Investigations of syphilis testing and of test cross reactions in Ghanaian blood donors

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By

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Dedication

This thesis is dedicated to my lovely wife Joana and my delightful children, Priscilla, Candace, and Aristarchus who through their firm support have suffered these long periods to make this dream a reality.

And to my parents (Pastor and Mrs. Alexander Gyening Mensah), who have demonstrated in my life to always trust in God and to believe that hard work and determination are keys to success.

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List of Acronyms and Abbreviations

TTI	Transfusion-transmitted infections
SSA	Sub-Saharan Africa
VNRD	Voluntary non-remunerated donors
STS	Serological testing for syphilis
KATH	Komfo Anokye Teaching Hospital
RPR	Rapid plasma reagin
RDT	Rapid diagnostic test
HIV	Human immuno-deficiency virus
HBV	Hepatitis B virus
HCV	Hepatitis C virus
WHO	World Health Organisation
EIA	Enzyme immuno assay
TPHA	*Treponema pallidum* haemagglutination assay
VDRL	Venereal disease research laboratory
NBSG	National Blood Service, Ghana
SOP	Standard operating procedure
MoH	Ministry of Health
NHIS	National Health Insurance Scheme

GHS	Ghana Health Service
FD	Family donors
SABC	Southern Area Blood Centre
TMU	Transfusion Medicine Unit
TTH-BB	Tamale Teaching Hospital Blood Bank
CABC	Central Area Blood Centre
NABC	Northern Area Blood Centre
HTC	Hospital transfusion committee
TTS	Transfusion-transmitted syphilis
CNS	Central nervous system
CFS	Cerebrospinal fluid
MSM	Men having sex with men
CDC	Centre for disease control
AIDS	Acquired immune deficiency syndrome
STI	Sexually transmitted infection
USA	United States of America
IgM	Immunoglobulins M
IgG	Immunoglobulins G

TPIA	*T. pallidum* immune adherence
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid
FTAAbs	Fluorescent treponemal antibody absorption
TPI	*T. pallidum* immobilization
CLIA	Chemiluminescence immunoassay
HRP	Horseradish peroxidase
CMIA	Chemiluminescent microparticle immunoassay
RLU	Relative light units
ICS	Immunochromatographic strip
TPPA	*T. Pallidum* Particle-agglutination Assay
NYCP	National Yaws Control Programme
RFLP	Restriction fragment length polymorphism
NIH	National Institutes of Health
QC	Quality control
LIA	Line immuno assay
KNUST	Kwame Nkrumah University of Science and Technology
CHRPE	Committee on Human Research, Publications and Ethics

REC	Research and Ethics Committee
LSTM	Liverpool School of Tropical Medicine
T-REC	Transfusion research capacity
EQA	External quality assessment
IQA	Internal quality assessment
LMIC	Low and middle-income countries
PPV	Positive predictive value
TPA	*Treponema pallidum* antibody
QA	Quality assessment
EDTA	Ethylenediaminetetraacetic acid
TCT	Total community treatment
TTT	Total targeted treatment

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Abstract

Blood transfusion is an important element of health care which saves millions of lives each year but has life-threatening risks. In low-resource countries in Africa, a significant proportion of donated blood remains unsafe if blood units are not screened appropriately for all major transfusion-transmitted infections (TTIs). Syphilis, one of the TTIs, remains a public health problem in developing countries including Ghana if testing is not performed in a well-controlled manner. Thus, there is the possibility of infecting recipients with syphilis which may have long-term sequelae.

The study investigated syphilis testing practices in transfusion facilities in Ghana, test cross-reactions between various techniques, and the potential infectivity of 'true' positive blood samples, in the Ghanaian blood donor population. The goal of the research was to guide transfusion policies and practices, and advance transfusion medicine research in Ghana by improving syphilis testing. Data collection was in three parts based on five specific objectives. The first part was a survey, carried out in 149 transfusion facilities in Ghana and found a syphilis seroprevalence of 3.7% for blood donors. The laboratories used predominantly non-approved test kits at variable costs. In the second part, using rapid diagnostic test, Fortress RDT, and rapid plasma reagin (RPR), syphilis testing was performed on 16,016 prospective blood donors who came to donate blood for the Komfo Anokye Teaching Hospital (KATH) blood bank. Positive predictive values of the RDT and RPR were found to be 90.9% and 97.1% respectively. The third part was a quality assessment to determine the performance of the frequently used syphilis test in Ghana, the ABON RDT, in four selected facilities. Sensitivity and specificity were found to be 99.2% and 82.5% respectively. Finally, we determined the relative proportions of confirmed syphilis antibody positive donors with clinical histories attributable to yaws rather than syphilis.

Our data from the surveys showed a considerable mismatch between recommendations and practice of syphilis testing in Ghana, with potential serious consequences for blood safety and public health. This study found that the combination of syphilis RDT and RPR which have a relatively good PPV, as a novel strategy, could contribute to improving blood safety when screening blood donors for syphilis. It was confirmed that quality systems for syphilis testing are generally weak in Ghana, but are important for any laboratory or testing site to ensure accuracy, consistency, and reliability of test results which directly contributes to the safety of blood supply.

Finally, the question, whether some of the syphilis positivity in blood donors could be attributable to yaws rather than syphilis remained speculative.

CHAPTER 1

INTRODUCTION

1.1 Transfusion of unsafe blood

Blood transfusion is an important element of health care which saves millions of lives each year. Every second, someone in the world needs blood for surgery, trauma, severe anaemia or complications of pregnancy. However, millions of people are exposed to avoidable, life-threatening risks from the transfusion of unsafe blood every year [1]. About 7.5% (6/81 million units) of blood collected annually in 178 countries are not tested for transfusion-transmissible infections (TTI) [2]. In low-resource countries in Africa, a significant proportion of donated blood remains unsafe as it is either not screened for all major TTIs or not in a quality-controlled manner [3]. Particularly in sub-Saharan Africa (SSA) blood screening programmes have limitations, and safety from infection risk can often not be guaranteed. Thus, while blood transfusion can be life-saving, there are associated risks, including TTIs.

Syphilis is a chronic, systemic infection, caused by an organism called *Treponema pallidum* subspecies *pallidum.* It affects large numbers of people through sexual transmission, from mother to offspring and via blood transfusion. Being one of the TTIs, it represents a major public health problem because of its worldwide occurrence in the most neglected and troubled regions. The past five years have seen a number of outbreaks in many countries involved in blood donations [4-8].

Serologic tests are the most common tests used for screening for syphilis. This is because *Treponema pallidum* cannot be cultured in vitro. Additionally, *T. pallidum* nucleic acid amplification tests are not widely available for use by clinical laboratories. There has been development of rapid treponemal-based syphilis tests which was driven by the need for simple,

point-of-care tests in resource-poor countries and this will be discussed further in the coming chapters.

1.2 Syphilis prevalence

Treponema pallidum, the aetiologic agent of syphilis [9], is prevalent but rarely transmitted via blood transfusions in developed countries because the blood supply is safe due to a combination of donor education, donor screening, willingness to donate, treatment, safe sex, and strict laboratory testing procedures. Thus, the risk of TTI is extremely low in these countries. However, syphilis infection is much more common in developing countries where prevalence can reach 23.5% amongst blood donors [10]. In Ghana, data on *T. pallidum* seroprevalence are scanty, although antibodies are thought to occur as frequently as HBV antibodies [11]. Published studies from Ghana show syphilis seroprevalence of between 7.5% and 13.5% [7,12]. For example, the Central Area Blood Centre in Accra, which serves the Korle Bu Teaching Hospital and its environs in Ghana, has shown an increasing trend in the prevalence rate of syphilis infection from 1.23%, to 2.89% and 4.86% respectively among blood donors in 2009, 2010 and 2011, in performance reviews respectively [13].

Yaws is a neglected non-venereal endemic treponematosis caused by the bacterium *Treponema pallidum* subspecies *pertenue* and spread by skin to skin contact [14]. It often starts as a single lesion of swelling on the skin but may progress to multiple lesions all over the body. In the late stages, if untreated, the disease can progress to cause destructive lesions of bones and cartilage leading to disabilities and disfigurement in 10% (figure 7.1) [14]. It is closely related to syphilis [15] and the cross-reactivity between them leads to problems for blood services since yaws may cause false positive screening tests for syphilis. Both syphilis and yaws are distinguishable by epidemiological characteristics and clinical manifestations. Yaws is mostly seen in children below the age of 15 years, whilst syphilis affects individuals of any age. A primary chancre on mucous membranes is

seen in individuals with syphilis but not in yaws, where the primary lesion is in the skin. This comparison between yaws and syphilis is important for blood services since they need to distinguish between these two conditions in the absence of a specific differentiating test, and will be explained further in the next chapter.

1.3 Problem Statement

Delivery of safe blood for transfusion is a big challenge for countries in SSA including Ghana. A contributing factor to this is high rates of donor deferrals, which adds to difficulties in retaining voluntary non-remunerated donors (VNRD). Blood donors who test positive for syphilis may contribute to the lack of safe blood in low resource countries so it is important to try and differentiate between donors who are infective and should be excluded, and those who are non-infective but screen positive during donor testing. Cost of reagents and labour must be considered when determining the best strategy for syphilis testing [16]. Although syphilis testing of blood donors is recommended and mandatory in many countries, the cost-effectiveness may be questionable. Firstly, blood donors who are positive for syphilis antibodies may not be infectious either because they do not carry active infections or because they are false positives, e.g. not infected with syphilis. Secondly *T. pallidum* is less infectious after cold storage, rendering the risk of transmission through transfusion via red blood cells or plasma components very low [17].

Serological testing for syphilis (STS) is a principle of measuring either specific (anti-*T. pallidum* antibody detection) or non-specific (anti-lipoid antibody detection) antitreponemal antibodies which will be explained further in the next chapter. The advantage of specific syphilis antibody testing is the relatively high sensitivity and specificity. However, for the screening of blood donors in an area with high prevalence of syphilis antibody reactivity, the disadvantage is that specific syphilis tests stay positive persistently after the infection has been cured. However, the

identification of donors carrying previous syphilis infections may be useful in many settings as syphilis positivity may be a marker of high-risk sexual behaviour. Unfortunately, in West Africa where yaws is prevalent [18], it can be assumed that previous yaws infections may also contribute considerably to the high proportion of blood donors who screen positive for syphilis. Treponemal tests are commercially available in the formats of rapid diagnostic testing with minimal cost, minimal training and equipment requirement and availability of results within 5-20 minutes [19]. However, positive results of these rapid syphilis tests need confirmation with quantitative non-treponemal testing to determine whether there has been recent infection and the response to treatment.

The present study investigates the processes and variations in syphilis testing and the associated testing costs in Ghana. Recently, there was a case of syphilis transmission through blood transfusion in Ghana where an 8-year-old girl with severe malarial anaemia showed seroconversion after receiving a syphilis reactive unit of blood that had been refrigerated for only 1 day [6]. With data from Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana, the project explores the value of rapid diagnostic testing of blood donors for syphilis. The approach to the study was as follows: the positive predictive values of syphilis tests in routine use in blood donor screening in Ghana (i.e. donors being truly syphilis antibody positive with potential active infections) was determined by the combination of subsequent composite gold standard testing and unspecific rapid plasma reagin (RPR) testing. Donors that tested positive on initial screening, but did not test positive in subsequent gold standard testing were considered false positives whereas donors testing negative in RPR testing but positive with initial testing were considered previously infected only. Finally, among donors that were truly syphilis antibody positive, the study examined

to what degree previous syphilis or yaws could be suspected as the cause of the positive result in the individual donors.

