effects on recipients in Africa. **Aim:** The aim of the study was to determine the level of bacterial contamination in donated blood in Ghana and its effects on transfusion recipients. **Method:** This was a prospective study conducted at the Komfo Anokye Teaching Hospital. Consent was obtained from the patients prior to starting transfusion. At the tail end of the transfusion, 5 mls of blood was taken aseptically from the blood bag and immediately inoculated into 20 mls of brain heart infusion broth. The broth was transported to the microbiology laboratory and incubated for up to 7 days. Blind sub-culture of the broth onto blood and MacConkey agar plates was performed on days 2 and 7. Patient's vital signs were monitored and documented hourly until the transfusion was complete. Patients were clinically reviewed immediately after the transfusion, 24 hours after the transfusion and each day until discharge. If bacteria were isolated from the blood unit after the patients discharge, the recipient of the blood was immediately recalled for re-evaluation and a blood culture taken. **Results:** Voluntary donations comprised 71.5% of the blood that was transfused and 34.0% of donors were females. The blood was stored in the fridge for a median of 3 days (range 0 – 21 days). 23 bacteria were cultured from 200 transfused units of blood, a positivity rate of 11.5%. Coagulase negative *Staphylococcus* was the most common isolate (47.8%). Other organisms were *Bacillus* species (21.7%), *Pseudomonas aerugenosa* (13%), *Shigella* species (8.7%) and *Alkaligenes* spp. and *Citrobacter freundii* (4.4% each). There were no overt clinical signs of sepsis in patients who received these contaminated units of blood. Follow up blood cultures from the recipients of contaminated units were negative. One patient, who received blood contaminated with *Pseudomonas aeruginosa*, although not showing any signs of sepsis required readmission for anaemia and was re-transfused. 64.5% of the transfusion recipients received antibiotic therapy during admission for underlying conditions. The mortality was 10.5%, none of which resulted directly from the blood transfusion.

Conclusion: There were no apparent clinical effects of bacterially contaminated blood on recipients of such transfusions. It is unclear whether these organisms were in fact transfused into the transfusion recipients. The bacterial isolates may have resulted from contamination during the process of obtaining samples, transport to the laboratory or in the laboratory. Further studies are required to determine the true occurrence and effects of bacterial contamination of blood in this setting.

Appendix 1.6: Presentation at the 31st International Congress of the ISBT, Berlin, Germany. 2010

Abstract 5D-S54-03: Syphilis screening of donated blood in a teaching hospital in Ghana

Background: Screening of syphilis is one of the WHO recommended strategies for reducing transfusion transmitted infections. It has however been suggested by some experts in the developed world that screening should be discontinued. Transfusion transmitted syphilis (TTS) is now a rare occurrence and has not been reported in the international literature for >25 years. Storage of blood in temperatures at 2-8^{0}C, which is lethal to *Treponema pallidum*, largely accounts for the elimination of TTS. In developing countries, however, where blood is in high demand and blood supply is largely by replacement donation, blood units are kept in the fridge for only a short time. In such conditions, it is possible that TTS still occurs. **Aim:** This aim of this study was to determine if syphilis sero-conversion occurs in transfusion recipients in a hospital where screening of blood units for syphilis is not routinely performed. **Methodology:** This study was conducted in Medicine, Obstetrics and Gynaecology and Paediatrics departments of the Komfo Anokye Teaching Hospital (KATH) in Kumasi Ghana. KATH does not screen its donated blood for syphilis. After obtaining informed consent, a pre-transfusion sample from the patient and another sample from the unit of blood being transfused were obtained. Plasma was aliquoted, stored in a -20 freezer and later tested in batches. Samples were screened for syphilis initially using an enzyme immunoassay (EIA, Bioelisa Syphilis 3.0, Biokit). Confirmation of positive EIA results was by the *Treponema pallidum* haemagglutination assay (TPHA, Biokit Syphagen TPHA). A rapid plasma regain test (RPR, Biokit RPR Redirect) was used to determine disease activity in EIA/TPHA positive samples. Recipients who were sero-negative prior to transfusion and who received a syphilis positive blood unit were recalled for follow up testing with EIA, TPHA and RPR to determine if sero-conversion had occurred. Patients with positive serology results prior to transfusion and those who received blood with positive serology results were given appropriate counseling and treatment. **Results:** Voluntary donors comprised 68% of donations. 27.5% of the donors were female. 200 transfusion recipients were recruited into the study; results are currently available for 150. 7.3% of donated blood units and 13.3% of transfusion recipients were EIA/TPHA positive. 36.6% and 30% respectively were also RPR positive. Two recipients were initially EIA/TPHA positive and went on to receive EIA/TPHA positive blood. Two patients who received EIA/TPHA positive blood died before subsequent samples could be obtained to test for sero-conversion. One patient became EIA/TPHA positive one month after receiving syphilis positive blood. The blood had been transfused after 1 day's storage in the blood bank fridge. Serum has been stored for further confirmatory tests. This donated blood came from a walk in voluntary donor. **Conclusion:** This study shows that transfusion transmitted syphilis could potentially be a problem in our hospital. Further patient follow up and testing for sero-conversion is planned. Discussions are ongoing with the hospital management and transfusion committee to institute syphilis screening of all donated blood.

Appendix 2.1: Informed consent form

INFORMED CONSENT FORM

INFORMATION SHEET

Title of the research:
Transfusion Transmitted Malaria and Bacterial Infections in Ghana.

Name(s) and affiliation(s) of researcher(s) of applicant(s):
Dr Alex Owusu-Ofori of the Komfo Anokye Teaching Hospital (KATH), Dr. Imelda Bates and Dr Chris Parry of Liverpool School of Tropical Medicine

Introduction:
I am a member of a research team carrying out this research in KATH and Bekwai and wish to explain this study to you and to obtain your consent to participate in this study.

Purpose(s) of research:
The purpose of this research is to determine whether donated blood for transfusion has bacteria (including syphilis) or malaria parasites, and what effects there are (if any), when such blood is transfused.