The data generated shall serve as evidence not only to guide transfusion policies, practices and ensure quality of transfusions, but would hopefully also be of importance in the advancement of transfusion medicine research in Ghana and beyond.

1.4 Justification for the Study

Over the years, much controversy has arisen over the need for syphilis testing of blood donors [20]. In most of the transfusion centres in Ghana, syphilis testing is not done, probably because it is believed that stored blood renders the organism that causes syphilis non-viable. At KATH, which was one of the study site, between 18,000 and 20,000 blood donors donate blood every year with 70% being voluntary non-remunerated donations and the rest being family/replacement donations. Routine pre-donation testing with rapid diagnostic tests (RDT) of all blood donors for human immuno-deficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) have been performed for more than 15 years [21,22], whereas syphilis testing only started in June 2013. It is recommended that syphilis unscreened blood is stored for period >72 hours [17] because *T. pallidum*, the causative agent of syphilis, cannot withstand the lower temperatures (between 2 and 6 degrees Celsius). Unfortunately, in many countries where blood shortages are common, units of blood that are collected from donors typically do not stay in the blood bank fridge for more than 48 hours because of the demand for it [6]. The consequence is that patients who receive these unscreened units of blood may get infected with syphilis.

Furthermore, other spirochaetes like *T. pallidum ssp. pertenue* causing yaws may be present in blood in the early latent seropositive stage, when the microbes just become detectable causing strong cross reactivity [23].

The WHO recommends that syphilis testing is performed before transfusion, and that enzyme immuno assay (EIA) and *T. pallidum* haemagglutination assay (TPHA) are used as specific tests, while venereal disease research laboratory (VDRL) and RPR are used as non-specific tests [24]. For quality and consistency, the policy of the National Blood Service, Ghana (NBSG), advocates streamlining of purchasing test kits and their validation before use, adherence to the standard operating procedures (SOPs) for laboratory testing and implementation of effective TTI guidelines of donor care [25]. This will increase proper documentation and effectiveness for blood safety. Almost all the blood banks in the country screen for HIV, HBV and HCV, but only a small and (prior to this study) unknown proportion screen for syphilis

1.5 Research Questions

1. What is the existing syphilis testing practice of transfusion facilities in various parts of Ghana?
2. What is the positive predictive value of the RDT that is used most commonly in KATH and Ghana for syphilis testing?
3. What proportion of syphilis reactive blood donors are really infectious through transfusion?
4. What is the performance of a predominant syphilis RDT as used in some selected transfusion facilities in Ghana?
5. Could some of the syphilis positivity in blood donors be attributable to yaws rather than syphilis?

1.6 Study Objectives

1.6.1 Main Objective

To investigate syphilis testing practices in transfusion facilities in Ghana, test cross reactions, the potential infectivity of 'true' positives in the Ghanaian blood donor population, and to make recommendations in guiding transfusion policies and practices.

1.6.2 Specific Objectives

1. To investigate syphilis testing practices of transfusion facilities in various parts of Ghana.
2. To determine the positive predictive value of the RDT that is most commonly used in KATH and Ghana for syphilis testing.
3. To estimate the proportion of blood donors in KATH, Kumasi who are really infectious through blood transfusion.
4. To determine the performance of a predominant syphilis RDT in selected transfusion facilities in Ghana.
5. In confirmed syphilis antibody positive donors, determine the relative proportions of those with clinical histories attributable to yaws rather than syphilis.

1.7 Summary

In this chapter, an overview of syphilis testing in blood donors worldwide and the current status of syphilis-related blood transfusion safety in SSA including Ghana have been presented. The problem statement, justification, research questions, and study objectives have also been presented. In the next chapter, a background and review of the literature on syphilis prevalence and testing worldwide with emphasis on Ghana is presented.

1.8 Thesis outline

As stated earlier, chapter one presented an overview of syphilis testing in blood donors worldwide and the current status of syphilis-related blood transfusion safety in SSA including Ghana.

Chapter two provides a background and review of the literature that this thesis is based upon. A brief summary of the study background, the aetiology of the causative organism, stages of infection of the disease, global syphilis epidemiology, syphilis detection by various techniques, prevalence of syphilis infection in blood donors, various recommendations for syphilis testing, pathophysiology of yaws, and finally the comparison of syphilis and yaws are presented. The review also examines how various screening methods and tests are applied in SSA.

Chapter three focuses on the conceptual framework of the study and ethical considerations. Chapter four surveys syphilis testing practices in transfusion facilities in Ghana, discussing policy and practice of syphilis testing in the country. Chapter five focuses on improving syphilis testing of blood donors with RDT and RPR in KATH, Kumasi, with emphasis on calculating positive predictive values of these test kits to improve blood safety. Chapter six is a quality assessment study of a selected syphilis RDT performed at four selected transfusion sites in Kumasi. Chapter seven focuses on the relationship of yaws and syphilis, potential transmission of yaws via transfusion, and describes the recalled medical history, clinical manifestations, and treatment of yaws and syphilis by syphilis seroreactive blood donors when these donors were interviewed. Chapter eight gives the general conclusion and recommendations of the study.

CHAPTER 2

Background and literature review

2.1 Study background

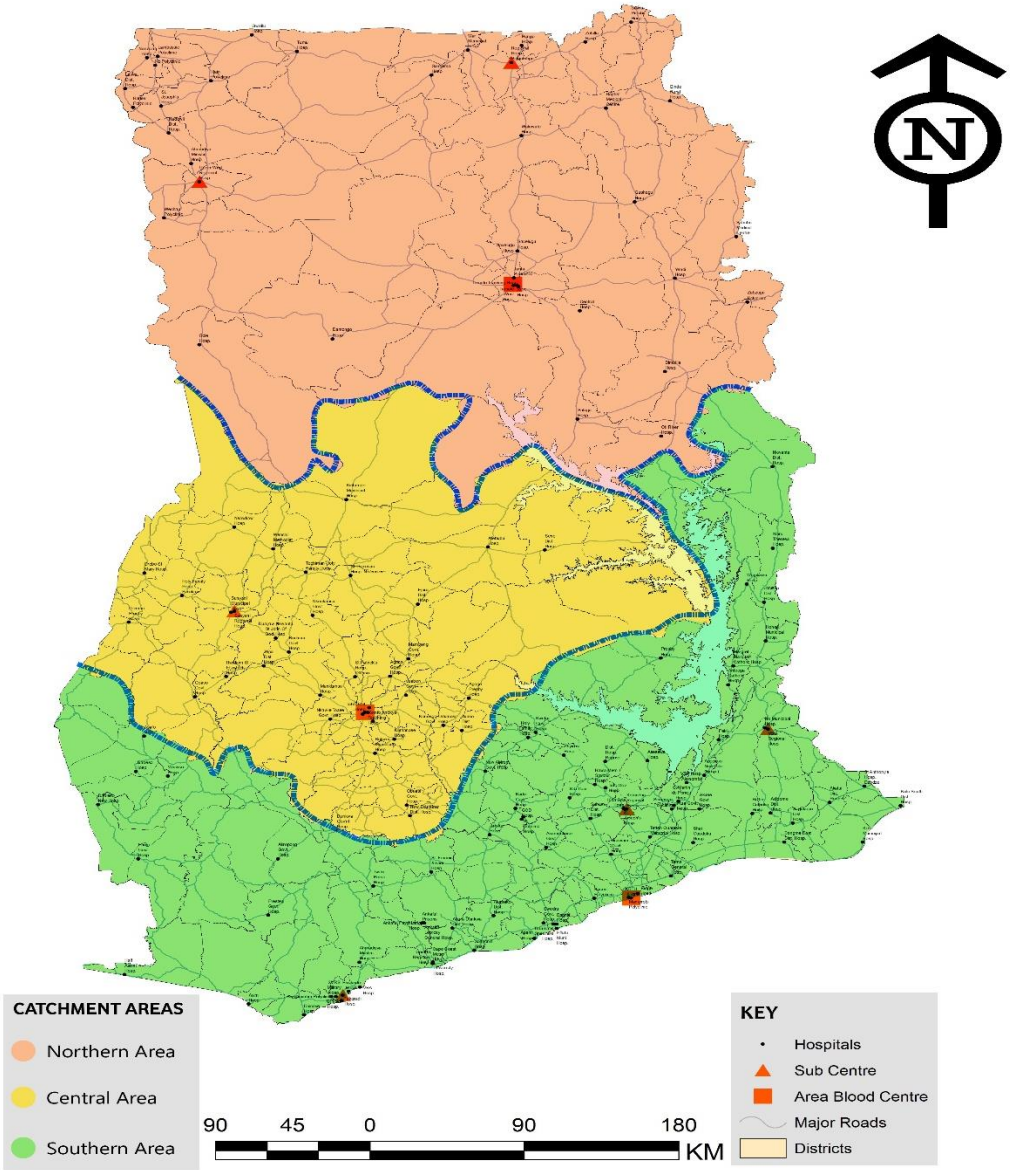
2.1.1 Ghana as study location

Ghana, a country of a population of 28,308,301 as of 2016 [26] officially called the Republic of Ghana, is a sovereign multinational state and unitary presidential constitutional democracy which is situated along the Gulf of Guinea and the Atlantic Ocean, in the sub-region of West Africa. It has a land mass of 238,535 km^2, with 2,093 kilometres of international land borders. It shares borders with Ivory Coast to the west, Burkina Faso to the north, Togo to the east and the Gulf of Guinea and the Atlantic Ocean in the south.

The Ministry of Health (MoH) is responsible for the health of Ghana. It is involved in providing public health services, managing Ghana's healthcare industry, and building Ghana's hospitals and medical education system. In 2013, the Government of Ghana spent US$63 annually on each person's health in order to provide basic health services [27]. The planned 2016 budget decreased this to US$31[28], less than half the target. Because the government does not spend enough, the burden of paying for health falls heavily on households. Despite the National Health Insurance Scheme (NHIS) which aims to accomplish universal health insurance coverage in Ghana [29], 67% of all health spending in the country was spent by households up-front, e.g. out-of-pocket expenditure, without insurance, in 2014 [30]. This has more than doubled since 2011[31], and the poorest in Ghana are likely to be excluded from care or pushed further into poverty by unsustainable payments. In addition, despite pregnant women in Ghana having a subsidized health insurance, a recent report has found that pregnant and postpartum women registered with the NHIS

continue to pay for services including medicines, ultrasound scans, and some laboratory tests including blood products for transfusion [32].

Figure 2.1 Map of Ghana showing catchment areas of Blood Centres



Under the MoH is the Ghana Health Service (GHS) a government body established in 1996 as part of the Health Sector Reform of Ghana. The Health service principally administrates the health

services provided by the government and in implementing government policies on health. Some of the functions of the GHS are to promote a healthy style of living and good health habits by people in Ghana and to establish an effective mechanism for disease surveillance, prevention, and control in Ghana. The MoH furthermore is ultimately responsible for the safety and adequacy of the blood supply in the country and takes measure to secure Government commitment and support for the National Blood Programme.

2.1.2 National Blood Service, Ghana

The National Blood Service, Ghana (NBSG) is one of the newest agencies under the MoH. The NBSG provides blood and blood products, as well as in relation to research and training. The NBSG is responsible for ensuring a coordinated national approach to the provision of safe, adequate and efficacious blood and blood products, making it timely, available and affordable to all patients requiring blood transfusion therapy in both public and private health care institutions in the country [25]. The strategy of the blood service is to be centrally coordinated and have a network of area centres, well-distributed in the country to provide effective coverage. There has been several TTIs that have been studied in Ghana, and these include HIV, HBV, HCV, Human erythrovirus B19, West Nile virus, GB virus, and malaria[33-38]. Currently, Ghana has 4 major TTIs that are screened for prior to transfusion namely HIV, HBV and HCV, which are screened at all blood transfusion facilities, whereas syphilis, is reportedly screened in 20% of the facilities [39]. According to the NBSG annual report in 2007, the average national prevalence rates in donors for HIV, HBV, HCV and syphilis were 1.27%, 10.14%, 0.99% and 1.48% respectively [39] with higher seroreactivity among family donors (FD) than voluntary non-remunerated donors (VNRD). Presently, the NBSG comprises a National Headquarters and three (3) Area Blood Centres (ABCs) – Southern Area Blood Centre (SABC) located at Korle-Bu Teaching Hospital (KTH), Accra;

Central Area Blood Centre (CABC) located at Komfo Anokye Teaching Hospital (KATH) in Kumasi; and Northern Area Blood Centre (NABC) at the Tamale Teaching Hospital (TTH).

The NBSG coordinates the activities of one stand-alone blood centre, the SABC which is located in Accra in the southern zone of Ghana and of over 149 hospital-based blood collection points. The SABC is responsible for procuring and supplying blood and blood products to the five southern administrative regions of Ghana with a total population of 13.04 million. The Transfusion Medicine Unit (TMU) of the Komfo Anokye Teaching Hospital (KATH) and the Tamale Teaching Hospital Blood Bank (TTH-BB), function as blood centres in the middle and northern zones of Ghana, and form the pivots for the proposed Central Area Blood Centre (CABC) and Northern Area Blood Centre (NABC) of the NBSG respectively.

2.1.3 Ghana national blood policy

The national policy for the blood service of Ghana provides directives to guide the MoH to ensure safety, efficacy, and adequacy of blood and blood products for all patients in all health institutions of the country both public and private, making it accessible and affordable. The Ghana national blood policy which was approved by cabinet in 2006 stated that all blood units collected must be tested prior to transfusion for TTIs and any other microbial diseases that may be thought to be relevant, using approved well-controlled techniques and procedures in accordance with WHO guidelines [25]. Furthermore, the NBSG is responsible for purchasing approved test kits, and for distributing them to the various transfusion facilities before use. The policy additionally indicated that detailed methods and standard operating procedures (SOPs) should be available for all activities of the NBSG.

2.1.4 Testing of donated blood in sub-Saharan Africa (SSA) with emphasis on Ghana

Blood units that are donated can be lifesaving for individuals who have lost blood because of accidents or surgery, as well as for people who have become severely anaemic through medical conditions and/or treatments.

Testing donated blood in SSA for infectious diseases helps to maximize the safety of blood donation for both the donor and the recipient. Laboratory testing of donated blood preceding transfusion is intended to ensure that recipients receive the safest possible blood products. Depending on the transfusion testing site requirements, all infectious disease screening assays must be nonreactive in order to release the blood unit or its components to hospitals for transfusion [1].

In some transfusion facilities in Ghana, syphilis testing is not done because it is believed that storing blood renders the organism that causes syphilis non-viable [40]. Most of the facilities also have challenges in terms of procuring reagents for testing. But if testing for diseases like syphilis prior to transfusion is not done or not accurately done, there is the possibility of infecting recipients with syphilis with consequent potential long-term sequelae.

2.1.5 Study areas

While Ghana is the area of study regarding chapters 1-4 and 7, and has been described in the foregoing, chapters 5, 6 and 8 relate to the Kumasi area.

Kumasi is the regional capital of Ashanti region. The population of Kumasi metropolis (1,730,249) depicts a broad base population pyramid which tapers off with a small number of persons who are elderly (60 years and older). The age dependency ratio for the Metropolis is 58; the age dependency ratio is 59.9 for males and 56.3 for females [41].

The main occupations in Kumasi are professional occupations such as services and manufacturing whilst the major export items are gold, hardwood, and cocoa. Of the employed population, 38.9 percent are in the service and sales work, 22.8 percent are in craft and related trades, 10.3 percent are into elementary occupation, 2.6 percent are skilled agricultural forestry, and the rest are fishery workers [41]. Public transport in the city is provided by transit buses, a mix of privately owned mini-buses known as Tro-Tros, taxicabs, and buses. However, when blood donation is organized by transfusion facilities, a majority of the blood donors rely on transit buses and Tro-Tros which are affordable.

2.1.6 Study site

The Komfo Anokye Teaching Hospital (KATH) in Kumasi, the study site, is the second-largest hospital in Ghana, and the only tertiary health institution in the Ashanti Region. KATH is the main referral hospital for the Ashanti, Brong Ahafo, Western, Central and Eastern regions of Ghana. The geographical location of the 1200-bed KATH, the road network and commercial nature of the city make the hospital accessible to all the areas that share boundaries with Ashanti Region and others that are further away.

The vision of the hospital is to become a medical centre of excellence which offers clinical and non-clinical services of the highest quality standards comparable to any international standards.

KATH manages blood transfusions which are required for several reasons;

- Most patients, who have a major surgical procedure, will have a blood transfusion to replace any blood loss during their surgery.
- Some patients have blood transfusion due to serious injuries from car crashes or natural disasters.

- Other individuals with an illness that causes anaemia, such as malaria, sickle cell disease, obstetric bleeding, leukaemia or kidney disease will often be the recipients of blood transfusion.

2.1.7 Transfusion Medicine Unit

The transfusion medicine unit (TMU) of KATH was created in 2002 as an autonomous entity operating under the Medical Director of the hospital. Although managed by the hospital, this unit is closely connected with the NBSG regarding regulations and recommendations applied to staff training, donor selection and quality of products. It provides blood units primarily to KATH but also to other public and private health care settings in Kumasi and its environs.

The functions of TMU are defined as follows:

1. To provide blood transfusion services to KATH and other health care facilities in Northern Ghana.
2. To provide clinical support regarding indications of blood products and monitoring adverse transfusion reactions.
3. To train medical and paramedical staff on blood relating issues.
4. To audit clinical use of blood products.
5. To conduct research with the aim of improvement of blood-related therapies at KATH and Ghana in its entirety.
6. It also has a role in surveillance of infectious diseases in the hospital such as HIV.

The TMU performs laboratory tests for multiple infectious disease markers on every unit of donated blood. These tests include: HBV, HCV, HIV 1, 2 and Syphilis (*Treponema pallidum*). The unit has been practicing pre-donation screening with viral RDTs for blood donors since 2000

[22,42], managing approximately 18 000 blood donations tested on site a year currently. KATH relies on two types of blood donors namely; voluntary non-remunerated donors (VNRD) constituting 70% of blood collection, and family/replacement donors (FD) constituting 30% of blood collection for over a decade now according to 2015 annual performance review. A high proportion of blood donors are students from secondary or tertiary institutions, and blood shortages can occur during vacations, examination periods in addition to high seasons of malaria.

2.1.8 KATH transfusion committee

The hospital transfusion committee (HTC) systematically scrutinizes blood supply, blood safety, donor care, clinical use of blood products, and costs. This committee has been influential in initiating and welcoming new screening strategies, showing the critical role such committees can play in the implementation of evidence-based measures to improve blood safety and availability even when resources are limited [43]. In July 2014, pre-donation screening of blood donors for syphilis was introduced with an anti-treponemal rapid diagnostic test (RDT; Fortress Quick Test, Fortress Diagnostics, UK). This was because it was realized that refrigerated storage times of donated blood was shorter than those recommended to inactivate syphilis organisms, due to the high demand and turnover, and because a study in the hospital had reported syphilis seroreactivity of 8% [6]. The introduction of this syphilis testing in a preliminary study, demonstrated a seroreactivity rate of 7.0% and the overall deferral rate for all TTIs increased from 9.5% to 16.5%. This had a critical and negative effect on the blood supply for the hospital. The TMU, therefore instituted a novel and pragmatic syphilis screening algorithm, where all initially syphilis seroreactive blood units are tested further with nontreponemal test to detect suspected active infections, to balance improved blood safety with the protection of the blood supply [44].

2.2 LITERATURE REVIEW

2.2.1 Pathogenic treponemes

Pathogenic treponemes are related with the following 4 diseases:

- Venereal syphilis, caused by *T. pallidum spp. pallidum*
- Yaws, caused by *T. pallidum spp. pertenue*
- Endemic syphilis (bejel), caused by *T. pallidum spp. endemicum*
- Pinta, caused by *T. carateum*

The treponemes responsible for these diseases cannot be distinguished serologically and morphologically, and they have not been successfully cultivated on artificial media because the bacteria cannot be cultured in vitro. Of the many spirochaete–like organisms, the only pathogenic one that has proven capacity to infect humans via transfusion is *T. pallidum.*

2.2.2 Aetiology of the disease

Syphilis, once known as the Great Pox, is a chronic, systemic infection, and the causative organism, *Treponema pallidum* subspecies *pallidum* is a spirochaete, under the Family Spirochaetaceae of the Order Spirochaetales. Pathogenic members of this genus consist of *T. pallidum, T. pertenue,* and *T. carateum*. The genus name, *Treponema*, is a derivative of the Greek term for "turning thread". The *T. pallidum* organism is a spiral-shaped, extremely mobile, Gram-negative bacterium and a helical to sinusoidal bacterium which ranges from 5 to 15 microns in length [45]. It consists of an outer envelope and a protoplasmic chamber, between which lie axial threads (flagella) [46]. It multiplies by binary transverse fission and normally enters the body through mucous membranes. Between 1905 and 1910, Schaudinn and Hoffman identified the organism *T.*

pallidum as the causative agent of syphilis, and Wasserman described it with the demonstration of spirochaetes in Giemsa-stained fluid from syphilitic lesions [47].

The disease is mostly transmitted by sexual contact with a person who has been infected or congenitally, but transmission has also been reported through blood and blood products and intravenous drug use [48]. Several hypotheses have been put forward to explain the low number of documented transfusion-transmitted syphilis (TTS). TTS was first described in 1915. By 1941, a total of 138 cases had been defined in the literature [49]. It was the first transfusion-transmitted disease described and was reported before 1950 [50]. Because of the inability to culture the organism and the restrictions of direct microscopy, serological testing has been the basis of laboratory diagnosis [51].

2.2.3 Pathophysiology of syphilis

Syphilis is usually contracted during sexual intercourse by the passage from genital lesions of one partner through the skin or mucous membranes of the other partner. The organism multiplies at the primary site of infection [52] in local lymph nodes within minutes and systemic dissemination within hours [47]. Within few days, the spirochaetes disseminate by the invasion of the local lymph nodes causing swelling (lymphadenopathy) followed by seeding to the bloodstream in large numbers [52].