Selection of participants:
This study involves all children, pregnant women and immuno-compromised patients who are to receive blood transfusion.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research:
You/your child/your relative is about to receive a blood/blood product transfusion. This blood has been investigated for HIV, Hepatitis B and C and is considered safe. However malaria parasites and bacteria have not been looked for because it is not routinely done in this country. What this study seeks to do is to look for the malaria parasites and bacteria (including syphilis) in the blood and investigate if it has any effects.
If you agree to be enrolled into this study, before the transfusion is started, we will take six millilitres (a little over 2 teaspoonful) of your blood. This will be tested and compared to any later findings we make. We will also check your vital signs before the start of the transfusion.

When the transfusion starts, we will monitor your vital signs regularly until the end of the transfusion. At the end of the transfusion, the leftover of the blood in the transfusion bag will be taken to the laboratory and tested for malaria and bacteria. We will know within 24 hours whether the blood you received had malaria or not. Based on this result, you will be allocated into either of two groups; those whose blood had malaria (‘cases’) or those whose blood did not have malaria (‘controls’). There will be a total of 200 cases and 400 control participants for this study. Participants allocated into both groups will be asked to stay in hospital for 72 hours during which we will monitor you intensively, including 8 hourly vital signs examination until you are discharged.

On discharge you will have to come back to see us after one week, two weeks and four weeks. We have a special clinic for you so that you can see us directly. When you come for each of these follow-up visits, we will ask you to tell us about your health since you went home and we will examine you fully. We will take a small amount of blood, equivalent to about half a teaspoon.
If at any point before you come back to the hospital we find some bacteria in the blood that was given you, we will call you back, examine you and refer you for treatment. If at any time before you are scheduled to come back you feel unwell, you can call us or come to the hospital. We will like you to always come to us when you don't feel well and not go anywhere else so we can investigate you properly and have well trained people treat you.
Once this study is completed, any of your sample left, will be destroyed.

Risk(s):
There is the possibility of feeling pain when we are taking your blood and the place from where we take the blood may be bruised.

Benefit(s):
The immediate benefit for you in this study is that you will have direct access to a medical team in the event that you develop any symptoms and at no extra expense to you.
The goal of this research is to find out if there are malaria parasites and bacteria in the donated blood and what happens when such blood is given to people. What we find will be able to help us provide safer blood and in the event of not finding anything, our current practice will continue.
Our findings may go on to affect how we give blood in the future so that in case you or someone else comes for blood, we will know that the blood we are giving is safe.
We will reimburse your transportation cost to the hospital for all your follow-up visits.

Confidentiality:
All the information collected from you in this study will be well kept by us and not given to anybody. However, the people who approved this study may sometimes carry out inspections, so if that happens in this study, we will show them the information collected but without your personal details.
Your name will be initially recorded to be able to trace you in case of any eventuality. However all names will be deleted from our records immediately the study ends.
Your name will not be published.

Voluntariness:
Your participation is entirely voluntary and can decide to participate in this study or not.

Alternatives to participation:
If you chose not to participate in this study, it will not affect the way you will be treated in this hospital.

Consequences of participants' decision to withdraw from research and procedure for orderly termination of participation:
You may choose to withdraw from this study at anytime. Please note that some of the information that has been obtained about you before you chose to withdraw may

have been used in reports and publications and cannot be removed anymore. However we promise to comply with your wishes as much as is practicable.

Dissemination of results:
Results of this study will be shared with the management of the hospital and the ministry of health. It will also be published in international journals.

Study contact person: In case you have any questions, difficulties, or emergencies, you can contact Dr Alex Owusu-Ofori, Directorate of Diagnostics, KATH, Kumasi; Phone number 0244 605 543.

CONSENT FORM

Statement of person obtaining informed consent:
I have fully explained this research to ______________________________
and have given sufficient information, including about risks and benefits, to make an informed decision.

DATE:____________________SIGNATURE: ___________________________

NAME: ____

Statement of person giving consent:
I have read the description of the research or have had it translated into language I understand. I have also talked it over with the interviewer to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have received a copy of this consent form and additional information sheet to keep for myself.

DATE:________________SIGNATURE/THUMBPRINT: __________________

WITNESS' SIGNATURE (if applicable): ______________________________

WITNESS' NAME (if applicable): ____________________________________

Tel number:____________________________________

Address:____
____
____
____
____

Appendix 3.1: Data collection form for survey on transfusion practices

Survey on Transfusion Practices

Age:	Sex:	Unit/Directorate: ....
Folder number:		Date:

The ff details required relate to the latest transfusion episode

Date of admission________________ Date of transfusion________________	Duration of current transfusion(hrs) _____________ Was the transfusion completed? [Yes] [No]

Frequency of observation of vital signs during transfusion:

Vital sign	Temperature	Pulse	Respirations	BP
Frequency				

1a. Pre-medication given? [Yes] [No] 1b)Name of medications:___________________
2. Pre-transfusion Hb____________________
3. Patient diagnosis? 1. ____
2. ____
4. Any transfusion prior to this admission? [No] [once] [twice] [thrice] [more than 3X]
5. How many transfusions have been received during this admission? [1] [2] [3] [4][>4]
6a. If pregnant, has patient taken any iPT during the pregnancy? [YES] [NO] [Not stated]
b. How many doses were taken? [1] [2] [3] [Not stated]
c. Name of drug __________________________ d. How long ago was it taken (weeks)?_____
7a. Has patient received antimalarials during this admission? [YES] [NO]
b. Which antimalarials were given?____
c. When was antimalarials given?
[A] Given at the time of requesting transfusion without a positive malaria test
[B] Given at the time of requesting transfusion; patient positive for malaria
[C] Given post transfusion due to fever, without a positive malaria test.
[D] Prescribed post transfusion, patient positive for malaria.
[E] Given post transfusion due to fever, without a positive malaria test.
[F] Other...

8a. Has patient received antibiotics during this admission? [YES] [NO]
b. If antibiotics were given, was it prescribed after the transfusion? [YES] [NO]

9a. Did patient have an increase in temperature within 24hrs of the transfusion? [YES] [NO]
b. If there was fever, what was the highest temperature difference from baseline? _____
c. If there was fever, did it subside within 24 hours? [YES] [NO] [NA]

10a. From the review of the case notes, is there any indication/ documentation that a transfusion reaction occurred? [YES] [NO]
b. Details: ....
11a. Is there any treatment in the notes that indicate that patient has been treated for a transfusion reaction? [YES] [NO]
b. Medications given______________________________

12. Outcome at 24 hour: [Alive] [Died].

Appendix 4.1: Classification of adverse events of transfusion

CATEGORY	DEFINITION*
Acute transfusion reaction (ATR)	Reactions occurring at any time up to 24 hours following a transfusion of blood or components, ***excluding*** cases of acute reactions due to incorrect component being transfused, haemolytic reactions, transfusion-related acute lung injury (TRALI), transfusion-related circulatory overload (TACO) or those due to bacterial contamination of the component. Examples of ATR are: **Isolated febrile** – a rise in temperature of > 1oC +/- minor rigors and chills. **Minor allergic** – skin +/- rash **Anaphylactic** – hypotension with one or more of: urticaria, rash, dyspnoea, angioedema, stridor, wheeze, pruritus, within 24 hours of transfusion. **Severe allergic reaction** – Severe allergic reaction with risk to life occurring within 24 hours of transfusion, characterised by bronchospasm causing hypoxia, or angioedema causing respiratory distress. **Hypotension** – a drop in systolic and/or diastolic pressure of >30mm Hg occurring within one hour of completing transfusion, provided all other adverse reactions have been excluded together with underlying conditions that could explain hypotension. **Febrile with other symptoms/signs** – rise in temperature of >1oC, with no features of an allergic reaction, but with one or more of myalgia, nausea, change in blood pressure or hypoxia.
Acute Haemolytic transfusion reaction (HTR)	**Acute** HTRs are defined as fever and other symptoms / signs of haemolysis within 24 hours of transfusion; confirmed by a fall in Hb, rise in LDH, positive DAT and positive cross match.
Delayed Haemolytic transfusion reaction (HTR)	**Delayed** HTRs are defined as fever and other symptoms / signs of haemolysis more than 24 hours after transfusion; confirmed by one or more of: a fall in Hb or failure of increment, rise in bilirubin, positive DAT and positive cross match not detectable pretransfusion. Simple serological reactions (development of antibody without positive DAT or development of haemolysis) are excluded. Cases with relevant features should be reported together with results of all laboratory investigations and antibody identification

	results if available. Cases will be included with no clinical or laboratory features as long as DAT is positive.
Transfusion related acute lung injury (TRALI)	Acute dyspnoea with hypoxia and bilateral pulmonary infiltrates during or within six hours of transfusion, not due to circulatory overload or other likely cause. (Suspected cases should be discussed with a Blood Service Consultant, and reported if there is a high index of suspicion, even if serological investigation is inconclusive).
Post transfusion purpura (PTP)	Thrombocytopenia arising 5 – 12 days following transfusion of red cells, associated with the presence in the patient of alloantibodies directed against the HPA (Human Platelet Antigen) systems. (Cases where the platelet count drops more than 50% following transfusion should be investigated and reported if complete or partial serological evidence is available).
Transfusion associated graft versus-host disease (TA-GvHD)	Characterised by fever, rash, liver dysfunction, diarrhoea, pancytopenia and bone marrow hypoplasia occurring less than 30 days after transfusion. The condition is due to engraftment and clonal expansion of viable donor lymphocytes in a susceptible host.
Transfusion transmitted infections (TTI)	Include as a TTI if, following investigation, the recipient had evidence of infection post transfusion, and there was no evidence of infection prior to transfusion and no evidence of an alternative source of infection. Plus; **Either** at least one component received by the infected recipient was donated by a donor who had evidence of the same transmissible infection. **Or** at least one component received by the infected recipient was shown to contain the agent of infection.
Transfusion associated circulatory overload (TACO)	Any four of the following occurring within six hours of transfusion: ° Acute respiratory distress. ° Tachycardia. ° Increased blood pressure. ° Acute or worsening pulmonary oedema. ° Evidence of positive fluid balance.

*Classification as defined by SHOT

Appendix 5.1: Transfusion monitoring form

Transfusion monitoring form	
Blood ID:	Study Number :

Date of transfusion (dd/mm/yy): ___/___/___
Type of component received: [whole blood] [packed cells] [FFP] [Cryoprecipitate]
Time transfusion was started (24 hr clock): ……………………………………..
Time transfusion was completed or stopped (24 hour clock): ……………………..
Duration of transfusion (in hours): ………………………………………………..
Monitor patients vital and complete the table below:

Time point	**Temperature**	**Respiratory rate**	**Pulse**	**Blood pressure**
Pre-transfusion T = 0				
15 min after start of transfusion				
30 min after start of transfusion				
1 hr after start of transfusion				
2hrs after start of transfusion				
3hrs after start of transfusion				
7-12 hrs after the start of the transfusion				

Did the patient develop any of the following during transfusion?

Sign/symptom	**Yes**	**No**	**Sign/symptom**	**Yes**	**No**
Fever			Urticaria		
Restlessness			Anxiety		
Rigors/chills			Headache		
Cyanosis			Pruritus		
Chest pain			Dyspnoea		
Loin/Back pain			Rash		
Palpitations			Dark urine		
Unexplained bleeding			Respiratory distress		
Wheezes/stridor					

Was the transfusion stopped before the unit finished? [Yes] [No]

If yes, estimate how much of the unit was transfused before it was stopped?
A. [Less than 10% transfused] B. [10-<25 % transfused]
C. [25-<50 % transfused] D. [50-<75 % transfused] E. [75-<100 % transfused]

Did the patient have a severe adverse reaction? [Yes] [No]

If yes, please complete the transfusion reaction form and the required investigations:

Take immediate post transfusion blood samples from the vein opposite the infusion site and send to the transfusion lab for:
Repeat blood group and cross matching with the offending unit
Direct Antiglobulin test
Return the 'offending' blood unit and giving set containing the remaining of the donor blood to the transfusion lab.
Take blood for FBC including retics
Take blood in appropriate bottle for blood culture
Blood for urea, creatinine and electrolytes.