If left untreated, the infection progresses, with varied and often delicate clinical manifestations, which can result in potentially life-threatening cardiovascular and neurological disease. Syphilis is untreated a lifelong infection that progresses in several stages based on its clinical presentation, infectivity, and progression. It can present in one of four different stages: primary, secondary, latent, and tertiary [53]. Blood donors at any stage of disease, including late, latent syphilis, can transmit the infection [54].

2.2.4 The primary stage

This stage is defined by an ulcer (also called a chancre) at the site of inoculation. The primary chancre can be found in up to 97% of infected individuals, although up to 60% may not remember having had a lesion [55]. The chancre is a firm painless ulceration, 5-15 mm, sharply demarcated, and usually appears 3 to 90 days (average, 21 days) after contact [56]. Chancre progression from primary to secondary disease occurs in approximately 46 days (range 30 - 70 days) [56]. The primary lesion, which contains infectious treponemes, arises within hours after infection and can persist throughout primary and secondary stage of the disease. However, lesions usually heal spontaneously without treatment within 6 weeks although lymphadenopathy persists [52].

2.2.5 The secondary stage

If untreated, the spirochaete begins migrating to the bloodstream, and the disease progresses to the secondary stage 6 to 12 weeks after exposure (2 - 8 weeks after development of the chancre), with a corresponding vigorous antibody response [57]. This stage is characterized by a polymorphic rash, lymphadenopathy, and other systemic manifestations, which develop 6 weeks to several months after untreated primary syphilis. This stage of rapid spirochaete multiplication and dissemination may bring an invasion of the entire body. Once the organism has invaded the bloodstream, it continues to infiltrate all organs and body fluids [58]. Peak spirochaetemia with high organism load and general infection occur during the secondary stage of syphilis infection [55]. The secondary stage is the most clinically apparent which may usually involve the scalp, palms, and soles and is characterized by an acute infection symptom, caused by multiplication and systemic dissemination of the organism. During secondary syphilis, spirochaetes are cleared from the bloodstream, perhaps by a lytic process that involves complement [55]. The untreated secondary disease lasts a mean of 3.6 months, with a range of 1 to 12 months [56]. In 50% of cases, the patient

will no longer be infectious or exhibit any symptoms and will be considered cured. The other 50% will go on to a latent phase [52].

2.2.6 The Latent stage

A variable asymptomatic latent period (serologic evidence without any signs) follows, which for epidemiologic purposes are divided into early (<1 year) and late (>1 year) stages. Early latent syphilis is when the persistent abrasions of secondary syphilis are most likely to occur. No relapses occur after the first year; what follows is late syphilis, which may be either asymptomatic (i.e. late latent) or symptomatic (i.e. tertiary). Late latent syphilis is associated with resistance to both reinfection and relapse where syphilis is non-infectious but the bacteria can remain inactive in the lymph nodes and the spleen. In this stage, a positive serologic test for syphilis will be the only indicator of infection [56]. There are no cutaneous manifestations or clinical symptoms, but the organism may be actively replicating in target organs like the heart, eyes, brain, nervous system, bones, and joints.

Although most patients with late latent syphilis remain asymptomatic, one-third progress to tertiary syphilis 10 years afterward [58]. This latent stage could persist for life or advance several years or decades later to tertiary syphilis.

2.2.7 The tertiary stage

Tertiary syphilis can manifest in various ways, marked by cardiovascular and neurologic sequelae and gummatous involvement of any organ system. The destruction of the nerve cells in the cerebral cortex at this stage leads to a combination of psychiatric manifestations and neurologic findings. The central nervous system (CNS) syphilis - infection of the brain or spinal cord caused by *T. pallidum* - is the most common presentation of late syphilis observed in current clinical practice [59]. It usually occurs in people who have had chronic, untreated syphilis, usually about 10 to 20

years after first infection and develops in about 25%–40% of persons who are not treated [60]. Neurosyphilis (CNS syphilis) begins with the invasion of the cerebrospinal fluid (CSF), a development that probably occurs shortly after the acquisition of *T. pallidum* infection [61]. The organism can be identified in the CSF from roughly a quarter of untreated patients with early syphilis [62]. The manifestation of tertiary syphilis also includes insanity, blindness, and paralysis [63]. At this stage, the person may no longer be contagious but the damage is irreversible.

2.2.8 Congenital Syphilis

Congenital syphilis is caused by trans-placental transmission of spirochaetes; the transmission rate reaches 90% if the mother has untreated primary or secondary syphilis. Fetal infection can develop at any time during gestation [64], though mostly after the first trimester. Manifestations are defined as early if they are seen in the first 2 years of life and late if they progress after age 2 years. According to the Centre for Disease Control and Prevention report, untreated syphilis, especially early syphilis, at the time of pregnancy can lead to deafness, neurologic impairment, bone defects, stillbirth, and neonatal death [65]. Most pregnant women with syphilis are asymptomatic and can only be identified through serological screening [66].

2.2.9 Syphilis global epidemiology

Approximately 349 million people are actively infected with this treatable sexually transmitted disease. The WHO [67] during 1999, estimated that more than 12 million new cases of adult syphilis occur worldwide each year and almost two-thirds of these cases were in SSA and south/southeast Asia [68]. In 2008, the total number of new cases of syphilis in adults was estimated to be 10.6 million which was the same figure as 2005 whilst during the same year, the number of adults infected was estimated to be 36.4 million [69].

Globally, syphilis affects between 700,000 and 1.6 million pregnancies a year that has resulted in spontaneous abortions, stillbirths, and congenital syphilis, contributing to approximately 20% of perinatal deaths [70]. Syphilis infection rates in pregnant women in Africa as a whole have been estimated to be between 3 and 15%. Of those, 30% of the untreated cases result in stillbirth and in another 30% the child will be born with congenital syphilis [71]. The disease congenitally affects 500,000 or more infants annually in SSA alone [66]. The current incidence of syphilis shows that there is still the need to improve on our control efforts.

Syphilis remains prevalent and a major public health problem in many developing countries with a resurgence in developed countries [72], including North America, Asia, and Europe, especially Eastern Europe. The prevalence of *T. pallidum* infection in the general population and blood donors has been increasing over the last two decades and varies by region which could be possibly due to sexual behaviour. Additionally, the poor quality of laboratory screening due to the lack of equipment, trained personnel, reagents and standard procedures compounds the problem.

The highest rates of syphilis were in South and Southeast Asia, followed closely by Africa. The third highest rates were in the regions of Latin America and the Caribbean [67]. In Western Europe, syphilis prevalence has declined considerably since the peak after the second world war, with incidence rates below 5 per 100 000 in the majority of countries[73-75].

In contrast with the decline in rates observed in Western Europe, since 1989 there was an alarming increase in rates in the newly independent states of the former Soviet Union where syphilis incidence increased from 5-15 per 100 000 observed in 1990 to as high as 120-170 per 100 000 of the population in 1996 [76]. In some regions of Siberia, as of 1999, syphilis prevalence was 1300 cases per 100,000 populations [77].

In the 2012 data shown below in table 2.1, the prevalence and incidence rates for the African region became relatively high compared to the rest of the regions globally. This could be as a result of improved lifestyle and technology in the developed regions compared to limited resources for testing and population growth in the African region. Some or most of the increased prevalence of syphilis in Africa is caused by population growth. From the table below, it is seen that the population increase in syphilis in Africa is very modest.

Additionally, particularly in low-resource countries, a substantial proportion of donated blood remains unsafe because it is either not screened for all major TTIs or not in a quality-controlled manner [78].

Table 2.1 Population growth and syphilis prevalence in Africa

Africa	1995	1999	2012
Syphilis prevalence	4.14	4.19	7.45
Total population	586	651	923
Proportion (%)	0.71	0.64	0.81

(All numbers in millions)

In Europe, between 2006 and 2009, the number of reported cases decreased in 10 countries and increased in 18, resulting in an overall decrease of 7%. This was mainly due to a substantial decrease of cases in a number of countries that have reported very high rates of syphilis in the past. In the Western Pacific, relatively high syphilis prevalence rates were found in Cambodia (4%), Papua New Guinea (3.5%) and the South Pacific (8%) [79].

The rise in the late 1980s and early 1990s coincided with the widespread use of crack cocaine and exchange of sex for money and/or drugs. Moreover, syphilis has acquired a higher potential of

morbidity and mortality with the increasing prevalence of HIV infection. But this has reduced with the introduction of anti-retroviral therapy (ART).

Table 2.2 Global and regional estimates of prevalent and incident cases of syphilis infection (in millions) among adults, 2012

Region	**Prevalence**		**Total**	**Incidence**		**Total**
	Male	Female		Male	Female	
America	0.992	1.003	**1.995**	0.466	0.471	**0.937**
Europe	0.368	0.366	**0.734**	0.221	0.219	**0.44**
Eastern Mediterranean	0.816	0.792	**1.608**	0.253	0.243	**0.496**
Africa	3.726	3.723	**7.449**	0.923	0.920	**1.843**
South and South-East Asia	1.856	1.791	**3.647**	0.451	0.435	**0.886**
Western Pacific	1.109	1.179	**2.288**	0.551	0.482	**1.033**
Global total	**8.938**	**8.783**	**17.721**	**2.825**	**2.769**	**5.626**

Another similar reason was that men having sex with men (MSM) with multiple partners as well as HIV infection in MSM was the cause of the surge in the late 80s and early 90s [80,81].

As syphilis rates declined in the Unites States of America (USA) in the late 1990s, the Centre for Disease Control (CDC) proposed a plan to eliminate syphilis in the USA [82]. It may, however, be noted that the decline in the late 1990s could partly be due to the control of HIV through safer sex and other acquired immune deficiency syndrome (AIDS) control campaigns [81].

Over the past decade, diagnosis of syphilis in the United Kingdom (UK) has increased greatly notably among males [83] which might partly be due to ongoing unsafe sexual behaviour. MSM

continue to experience high rates of sexually transmitted infections (STIs) and remain a priority for targeted HIV and STI prevention and health promotion work [84].

A quarter of an estimated 12 million cases of syphilis in the world each year occur in Africa [85]. Infection rates in major African cities of Zambia and Cameroon were reported at 10% and 6% in both genders [86]. In addition to this, syphilis prevalence rates amongst pregnant women vary from 2.5% in Burkina Faso to 17.4% in Cameroon [87,88]. In circumstances like this, if 90% of children are born with untreated syphilis as stated in page 21 under congenital syphilis, then situations can be worse in terms of disease prevention. In Ethiopia, the seroprevalence of antibodies to syphilis among blood donors is 12.8% [89]. A similar prevalence rate (12.7%) of syphilis antibodies has been reported in blood donors from Dar es Salaam, Tanzania [90].

There was little information available regarding the seroprevalence of syphilis and other potential transfusion-related pathogens in Ghana [91]. Published studies from Ghana show seroprevalence of syphilis ranging between 3.7% and 13.5% [7,92,93]. A recent study [6] in Ghana demonstrated syphilis prevalence of 8% among blood donors in Kumasi, Ghana and concluded that syphilis is prevalent among healthy blood donors in Ghana and that there is a need to introduce syphilis testing all donated blood.