TRANSFUSION REACTION INVESTIGATION

Has the unit being transfused returned to blood bank? [Yes] [No]
Has patient sample been taken for culture and sensitivity? [Yes] [No]
Has blood culture from the transfused unit been taken? [Yes] [No]
Has sample for biochemistry been sent? [Yes] [No]
Has sample for full blood count been sent? [Yes] [No]

Laboratory results

Investigation	**Result**	**Comment**
ABO		
RhD		
Direct Antiglobulin Test		
Urea and Creatinine		
Full Blood Count		
Blood culture (patient)		
Blood culture (transfused unit)		

Any other investigations and Results:
____
____
____
____

What type of transfusion reaction occurred? *(Support diagnosis with reasons why. Please see appendix 3 for a classification of the adverse events of transfusion)*
____
____
____
____
____
____

Appendix 6.1: Case report form for Transfusion transmitted malaria study

Name:____Department/ward______________
____

Sex: [M] [F]   Age (yrs):_______________ Hospital number:__________________

Date of admission (dd/mm/yy): ___/___/___Date of transfusion (dd/mm/yy): ___/___/___
Initial diagnosis:____
Indication for transfusion:____

This section to be filled if patient is pregnant

LMP:___/___/___	Is patient a regular ANC attendant [Yes] [No]
Gestation: no.of weeks ………………..[1st trimester][2nd trimester][3rd trimester][puerperium]	
Parity: [Nullip] [Primip] [Multip (no) …….] [Grand multip (no)………..]	
Is patient on IPT (antimalarials)? [Yes] [No] How many doses [1] [2] [3] [dont know] *If yes*, name of antimalarial…………………………how long ago was it taken?(weeks)..........	

Monitoring of signs and symptoms after transfusion; *Please indicate yes or no in each box*

Sign/Symptom	**Pre-transfusion (day 0)**	**Day 1**	**Day 3**	**Discharge (Day……)**
Headache				
Chills/rigors				
Coca-cola urine				
Palpitations				
Urticaria				
Bodily pains				
Fever				
Restlessness				
Wheezes/stridor				
Other…………..				

Monitoring of vital signs after transfusion

Day	**Time point**	**Temperature**	**Respiratory rate**	**Pulse**	**Blood pressure**
Day 1	Morning				
	Afternoon				
	Evening				
Day 2	Morning				
	Afternoon				
	Evening				
Day 3	Morning				
	Afternoon				
	Evening				

Discharge summary

Did the patient receive antimalarials? [Yes] [No]

Name of antimalarials……………………………………………………………………..

If yes, when was it given?

i) [A] before transfusion [B] after transfusion

ii) Number of days……………………………………………………………………

Did patient receive antibiotics during this admission? [Yes] [No]

Name of antibiotics……………………………………………………………………..

How many units of blood/blood components did patient receive? ____________________

Discharge diagnosis____

Date of discharge (dd/mm/yy): ___/___/___ Days on admission________________

Outcome: [alive] [died]

FOLLOW UPS

Day 7

Did patient come for visit? [Yes] [No] [Still on admission]

Has patient had fever since day 3 [Yes] [No]

Has patient taken any antibiotics or antimalarials since discharge? [Yes] [No] [NA]

If yes, give details: ____

Does patient have any of the following symptoms?

i) Headache [Yes] [No] ii) Fever [Yes] [No]

iii) Vomiting [Yes] [No] iv) Chills/rigors [Yes] [No]

v) Bodily pains [Yes] [No] vi) loss of appetite [Yes] [No]

vii) Coca cola urine [Yes] [No] vii) others…………………………………………..

Has blood for Hb and malaria parasites been taken? [Yes] [No]

Has patient been referred for treatment? [Yes] [No]

Day 14

Did patient come for visit? [Yes] [No] [Still on admission]

Has patient had fever since day 7 [Yes] [No]

Has patient taken any antibiotics or antimalarials since discharge? [Yes] [No] [NA]

If yes, give details: ____

Does patient have any of the following symptoms?

i) Headache [Yes] [No]		ii) Fever [Yes] [No]
iii) Vomiting [Yes] [No]		iv) Chills/rigors [Yes] [No]
v) Bodily pains [Yes] [No]		vi) loss of appetite [Yes] [No]
vii) Coca cola urine [Yes] [No]		vii) others…………………………………………..

Has blood for Hb and malaria parasites been taken? [Yes] [No]
Has patient been referred for treatment? [Yes] [No]

<u>Study Conclusion</u>

[A] Patient completed study without getting malaria
[B] Patient developed malaria during the study
[C] Lost to follow up
[D] Consent withdrawn/refused to return for visit
[E] Patient still on admission
[F] Patient died
[G] Other:…………………………………………………………………………………….

Appendix 6.2: Results sheet for transfusion transmitted malaria study

Results sheet for Transfusion transmitted malaria	
Blood ID:	Study Number :

Donor characteristic

Age of donor ______Sex of donor: [M] [F]   Blood Grp:____________

Date of was donation: ____/____/_____Date of transfusion: ____/____/______

How many days was blood kept before being given out? ______________

Type of donor: [voluntary] [replacement] [other…………………………………]

Malaria microscopy

Date done (dd/mm/yy): ____/____/______ Result: [Positive] [Negative]

Count:____________________ Density_____________ Species___________________

RDT

Date done (dd/mm/yy): ____/____/______

Result: [positive] [negative]   Species_____________________   [ ] Not done

Enzyme Immunoassay

Date done (dd/mm/yy): ____/___

PCR

Date done (dd/mm/yy): ____/____/______

Result: [positive] [negative]

Genotyping____

General comment:____

____

____

Patient results:

Malaria

		Microscopy			**PCR**	
Time points	**Hb**	**Parasite count**	**Parasite density**	**Species**	**Positive/ negative**	**Genotyping**
Pre - transfusion						
Day 1						
Day 3						
Day 7						
Day 14						
Unschedule (Day…..)						

Appendix 6.3: Thick and thin film preparation

Preparation of thick and thin films preparation for malaria microscopy

Thick and thin blood films were prepared from venous blood that had been collected from the patient or blood bag into EDTA tubes. A capillary tube was used to transfer blood samples from EDTA tubes unto the glass slides. The same glass slide was used to prepare both thick and thin films. Slides were labelled appropriately with the study number, date and study day.

Thin films

A drop of blood was placed in the middle of glass slide.

Another glass slide was placed in contact with the drop of blood but at an angle of 45° such that the blood spreads along the edge of the second glass slide which acts as the spreader.

The spreader was pushed forward quickly to the end while maintaining the angle. This ensures that the blood is spread thinly.

The slide was air dried and fixed by dipping into 100% methanol for a half a minute. The slide was placed on its side for the alcohol run off and dry.

The smear was then stained with 10% Giemsa stain for 15 minutes and washed with the clean water.

The slide was then placed on a drying rack and allowed to dry at room temperature.

Thick films

A drop of glass was placed on one side of the glass slide.

Using the corner of another glass slide, the drop of blood was spread evenly in a circular fashion to ensure an even spread the blood with moderate thickness.

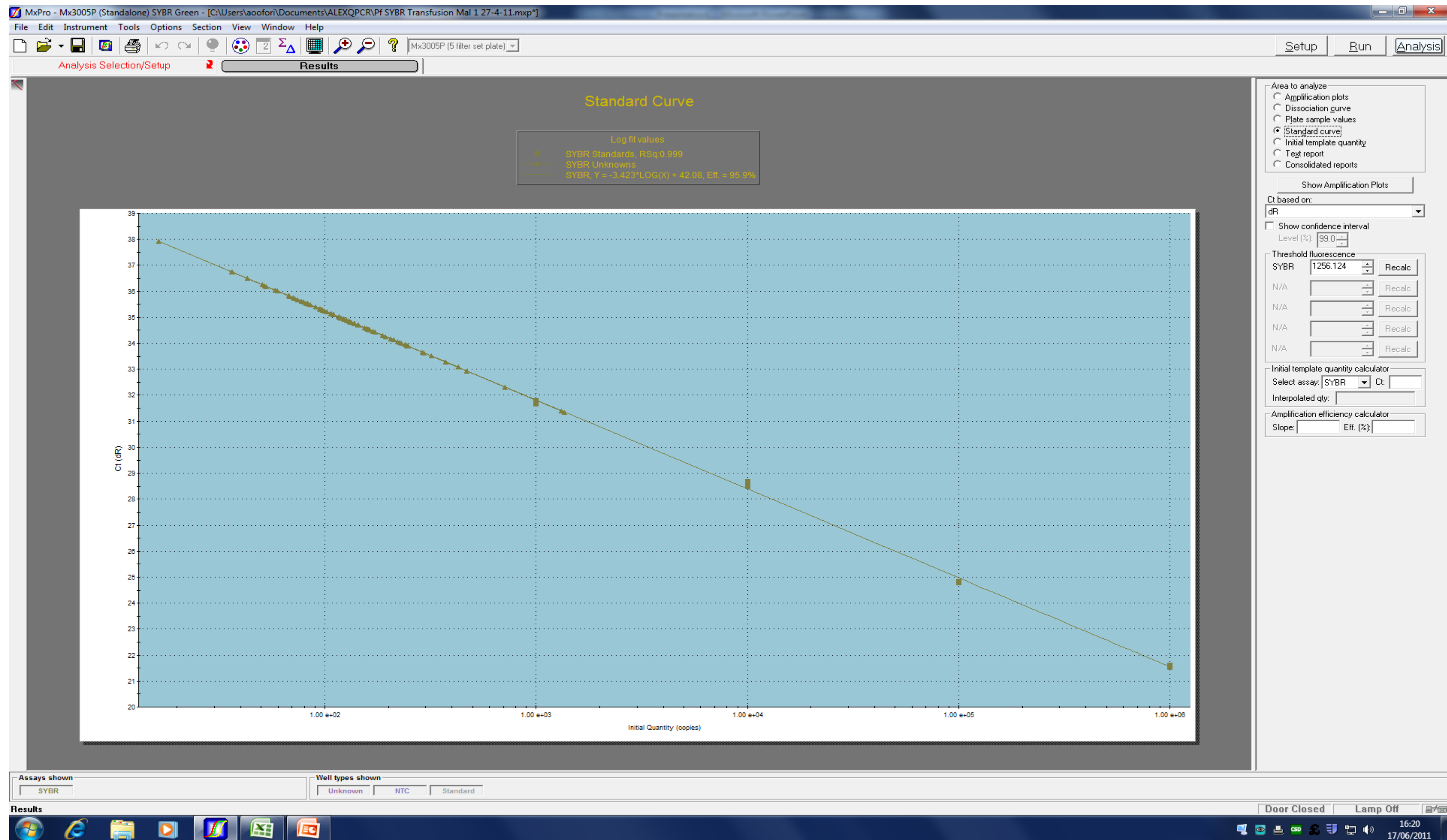
The blood was air dried but not fixed in alcohol.

The smear was then stained with 10% Giemsa stain for 15 minutes and washed with clean water.