2.2.10 Transfusion-transmitted syphilis (TTS)

T. pallidum, the spirochaete that causes syphilis, can be transmitted by transfusion of blood or blood components from donors with active syphilis [94]. The first case of TTS was reported in 1915 [95]. The numbers increased to 138 by 1941, but since then the number of cases has declined all over the world. A case of TTS was reported more than forty years ago in the USA [96], but in the past 35 years, and since then, only three cases of TTS have been originally reported. Recently, there has been a case of TTS reported in Ghana [6]. The absence of TTS in many developed countries

challenges the justification for continuing syphilis testing of blood donors [55]. This is probably because the treponemes are relatively fragile and sensitive to cold; hence the risk of transmission through transfusion of blood stored below 20^0 C for more than 72 hours is very low[55,97,98]. Units of blood directly transfused few hours after collection without testing comprises a higher risk of transmitting syphilis. This is the case in many developing countries with limited blood supply, where blood is collected from family donors and frequently transfused in the following hours or days [6]. But, the high prevalence of syphilis seropositivity in blood donors and seroconversion of a transfusion recipient shows that in centres where screening is not conducted, patients who receive blood through transfusion are at risk for contracting syphilis [6]. Universal testing of blood donors played a role in the abolition of TTS.

Various strategies have been proposed by the WHO, International Society of Blood Transfusion, and American Association of Blood Banks to prevent TTS. These include:

(i) Selection of low-risk donors and screening for *T. pallidum* using well-organized laboratory methods,
(ii) Application of pathogen reduction technology; and
(iii) Rational use of blood products.

However, blood safety begins with the implementation of organized blood centres, a quality system, hemovigilance programmes and adherence to SOPs [99].

2.2.11 Risk factors of syphilis infection in blood donors

The risk of TTS is closely related to risk factors in the blood donor, in particular, sexual behaviour since the disease is primarily transmitted by the sexual route, thereby compromising the safety of blood used for transfusion. Donor selection is important because donors with high-risk behaviours and other risk factors may be infected by syphilis and compromise the safety of blood used for

transfusion. The rates of infection are high among homosexual men [100]. Older age, MSM, two or more sexual partners, a past history of syphilis treatment and HIV seropositivity are closely related to TTS [101]. Other risk factors associated with TTS include prostitution, bisexuality (men having sex with both men and women), intravenous drug use and skin scarification (tattooing, blood rituals) [102]. Donors can be deferred during selection, which is particularly useful in the early period of infection when laboratory tests are not efficient [103].

2.2.12 Prevalence of syphilis among blood donors

The global incidence of syphilis among blood donors is variable. In developed countries, the prevalence of *T. pallidum* infection has declined in blood donors probably because there have been an improved donor selection criteria coupled with current testing methods with improved laboratory equipment [104]. However, there is a different situation in developing countries of the sub-Saharan region where the syphilis prevalence may reach 25% [105]. The prevalence of syphilis antibodies in healthy African blood donors is relatively high compared to other regions [90]. For example, the prevalence of syphilis among blood donors in India was recently reported to be 0.7% [106]. An incidence of 12.7% was noted among Tanzanian donors by Matee *et al.* [107] whereas Bhatti *et al.* [108] found an incidence of 0.75% among Pakistani donors. In such cases in developing countries, the inefficient manner of laboratory screening due to the lack of equipment, trained personnel, reagents and standard operating procedures makes it complex, thus, there is the need for systematic and better screening for syphilis to help ensure a safer blood supply [101].

2.2.13 Sources of blood supply and syphilis infection in SSA

In SSA, blood donations are collected from two main donor categories which are VNRD and FD. Family donors – who are individuals prompted to provide blood units to replace blood transfused to their relatives or friends [109] – remain dominant on the African continent as a response to

difficulties in recruiting and attracting VNRD [110]. There are published data indicating that the prevalence of syphilis is higher among family donations than in voluntary donations [106]. In Ghana, as elsewhere, there is a higher proportion of syphilis seroreactive donations among FD possibly because they are mostly older than VNRD and therefore have had longer exposure to sexual risks [111]. A study carried out, as part of this research, found that the higher prevalence among FD was partly due to higher age and more males. However, FD status which may also have riskier lifestyles contributing to syphilis risk, was an independent positive predictor of syphilis reactivity in a logistic regression analysis [112].

2.2.14 Antibody response to *T. pallidum*

There has been a wider study of serum immunoglobulin (IgM and IgG) antibody responses to *T. pallidum* in experimentally infected animals and humans [113]. Around 2 weeks after exposure, antitreponemal IgM antibodies are produced followed by IgG antibodies 2 weeks after IgM production [114]. Both antitreponemal IgM and IgG antibodies may be detectable within 3 days of lesion onset in primary syphilis [115].

T. pallidum disseminates from the primary chancre through the blood, although the timing of spirochaetemia in primary syphilis and its relationship to the serological window [116] is not well characterized. However, spirochaetemia can occur early, because transfusion-transmission has been reported in this serological window [56].

Early antibody responses (IgM and IgG) are against TpN15, followed by TpN17 and TpN47 [57]. IgG antibodies appear to be highest in patients with a longer duration of symptoms [115]. After therapy in primary and secondary syphilis, *T. pallidum* IgM antibodies decrease rapidly, becoming undetectable within 6–12 months. Several studies suggest that decreasing IgM levels indicate adequacy of treatment [117]. Merlin *et al* [118] demonstrated the absence of IgM antibodies in 84% of

patients with previously treated syphilis. In patients with syphilis, the level of IgM antibodies against *T. pallidum*, and the decline after treatment, is currently used as a marker for evaluating the effects of treatment [119]. However, levels of IgG antibodies do not change significantly with treatment in most instances, and they are generally thought to have little bearing upon the assessment of the efficacy of treatment [120-123].

Tanaka et al in 1990, followed patients with syphilis from the beginning of treatment by measurements of levels of IgM and IgG, by the *T. pallidum* immune adherence (TPIA) test, and their data showed a decrease in levels of IgG antibodies after treatment, even though the decrease was not as rapid and complete as in the case of IgM antibodies [120].

2.2.15 Recommendations for syphilis testing for blood donors

The WHO recommends that, to minimize the risk of syphilis infection through the route of transfusion:

1. Screening should be performed using a highly sensitive and specific test for treponemal antibodies: either TPHA or EIA.
2. In populations where there is a high incidence of syphilis, screening should be performed using a non-treponemal assay: VDRL or RPR [124]. This is probably because these assays are primarily used as qualitative assays for screening in the traditional algorithm or as quantitative assays to help stage infection and to assess the response to treatment. Additionally, all nontreponemal assays detect both IgM and IgG antibodies, which are commonly detectable as early as 6 days' post-infection [125-127].

According to the guidelines published by the U.S. Centres for Disease Control and Prevention, the diagnosis of syphilis should be based on the results of at least two tests: one treponemal and the

other non-treponemal [128]. VDRL and RPR are sensitive for recent syphilis infection, but not for past infection. Although the traditional RPR has been used successfully in many settings, its accuracy is often improved by coupling with a treponemal haemagglutination (TPHA) or particle agglutination (TPPA) [129]. Additionally, false negatives may occur both in early primary cases and in patients with secondary syphilis, as a result of prozone reactions [130]. This may limit the sensitivity of the test.

The Ghana national blood policy for the Health Sector of MoH, which was approved by the Cabinet in February 2006 reiterated that, all units of blood collected must be tested prior to transfusion for HIV I & II, HBV, HCV and syphilis, and any other transfusion transmissible microbial diseases that may be thought to be relevant by the NBSG, using approved well-controlled techniques and procedures according to WHO guidelines [25].

2.2.16 *T. pallidum* detection by PCR

The diagnosis of syphilis is complicated because *T. pallidum* is one of the few major bacterial pathogens of humans that cannot be cultivated on an artificial medium. Current methods for detection of *T. pallidum* in clinical specimens are either insensitive, as in the case of dark-field microscopy [131], or impractical, as with rabbit intratesticular inoculation (rabbit infectivity testing or RIT) [132,133].

At present, deoxyribonucleic acid (DNA) amplification by the polymerase chain reaction (PCR), the most sensitive and specific in vitro technique, has found growing application in the laboratory diagnosis of infectious diseases [134]. PCR is a technique which is used to amplify nucleic acid sequences of microorganisms which may be present in the blood or other body fluids. PCR can selectively amplify the copy number of a target gene more than 10^6-fold [135]. PCR, therefore, has great potential for improving the ability to diagnose infectious diseases caused by fastidious or

slowly growing microorganisms [134]. In addition to its obvious diagnostic applications, detection of *T. pallidum* by PCR may prove to be a valuable tool for clinical and laboratory investigation of syphilis.

A number of PCR-based methods have been developed worldwide for the detection of the organism *T. pallidum* DNA or RNA in clinical specimens. These assays are based on the detection of various target genes including bmp, 39-kDa basic membrane protein [136]; tpp47, *polA*, DNA polymerase I [137]; tmpC, a 35kDa membrane protein [138], and 16SrRNA [139].

There has been a report on the development of an exquisitely sensitive assay for *T. pallidum* that is based upon amplification of the gene encoding the pathogen-specific and highly conserved 47-kDa membrane immunogen (tpp47) [140,141]. Detection of *T. pallidum* by amplification of a portion of the tmpA gene (a 45kDa membrane protein) was reported by Hay *et al* [142] which was the first application of PCR in clinical samples from patients with syphilis.

There is also a sensitive assay for *T. pallidum*, the agent of venereal syphilis, based upon PCR where a 658-bp portion of the gene encoding the 47-kDa membrane immunogen was amplified, and the PCR products were also probed by DNA-DNA hybridization with a 496-bp fragment internal to the amplified DNA [143]. The assay detected approximately 0.01 pg of purified *T. pallidum* DNA, and positive results were found normally from suspensions of treponemes calculated to contain 10 or more organisms and from some suspensions calculated to contain a single organism. Specific PCR products were obtained for the closely related agent of yaws, *Treponema pallidum* subsp. *pertenue* [144].

In addition to *T. pallidum* subsp. *pallidum* of the Burstain *et al* study, specific PCR products were obtained only from genomic DNA of T. *pallidum* subsp. *pertenue* [56]. Their result was not

surprising given the extraordinarily close genetic relatedness of the pathogenic treponemes [145] and the highly conserved antigenicity of their 47-kDa immunogens [146]. Amplification of tpp47 as performed in the Burstain *et al* study, therefore, was not able to distinguish syphilis and yaws any better than conventional diagnostic modalities [143].

It has been determined that whole blood in heparin or EDTA (but not serum), lesion exudate, and punch biopsy as well as swabs of lesions are useful specimens for examination by the PCR or the detection of *T. pallidum* [58].

The suitability of whole blood as a potential specimen to be tested by PCR for *T. pallidum* was also examined by Wicher *et al*. where extraction of larger volumes of samples, e.g. 250µl yields an increase in the sensitivity. The sensitivity in their experiments was similar to that reported by Hay *et al* and Burstain *et al*. [142,143] who reported that 1 to 10 organisms per 50 ml of sample was needed for a positive PCR.

At early stages of *T. pallidum* infection where serological tests are non-reactive, PCR has the ability to identify *T. pallidum* infection and further differentiate *T. pallidum* from other treponemes, making it most advantageous over serological testing in terms of its high level of specificity [147].