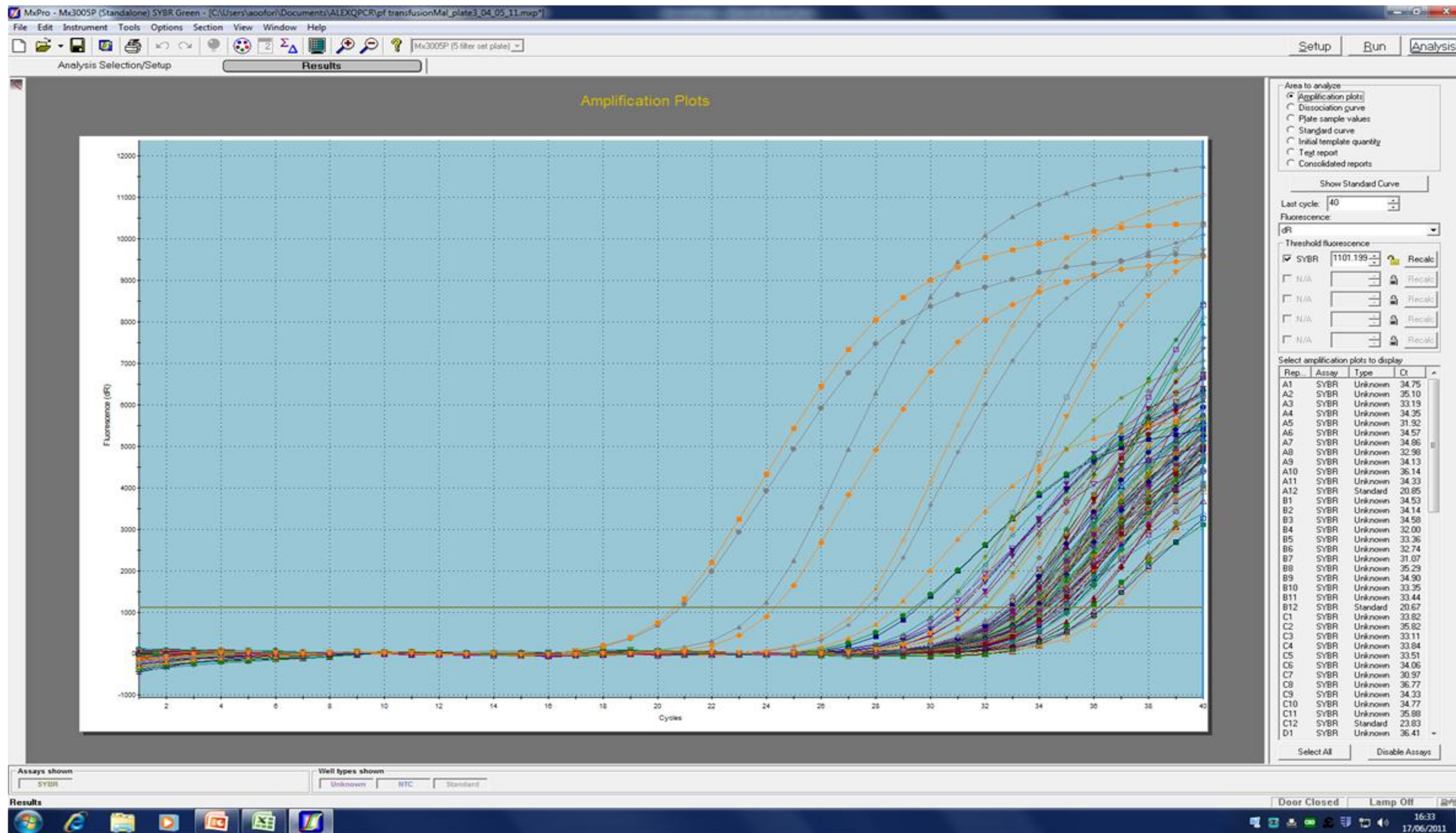
The slide was then placed on a drying rack and allowed to air dry at room temperature.

The stained blood smears were examined with 100X oil immersion objective for both think and thin films.

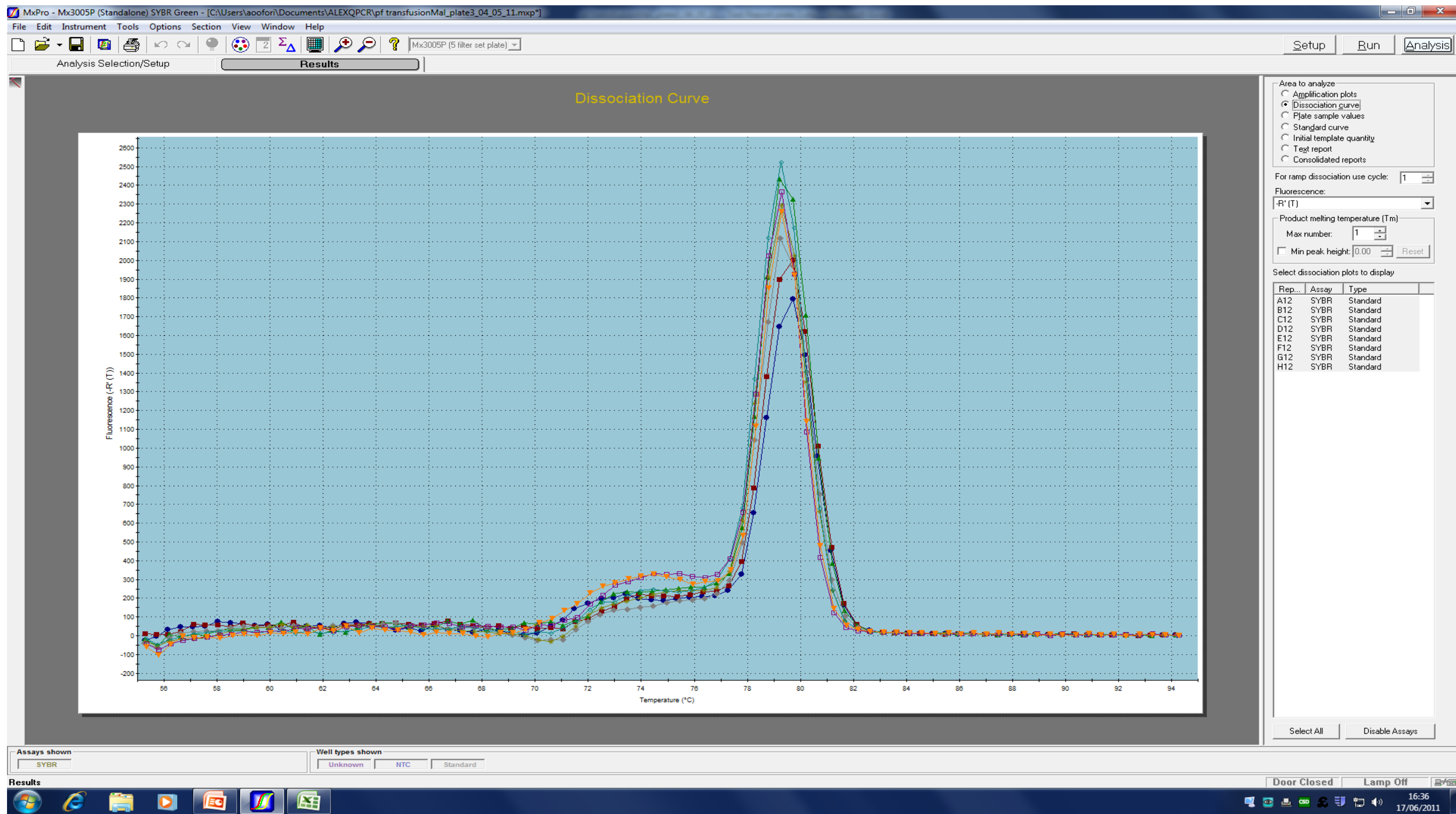
Appendix 6.4: Standard curve derived from real-time PCR assay



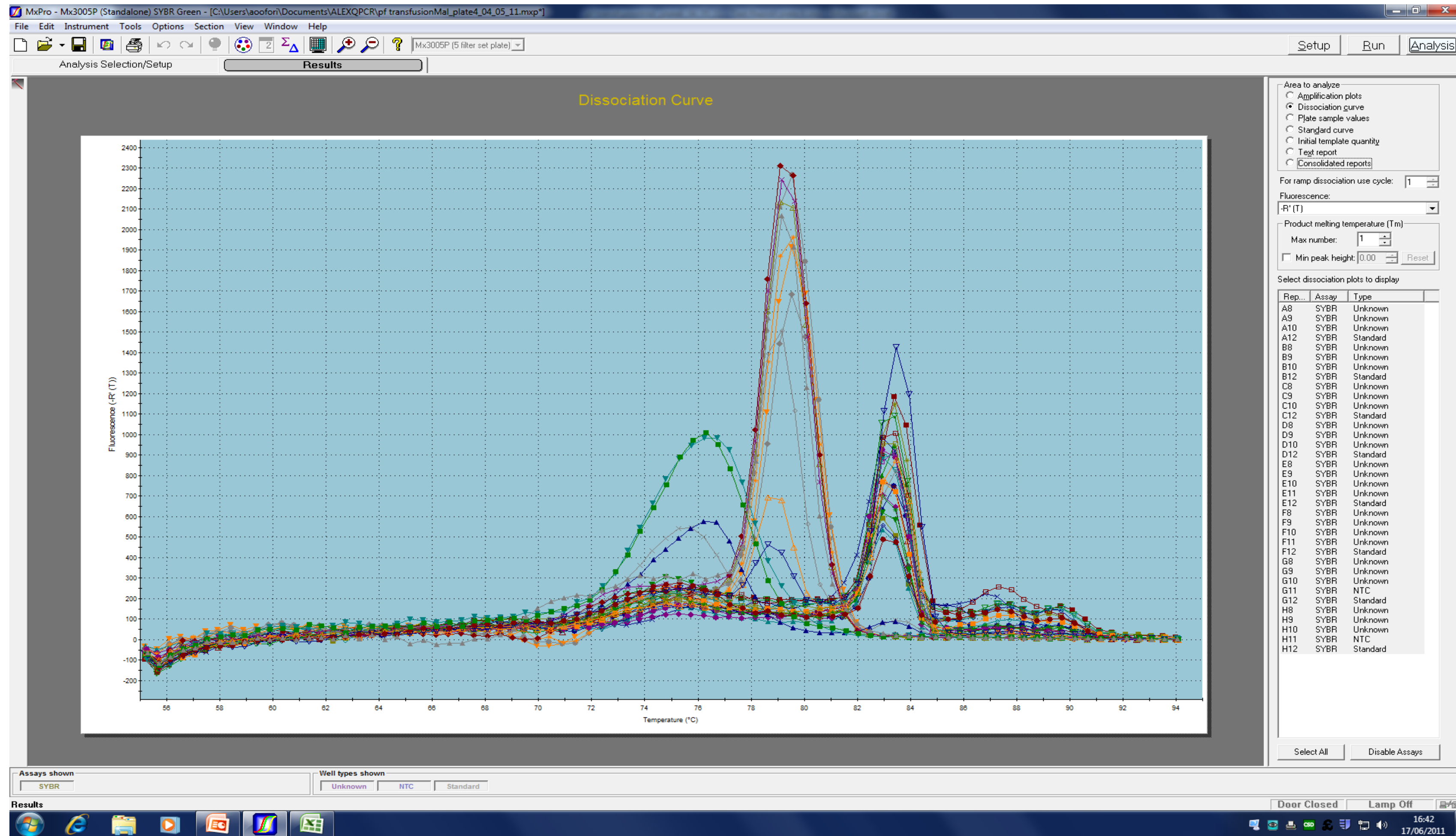
Appendix 6.5: Amplification plots for a *P. falciparum* real–time PCR assay



Appendix 6.6: Dissociation curves for standards in a *P. falciparum* real–time PCR assay



Appendix 6.7: Dissociation curve for blood samples in a *P. falciparum* real–time PCR assay



Appendix 7.1: Case report form for bacterial contamination study

Patient demographics

Name:______________________________________Department/ward_______________

Sex: [M] [F]   Age (mths):_____   Hospital number:_________________

Date of admission (dd/mm/yy): ___/___/__Date of transfusion (dd/mm/yy): ___/___/___

Initial diagnosis:____

Indication for transfusion:____

Pre-transfusion Hb or PCV:____

Donor characteristics

Date unit was collected (dd/mm/yy): ____/____/______Blood ID:___________
Date of issue (dd/mm/yy): ____/____/______
Type of donor: [voluntary] [replacement]   Sex: [M] [F]
Was blood kept in fridge before being given out?   [Yes] [No]
How many days was blood kept before being given out? __________

Monitoring of patient vital signs after transfusion

Timepoint	**Temperature**	**Respiratory rate**	**Pulse**	**Blood pressure**
Pretransfusion				
30 min post transfusion				
1 hr after transfusion				
2hrs after transfusion				
3hrs after transfusion				
4hrs after transfusion				
24hrs post transfusion				

Monitoring of signs and symptoms after transfusion; *please indicate yes or no in each box*

Sign/Symptom	**Pre-transfusion**	**Immediate Post transfusion**	**Day 1**
Headache			
Chills/rigors			
Coca-cola urine			
Palpitations			
Urticaria			
Bodily pains			
Fever			
Restlessness			
Wheezes/stridor			
Other…………..			