New molecular tests for syphilis are unlikely to replace serology in the short term because they require sophisticated equipment [58]. Although PCR has shown to be effective at diagnosing primary syphilis with sensitivities between 73% and 95% and specificities 95% [148,149], sensitivities of blood samples in primary syphilis are as low as 18% [148].

PCR assay which is more sensitive than standard diagnostic tests for the detection of *T. pallidum* and other TTIs is rather costly and not affordable for most sub-Saharan African blood banks and

requires an extensive capital investment [150]. Advanced set-up is required for training personnel, quality control, and maintenance of equipment [151]. However, PCR testing for blood donors is not able to differentiate between viable and dead spirochaetes [51]. This is a limitation because it is the viable and active ones which are potentially infectious to be transmitted through blood transfusion.

2.2.17 Serologic syphilis testing

Serological tests for syphilis contribute greatly to the detection of *T. pallidum* infection in blood donors and especially in those who are not identified during donor selection which is important because donors with high-risk behaviours and other risk factors are deferred before laboratory testing. Wassermann [152,153] developed the first test (nonspecific reaginic) of syphilis in 1906. Although it had some false positive results, it has been a major advancement in the prevention of syphilis because it has helped to diagnose the disease before the clinical manifestations appear and thus prevent its spread. In the scientific literature, the ranges of stage-dependent sensitivity and specificity of diagnostic assays for the serological detection of syphilis have been reported to be 70 to 100% and 97 to 99%, respectively [51]. Serologic testing remains the mainstay of laboratory diagnosis for secondary, latent, and tertiary syphilis as efforts to cultivate the organism in vitro have been largely unsuccessful. In addition, PCR is too insensitive, and is only able to confirm about 25% of infections[154]. Thus, serologic tests are the foundation of syphilis screening, and knowledge of their diagnostic limitations is critical for clinicians and blood transfusion services. Two main categories of serologic tests for syphilis are available, tests for nonspecific reaginic antibody and, tests for specific treponemal antibody [155].

None of the serologic tests (non-treponemal and treponemal) are sufficient for definitive diagnosis. Antibody detection by non-treponemal tests (anti-lipoid antibody detection) and treponemal tests (anti-*T. pallidum* antibody detection) is still regarded as the basis for diagnosing syphilis and for

monitoring the success of subsequent antibiotic treatment [156]. However, the quality of routine serological diagnosis of syphilis has been questioned by several studies that found significant inter- and intra-laboratory variability of test results [157,158].

2.2.18 Non-treponemal tests

Non-treponemal tests are based on non-treponemal lipid antigens (cardiolipin), frequently using the flocculation technique as initiated by Wassermann since 1906. In 1941, Pangborn [159] successfully isolated the active antigenic component, a phospholipid, cardiolipin from beef heart. Cardiolipin, - (IUPAC name "1, 3-bis (sn-3'-phosphatidyl)-Sn-glycerol") - is an important component of the inner mitochondrial membrane, where it constitutes about 20% of the total lipid composition. It can also be found in the membranes of most bacteria. When combined with lecithin and cholesterol, it forms a serologically active antigen for the detection of syphilitic antibody. With the advent of these new purified antigens, microflocculation tests, such as the VDRL test were developed [160].

The RPR teardrop card test [161], was developed as a screening procedure to be used in the field [162]. Laboratory equipment was not essential because all test materials were contained in a disposable kit. Plasma from blood obtained by finger prick is collected on a plasma collection card and then placed in a teardrop-shaped test area on a plastic-coated card. Next, a stabilized modified RPR antigen suspension, incorporating charcoal particles to aid in reading the reaction, is added to the plasma, and the test card is rocked by hand. The results are read macroscopically as reactive or nonreactive. The RPR teardrop card test is still employed in field work and as a screening procedure in some laboratories, but recent reports have found the test to be less sensitive than the currently used RPR 18-mm circle card test with serum as a sample source [163]. The RPR 18-mm circle card test measures IgM and IgG antibodies to lipoidal material released from damaged host

cells as well as to lipoprotein-like material, and possibly cardiolipin released from the treponemes[164,165]. If antibodies are present in the donor sample, they combine with the lipid particles of the antigen, causing them to agglutinate. The charcoal particles co-agglutinate with the antibodies and show up as black clumps against the white card. If antibodies are not present, the test mixture is uniformly grey. Qualitative results are reported as either reactive (clumps) or nonreactive (no clumps). However, quantitative results are reported based on serial dilutions ranging from undiluted (1:1), and in 1:2, 1:4, 1:8, and 1:16 dilutions. Thus, quantifiable titres can establish a baseline to evaluate treatment response [166]. Without some other evidence for the diagnosis of syphilis, a reactive nontreponemal test does not confirm *T. pallidum* infection [51] but rather a suggestion.

The VDRL and RPR tests are the most commonly used. These tests are inexpensive, fast, simple to perform and more sensitive than treponemal specific tests [167]. They are able to identify infected blood donors few days before the treponemal test and thus useful for acute infection. However, VDRL and RPR cannot be automated and are time-consuming if used for large scale testing. Additionally, the results may be difficult to interpret and they require training of health personnel to ensure testing is carried out and results are read correctly. Compared to treponemal tests, false-positive test results for non-treponemal tests are associated with viral infections, pregnancy, malignant neoplasms, autoimmune diseases, and advanced age [51]. Still, these tests are routinely used to screen blood donors. After effective treatment, non-treponemal tests usually become nonreactive [168,169] within a period of three years, in contrast to many treponemal test, which is a major strength of these tests.

For these reasons, classical syphilis screening uses nontreponemal RPR or VDRL tests. Reactive specimens are confirmed using treponemal assays like TPHA or fluorescent treponemal antibody

absorption (FTAAbs) tests. Most high-volume laboratories have adopted the "reverse algorithm" - screening with treponemal assays where reactive specimens are confirmed with nontreponemal RPR or VDRL - syphilis screening to improve efficiency and lower costs [170,171].

Despite the advantages and disadvantages of each diagnostic algorithm, the choice to use a treponemal or nontreponemal assay as the first screening test should be based on a combination of factors: local syphilis prevalence, the expected workload, the requirement for automation, and the available budget for labour and consumables [172].

2.2.19 Treponemal antibody tests

2.2.19.1 Treponemal manual

Initial attempts to develop a test using an antigen derived from the treponeme itself were unsuccessful until 1949, when Nelson and Mayer developed the first treponemal antibody test, the *T. pallidum* immobilization (TPI) test [173]. It was rapidly accepted as a specific test for syphilis although it was complicated, technically difficult, time-consuming, and expensive to perform. A collection of treponemal tests was later developed, some of which became popular in a short period of time [174,175].

In 1957, a major breakthrough in treponemal antigen tests occurred with the development of the fluorescent treponemal antibody (FTA) test [176]. This was later developed into a more specific and sensitive FTA absorption (FTA-ABS) test [177] and this remains the standard treponemal tests for syphilis today.

Treponema pallidum haemagglutination assay (TPHA) detects the presence of treponemal antibody in the patient's serum but is simpler to perform than the fluorescent-antibody tests. TPHA tests have detectable reactivity approximately 4 weeks after exposure.

Currently, some of the tests that are considered standard treponemal tests are FTA-ABS, FTA-ABS double staining, TPHA and MHA-TP. All of these tests use *T. pallidum* as the antigen and are based on the detection of antibodies directed against treponemal components.

2.2.19.2 Treponemal automated (Enzyme immunoassay)

In the 1990s, various enzyme immunoassays (EIA) that could detect both anti-treponemal IgG and IgM antibodies became commercially available. EIA reported in the literature have used different approach to determine sensitivities and specificities. These assays used microtitre plates of wells that were coated with wild-type *T. pallidum* antigens and had a clinical sensitivity of 98.4% and specificity of 99.3% when compared with TPHA and FTA-ABS [178].

2.2.19.3 Immunochemiluminescence assay (CLIA)

Since 2000, the options have expanded to include automated chemiluminescence immunoassay (CLIA) [179] and multiplex flow immunoassay [180] which have higher testing output and objective interpretation. As part of this study, Vitros Syphilis TPA assay (a CLIA using the Vitros ECi/ECiQ Immunodiagnostic Systems) was used as gold standard to confirm initially syphilis sero-reactive blood donor samples. This CLIA is a qualitative assay that detects total antibodies (IgG and IgM) to *T. pallidum* reacting with biotinylated and horseradish peroxidase (HRP)-labelled recombinant TP antigens TpN15, TpN17, TpN47 and is bound to streptavidin-coated wells.

It has a specificity of 99·8% (CI 98·7–100%) and a sensitivity of 100% using Syphilis Mixed Titre Performance Panel PSS202 (BBI Diagnostics, Bridgend, UK) and clinical samples from known syphilis-treated patients of both Caucasian and African origin [181].

2.2.19.4 The Abbott ARCHITECT Syphilis TP assay

The Abbott ARCHITECT Syphilis TP assay - which has also been used in this study as a secondary gold standard - is a two-step sandwich chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of antibody to *T. pallidum* in human serum or plasma based on recombinant antigens TpN15, TpN17 and TpN47. Antibody present in the sample binds to *T. pallidum* recombinant antigen coated paramagnetic particles. After wash step, murine anti-human-IgG/anti-human-IgM acridinium-labelled conjugate is added. Following a further wash step, pre-trigger solution (hydrogen peroxide) and trigger solution (sodium hydroxide) are added. The resulting chemiluminescent reaction is measured in relative light units (RLUs) which are directly proportional to the amount of anti-*T. pallidum* present in the sample. The Architect Syphilis is highly sensitive in detecting primary syphilis (97.5%) with a specificity of 99.1% [182] and good performance on reproducibility and intra-assay coefficient variation [183].

The advantages of EIA include the production of objective results, ability to link EIA readers directly to laboratory computer systems (reducing the potential for errors in transcription of results), and the facility for automation. They also have the capacity to process large numbers of samples. One limitation of treponemal tests is that they can remain positive even after treatment [184,185], in contrast to non-treponemal tests, so that someone previously treated for syphilis may be misdiagnosed as having a new, untreated case of syphilis if only treponemal tests are used and overtreatment could occur. Additional limitations of the EIAs are time and costs when small numbers of samples are to be processed.

2.2.19.5 Western Blot (WB) and Immuno-blot assays

Immunoblotting allows for the detection of antibodies to individual proteins. In the Treponemal Western blot (Tp-WB), solubilized *T. pallidum* proteins are separated by gel electrophoresis

according to their molecular size. The separated proteins are then transferred onto a nitrocellulose membrane which is dried and cut into strips. After incubating the strips with patient's serum, antigen-antibody complexes are visualized by adding enzyme-conjugated anti-human globulin followed by substrate, which causes a colour reaction. It is generally agreed that detection of antibodies to immuno-determinants with molecular masses of 15, 17, 44.5 and 47kDa (TpN15, TpN17, TpN44.5 and TpN47) are diagnostic for acquired syphilis [186].

2.2.20 The INNO-LIA Syphilis test (Innogenetics)

There are other line immunoassays (INNO-LIA syphilis test, Innogenetics) (Furijebio, Ghent, Belgium) detecting individuals binding to the same recombinant TP antigens TpN15, TpN17 as well as to TpN47 and a synthetic peptide TmpA derived from *T. pallidum* proteins [187]. The INNO-LIA Syphilis test had sensitivity of 99.6% and specificity of 99.5% [187].

Treponemal tests are conventionally used primarily to verify reactivity in the nontreponemal tests. Unfortunately, treponemal tests are technically more difficult and costly to perform than nontreponemal tests and cannot be used to monitor treatment as mentioned above. However, a reactive treponemal test result on a sample that is also reactive in a non-treponemal test is highly indicative of active-ongoing infection. Currently, treponemal tests in the form of rapid diagnostics are easy to perform and cheaper worldwide which is discussed below.

2.2.21 Syphilis rapid diagnostic tests (RDT)

In developing countries and areas with limited resources, laboratory facilities are often unavailable for standard syphilis tests. These countries are characterized by a difficult epidemiologic, sociological and economic environment, which limits the implementation of a high quality of

blood safety. A rapid serologic test for blood banks could greatly enhance public health efforts to decrease the spread of this infection through transfusion as this technique does not require sophisticated laboratory materials [103]. The availability of individual *T. pallidum* antigens through recombinant DNA techniques [188] has resulted in the use of these antigens for serologic tests by lateral-flow technology. Antibodies in the specimen bound at antigen site on the strip and are revealed with dye bound anti-immunoglobulin or dye bound antigen and a positive reaction appears as a coloured line. Although several different manufacturers have developed rapid tests using lateral-flow technology and recombinant antigens in the late 1990s, there are few published evaluations of these tests [189]. These assays are visually read, qualitative immunoassay for the detection of antibodies to the antigens of *T. pallidum*. The syphilis RDTs use serum, plasma, or whole blood specimens to detect IgM, IgG, and IgA antibodies [190] and involve immunochromatographic strips (ICSs) in which one or multiple *T. pallidum* recombinant antigens are applied to nitrocellulose strips as capture reagents [191]. Abbott Determine rapid syphilis TP assay had a high sensitivity (95.6 to 98.4%) and specificity (95.7 to 97.3%) with serum samples and TPHA as the reference test [192].

Over twenty rapid tests for the serological diagnosis of syphilis are commercially available [188],with lower costs compared with the traditional tests, and these provide opportunities to scale up syphilis screening in many transfusion settings where traditional tests are unavailable.

More syphilis rapid tests are commercially available, primarily because of less stringent procedures for their development internationally than automated ones [193]. Development of rapid treponemal-based syphilis tests was driven by the need for simple, point-of-care tests in resource-poor countries [194]. Overall, syphilis RDTs are highly sensitive and specific [166]. However, less sensitivity has been reported in field settings using whole blood rather than serum [195,196]. The WHO

compared the performance of eight rapid syphilis tests to a combined reference standard of TPHA and *T. Pallidum* Particle-agglutination Assay (TPPA), reporting sensitivities of 84.5%–97.7% and specificities of between 92.8%–98% [197,198].

The advantages of rapid syphilis tests include their affordable costs (ranging from US$ 0.2 to US$ 2.0.) [93] clear interpretation of results, availability of results within 5–20 minutes [198], and requiring minimal equipment and training, which is ideal for clinical and non-clinical settings. However, they cannot distinguish between active and treated syphilis, and false-positive reactions can occur [199]. Positive results would have to be confirmed with non-treponemal test to determine recent infection and response to therapy [101].

2.2.22 Yaws

Yaws is a communicable, non-venereal treponematosis caused by the bacteria *Treponema pallidum ssp. pertenue* [200] which is a gram negative spirochaete. It predominantly affects children under 15 years of age in the most underprivileged, remote, tropical rural communities. Favourable climate conditions such as humidity and a constant warm temperature appear to be especially important factors for yaws to flourish [201].

For decades, Ghana has been a major yaws endemic country in West Africa. It is the only country in Africa to maintain its yaws control programme after the early 1990s when many yaws programmes were discontinued. In 2008, the National Yaws Control Programme (NYCP) was reconstituted as the National Yaws Eradication Programme with the principal aim to eliminate yaws by 2012. Surveys carried out in 2008 by the MoH, Ghana, among children in schools and communities, showed prevalence of yaws in Ghana at 0.681% with prevalence in some rural communities up to 20% [202] although the mode of detection was unclearly defined. According to

the MoH in Ghana, all the 10 regions and nearly all the 170 districts reported yaws. A total of 28,000 cases were reported in 2008 and 25, 000 in 2010 [202].

2.2.22.1 Pathophysiology of Yaws

Children aged 2–15 years are the most vulnerable to yaws infection, which targets the skin, bones and cartilage, causing destruction of tissue and deformities in the late stages [203].In similarity with syphilis, the evolution of yaws is revealed in three stages of clinical manifestations [204]. Early yaws encompass both the primary and secondary stages (active yaws cases). During the primary stage (3–4 weeks incubation), patients display papillomatous raspberry-like lesions located mainly on the feet and legs. They also have ulcerations of variable size on other sites of the body that are highly contagious. Patients may spontaneously recover from the primary stage or progress to the second stage. The secondary stage consists of several episodes, lasting three to six months, of both contagious and non-contagious lesions. These primary and secondary stages usually last up to five years from the time of infection, with periods of latency in between symptomatic episodes [201]. About 10% of patients with untreated secondary stages pass into the tertiary stage (late yaws), which can occur up to 15 years after the secondary stage [205]. Late lesions may involve the skin and subcutaneous tissues, the mucosa, the bones, and the joints. Tissue destruction is common and characteristic. Third stage lesions are not contagious but disable people in their daily activities [201,206]. There is evidence that any potential abnormality resulting from latent yaws include congenital, visceral, and tertiary CNS lesions [206,207]. Thus, this may increase significant osseous, neurological, and ophthalmologic complications. Since the 1940s, a single intramuscular injection of long acting benzathine penicillin has been successfully used for treatment [208]. It is suggested that by using a modified strategy to focus investigation and control efforts on infectious yaws cases and their contacts, and by taking advantage of effective methods to obtain more accurate diagnosis

of yaws cases in the field, it should be possible to control yaws more effectively and efficiently, and perhaps to eradicate it [209].

2.2.22.2 Yaws global epidemiology

In the early 1950s an estimated 50 to 100 million people were infected in tropical areas particularly in Africa, Southeast Asia and South America [210]. In 1995, the last WHO estimate was that, there were 2.5 million cases of endemic treponematoses (mostly yaws) globally, including 460 000 infectious cases [211]. The epidemiological status of yaws globally today is unknown; however, there is growing evidence that the number of cases of yaws and other endemic treponematoses is increasing in some countries, while they have disappeared in other previously endemic countries. Yaws is transmitted by skin-to-skin contact with fluid from a lesion on an infected person among people with poor hygiene practices living in warm and humid tropical areas of Africa, the Americas and Asia. Because of latency and late recurrence, up to 15 years after primary infection, as described above, yaws may also have implications for potential blood donors, including in Ghana

It remains one of the most neglected tropical diseases, affecting primarily the poorest and most vulnerable populations: tribal and indigenous people living in remote, rural areas. The disease has not attracted global attention recently, although it can be highly controlled in eradication epidemiologically, technologically and in terms of cost-effectiveness.

In 1952, a yaws control programme was started in India with assistance from WHO and UNICEF. This campaign from 1952 to 1964, administered >50 million anti-yaws treatments in 46 countries, reducing the prevalence of the disease by about 95% [212]. In the context of neglected tropical diseases, WHO launched the global yaws elimination initiative in 2007 to address the persistence and resurgence of this disease [213]. In May 2016, WHO declared India free of yaws [214].

2.2.23 Relationship between syphilis and yaws

Among the pathogenic treponemes, most comparisons have been made between *T. pallidum* and *T. pertenue*; several experiments have demonstrated variable degrees of cross-immunity based upon symptomatic reinfection to challenge with heterologous organisms [215] and serological cross-reactivity [216]. Although T. *pallidum* subspecies differ pathogenically, they are >95% homologous by DNA-DNA hybridization [217]. Yaws and venereal syphilis can only be distinguished by epidemiological characteristics and clinical manifestations as the commonly used serological tests cannot discriminate one disease from the other [218]. However, there are minor [219-221] or substantial [222] genetic differences existing between both pathogens that have been reported. Subspecies-specific genetic signatures permit molecular differentiation using methods that involve PCR, restriction fragment length polymorphism (RFLP), and DNA sequencing [223]. This is another critical usage of PCR that enables to make differential diagnosis of treponemal species. Recently, however, a genetic signature was defined in the 59-flanking region of the 15-kDa lipoprotein gene (tpp15) that distinguishes *T. pallidum* subsp. *pallidum* from *T. pallidum* subsp. *pertenue* and *endemicum* [223]. Although PCR method can be useful for blood banking, they are costly, laborious, and time-consuming because of demand for blood. It is the usage of PCR only that enables to make differential diagnosis of treponemal species. Despite differences in the mode of transmission, geographic distribution, tendency to invade the central nervous system, or infect the foetus, the two subspecies share striking similarities at both the genomic and antigenic level [15,223,224]

When PCR is not used routinely for screening blood donors, as remains the case in Ghana, yaws and syphilis remain indistinguishable in practice. Consequently, these methods are not suitable for rapid screening applications. Whether donor history may be used to differentiate between sero-reactive donors is among the research questions for this thesis.

2.2.24 Challenges of syphilis testing in relation to blood donation

Syphilis may be transmitted via blood and blood products, and intravenous drug use [225]. Transfusion syphilis, now rare, does still occur [226,227], and although questions have been raised about routine screening of blood donors for syphilis [228], it is still recommended. There have also been several conferences that sought to include discussions on the extent to which tests for syphilis contributed to transfusion safety and whether their use as in current practice should be continued or modified. The resulting recommendation from the National Institutes of Health (NIH) consensus conference (1995) was to continue syphilis screening of all blood donors until more information was available regarding the effect of component storage conditions on *T. pallidum* survival and molecular techniques could assess the absence of *T. pallidum* in serologically positive donated blood [229]. Furthermore, there is an increasing requirement to use blood products like platelets which are held at 22°C, at which temperature, *T. pallidum* can survive for longer periods than at the normal refrigeration temperature of 4°C. There is thus a continuing need for an effective, practicable, and economical serological test to not only provide evidence for public health practice but also improve safety of stored blood for transfusion purposes.

Although syphilis testing is a recommendation from the WHO, not all blood transfusion centres in Ghana and other countries test blood donors for syphilis which is problematic in public health if undetected in blood donors. Ampofo [92] recommended that routine blood screening prior to transfusion should include tests for *T. Pallidum* antibodies and HCV, and that periodic study to investigate transfusion-transmissible infectious diseases is required to enable safety reviews of the blood supply.

Better methods of testing can make the process of identifying and curing infected individuals more cost effective and consequently more feasible. WHO reported in 2006 that 56 out of 124 countries

surveyed did not use appropriate basic tests on all blood donations [230], thereby increasing the likelihoods of contracting infectious diseases from blood donors via transfusion. In blood donor populations with high prevalence of HIV or HBV, the surrogate value of syphilis testing will be easier to justify due to the fact that HIV and syphilis co-facilitate transmission of each other [231]. Syphilis is also likely to be more common in these high HIV and HBV-prevalence populations, and thus the value of syphilis testing to prevent TTI may also be clearer [232] .