Total duration of transfusion (hours)____
Was sample obtained from transfusion bag?   [Yes] [No]
Has patient received antibiotics during this admission?   [Yes] [No]
If so, name of antibiotics ____

How many units of blood did patient receive? ____________________________________

Discharge diagnosis:____
____

Outcome: [alive] [died] [alive but with some sequele]
Date of discharge (dd/mm/yy): ___/___/___
How long was patient on admission (days)?_______________________________________

Did patient have a blood culture done prior to transfusion? [Yes] [No]
Was there an isolate? [Yes] [No]
What was the isolate?____
Sensitive to:____
Resistant to: ____

Day 7

Is patient still on admission? [Yes] [No]
Did patient come for visit? [Yes] [No]
Did patient receive more than 1 pint of blood during admission? [Yes] [No]
Has patient taken any antibiotics since discharge? [Yes] [No] [NA]
If yes, give details: ____
Did patient have a fever after discharge? [Yes] [No]
Is patient well? [Yes] [No]
Does patient require any further treatment? [Yes] [No]

RESULTS

Isolate from blood culture? [Yes] [No]
Name of isolate____
Sensitive antibiotics____
Resistant antibiotics____
Day of isolate: ____

Comments:____
____
____

STUDY CONCLUSION

Patient called back earlier than day 7 and referred for management due to isolate.
Patient came for follow up visit on day 7. No further action/treatment needed
Patient referred to his/her primary care physician/obstetrician/paediatrician
Contact made with patient but he/she refuses to come to hospital
Patient lost to follow-up/cant be traced
Patient came back after day 7. No further action.
Other (specify)____

Appendix 8.1: Case report form for syphilis

Patient demographics

Name:____Department/ward_______________

Sex: [M] [F] Age (yrs):_____ Hospital number:___________________

Date of admission (dd/mm/yy): ___/___/___Date of transfusion (dd/mm/yy): ___/___/___

Initial diagnosis:____

Indication for transfusion:____

Pre-transfusion Hb or PCV:____

Donor characteristics

Date unit was collected (dd/mm/yy): ____/____/______Blood ID:___________

Date of issue (dd/mm/yy): ____/____/______

Type of donor: [voluntary] [replacement] Sex: [M] [F]

Was blood kept in fridge before being given out? [Yes] [No]

How many days was blood kept before being given out? __________

Monitoring of patient vital signs after transfusion

Timepoint	**Temperature**	**Respiratory rate**	**Pulse**	**Blood pressure**
Pretransfusion				
Immediate post transfusion				

Monitoring of signs and symptoms after transfusion; *please indicate yes or no in each box*

Sign/Symptom	**Pre-transfusion**	**Post transfusion**	**Comment**
Headache			
Chills/rigors			
Coca-cola urine			
Palpitations			
Urticaria			
Bodily pains			
Fever			
Restlessness			
Wheezes/stridor			
Other…………..			

Total duration of transfusion (hours)____

Was pretransfusion sample obtained? [Yes] [No]

Was sample obtained from transfusion bag? [Yes] [No]

Has patient received antibiotics by the end of the transfusion? [Yes] [No]

If so, name of antibiotics ____

Discharge diagnosis____

Outcome: [alive] [died] [alive but with some sequele]
Date of discharge (dd/mm/yy): ___/___/___

Day 30

Did patient come for visit? [Yes] [No]
Did patient receive more than 1 pint of blood during admission? [Yes] [No]
Has patient taken any antibiotics since last admission? [Yes] [No]
If yes, give details: ____
Does patient require any further investigations? [Yes] [No]
Has blood been taken for syphilis testing? [Yes] [No]

RESULTS

Test	**Date of test**	**Donor blood**	**Pre-transfusion**	**30-day post transfusion**	**Comment**
ELISA					
TPHA					
RPR					

STUDY CONCLUSION

Patient called and told not to come for 30 day follow up
Patient came for follow up visit. No further action taken
Patient referred to his/her primary care physician/obstetrician/paediatrician
Contact made with patient but he/she refuses to come to hospital
Patient lost to follow-up/cant be traced
Patient dead
Other (specify)____

Appendix 8.2: Syphilis recall case report form

Syphilis-Recall Data Sheet

(The patient has been called back because he/she received a blood transfusion that has been identified to be serologically positive for syphilis. The patient is therefore to be assessed for any clinical evidence of syphilis)

Date (dd/mm/yy) ___/___/___ Study number____________________

Have you been unwell since your last visit? [Yes] [No]

Have you visited any clinic since you last visit here? [Yes] [No]

Have you had any of the following symptoms since your last visit?

Fever [Yes] [No] Skin lesions [Yes] [No] Itching [Yes] [No]
Headache [Yes] [No] Bone pain [Yes] [No]

Any other complaint (give details):..

Clinical examination

Muco-cutaneous lesions? [Yes] [No]
If yes, give details:
....

Generalised lyphadenopathy? [Yes] [No]
If yes, give details:
....

Any other clinical findings? [Yes] [No]
If yes, give details:
....
....
....

Sample taken for syphilis confirmation? [Yes] [No]
Patient referred to specialist for treatment? [Yes] [No]

Final Diagnosis...