2.2.25 Quality systems in laboratory testing for syphilis

Laboratory testing consists of pre-examination, examination, and post-examination processes, which require strict implementation of a quality management system. The maintenance of a quality management system is crucial to a laboratory for providing the correct test results every time. Important elements of a quality management system include:

- Documentation
- Standard operating procedures (SOP's)
- Quality control samples
- Internal and external quality assessment scheme [233].

Quality control (QC) are procedures used in each assay to assure a test run is valid and results are reliable especially the kit controls and quality control samples [124]. QC measures in serological syphilis testing are designed to ensure that reliable and reproducible test results are obtained within a laboratory and among different laboratories performing the same tests [234]. Strict adherence to recommended technique and the use of standardized reagents eliminates most technical errors [234]. Departures from predetermined reactivity of control serum samples detects day-to-day variability in testing and indicates any need for corrective action. Occasionally deterioration of test kits causes the variation, but other factors like storage and shelf-life of reagents under tropical conditions are more frequently responsible. Longer shelf-life reduces the pressure on the supply chain and the

probability of wastage of expired tests; a minimum of 18 months is recommended in remote, poorly resourced areas [235]. Testing facilities should make sure proper storage conditions are met (4-30ºC) in additions to using the kits within required shelf life so as not to affect kit performance.

Results of laboratory samples can be properly interpreted based upon how complex the sample is, stability factors based on transport and timing, and the competency of the laboratory staff. Test kits' efficacy is difficult to describe or follow if they are not daily controlled internally as they are prone to errors. This is because results of clinical testing obtained from laboratories or testing sites at or near the point of care must be as accurate as possible as they have a direct impact on care and treatment, prevention, and control of diseases [236]. The components of a quality system include internal quality (process) control, proficiency testing (PT), and quality improvement [237]. It is only when these components are implemented together that quality improvement or the highest attainable quality of testing can be achieved and consequently can improve health outcomes in terms of disease prevention and control, care and treatment [237].

CHAPTER 3

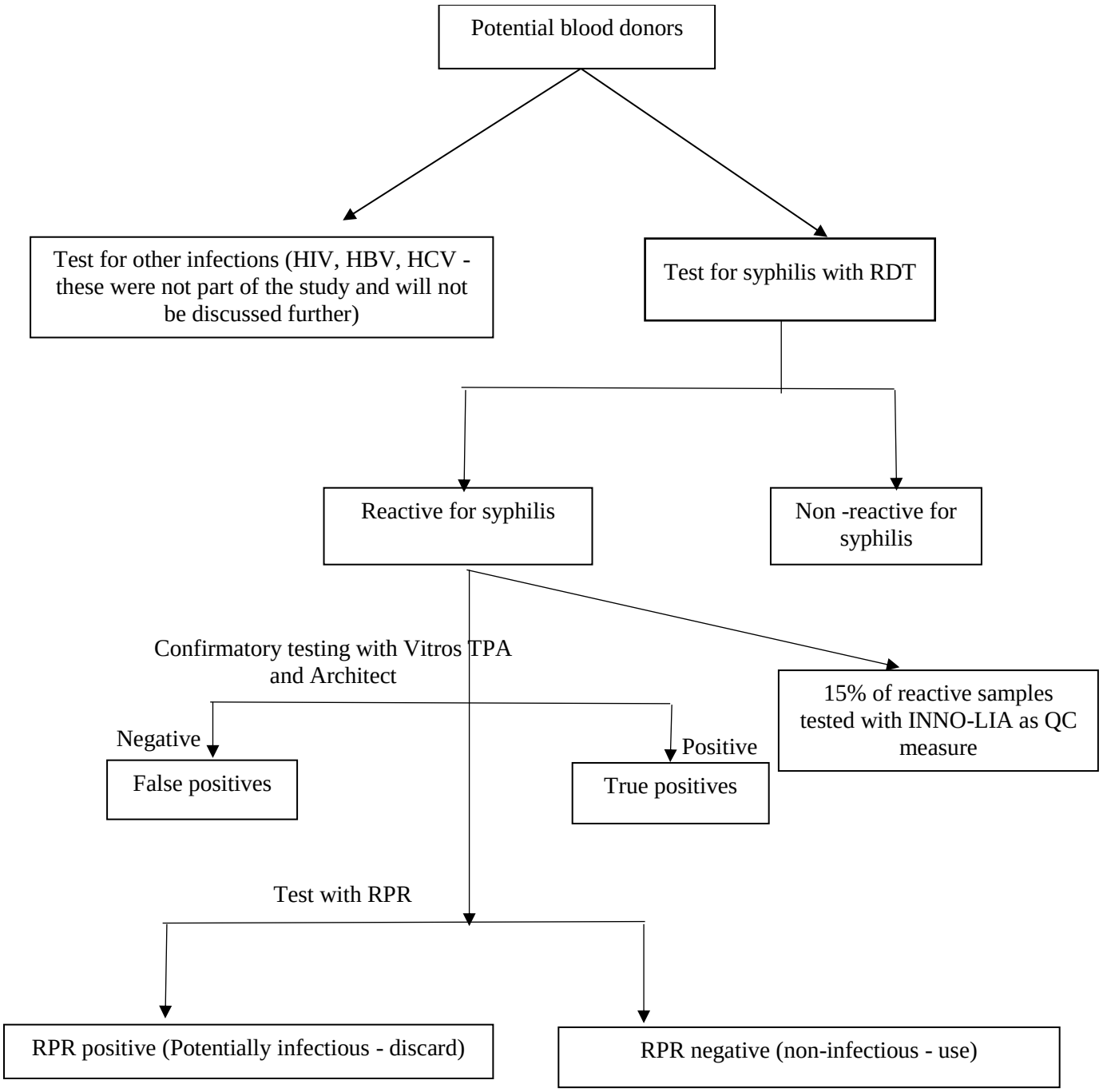
Conceptual framework and ethical considerations

3.1 Conceptual Framework for the study

Laboratory testing across the transfusion centres in Ghana is performed on all the blood samples that are collected from the prospective blood donors prior to transfusion. This is to ensure that recipients receive the safest possible blood products. Testing measures help to maximize safety of blood donation for the donor and the recipient. The concept for this study was to document the current processes for screening blood donors for syphilis in Ghana, and to build on this to arrive at a screening strategy that would be sensitive enough to detect the true positive blood donors while minimizing deferrals and consequently the impact on the blood supply, due to false positive reactions.

The methods outline is that blood samples were taken from potential blood donors and tested for markers of other TTI and syphilis with RDT (fig. 3.1). The seroreactive samples were tested using RPR to detect potentially active infectious blood donors. The RDT seroreactive samples were also tested with Vitros TPA and Abbott Architect TP, which were used as gold standards to detect true positives. Furthermore, a specific immunoblot called Inno-LIA was also included as a quality control measure for a sub-set of the syphilis RDT reactive samples. The Vitros TPA, Abbott Architect TP, and the LIA were used as gold standards because they detect antibodies binding to the recombinant TP antigens TpN15, TpN17 and TpN47, with higher performance, as used in developed countries like Denmark.

Figure 3.1 The algorithm for the syphilis study at KATH



TPA*; T. pallidum* assay, RPR; rapid plasma reagin, QC; quality control

3.2 Ethical clearance

Ethical approval for the study was obtained from the Research and Ethics Committee (REC), Liverpool School of Tropical Medicine (LSTM), University of Liverpool, UK (18/02/2014) (appendix 3.1). We additionally obtained another approval from the Committee on Human Research, Publications and Ethics (CHRPE/AP/423/13), School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi (appendix 3.2). An introductory letter for the survey of the syphilis testing was sent to the respondents from the head of the NBSG, explaining to them the aims of the study, collaborations and benefits (appendix 3.3). After the explanation of the study to the respondents, they were assured of their anonymity in the use of their data. Syphilis sero-reactive blood donors included in the study were informed about the study aims and objectives, risks, and possible benefits which included referral to an appropriate clinic for treatment in case the screening tests detected active infection with syphilis. After the explanation of the study and assessment of their understanding by a set of standard questions, participants were requested to sign or thumbprint an informed consent form (appendix 5.1). The study was thus, conducted in accordance with ethical principles.

Ethical considerations and blood donors

Donors are conventionally classified as voluntary non-remunerated (donors considered to have received no compensation for their donation), family replacement (donors donate with the intent of replacing or directly contributing to blood used by a specific patient), and paid (donors openly compensated monetarily for their donation).

In Ghana blood has been collected from both voluntary, family replacement and paid donors, but now is limited to voluntary and family replacement. This is because the nation does not have 100%

voluntary donation and sometimes has to rely on family donors especially when the schools are on vacation.

The issue of compensating donors has been a controversial and emotive one in blood transfusion for many decades. Recent statements from the WHO reiterate the belief that uncompensated donation contributes to safety from transfusion transmitted diseases[238,239]. It has also been shown that, In localized, controlled environments, viral marker rates for paid and unpaid donors were similar for the established transfusion transmitted infections[240].

Ethical considerations/limitations of using family members as donors

Family replacement donors (FRDs) are still a significant and sometimes predominant part of the blood supply in developing countries. These blood donors for many years, have been considered less safe than volunteer non-remunerated blood donors (VNRD) and actively discouraged by international organisations and well-off countries support agencies for developing countries. In addition to safety, it was considered unethical based on pressure and coercion. However, these beliefs were not evidence based. In comparing viral marker data confirmed seroprevalence in first-time VNRD and FRD corrected for gender and age, it showed no significant difference between the two groups[42]. Evidence has been provided that for both volunteer and family donors, compassion is more appropriate than altruism. The two groups join for psychological attitude to donation for which knowing someone needing transfusion is a powerful incentive to give blood. Excluding a life or death situation found in areas where severe blood shortage justifies replacement donation, pressures are exerted on both volunteer and family replacement donors. There is no evidence of putting pressure on FRD. FRDs thus meet all criteria for VNRD and are willing to become VNRD and to repeat donation. Banishing FRD is illegitimate and damaging to the blood supply in resource poor areas. In some countries, it makes no difference between the two groups

of donors which represents similar populations when asked to give blood in different circumstances. FRDs therefore, remain a critical source of volunteer, non-remunerated, blood meeting all standard principles of VNRD and are considered ethical and essential at this point in time instead of discouraged. FRDs, as well as VNRDs, are both altruistic, and both submitted to pressures of various kinds but should not be considered as coerced.

A study conducted in Kumasi, Ghana, clearly illustrated this point by showing that most FRDs gave blood "because they were asked" by patients or family members[241]. Thus, there is no ethical or safety reason to exclude FRD from participating in the blood supply. FRDs have the right to have clear and appropriate information, including the purpose of donor selection, and the consequences of failure to provide the relevant information to the blood service.

There are ethics governing paid donors and this discourages blood facilities from using their blood units. A level of stigma, based on safety and ethical considerations, has been attached to paid donation. This is because commercial supply of paid blood discourages altruistic, voluntary donation, hence leading to supply shortfalls[242] and increasing cost. Additionally, Paid blood donation is inherently unsafe as the financial motive makes people in high-risk groups for certain diseases lie about their status to get money.

Considerations about disclosing test results for blood donors

The blood service in Ghana provides safe and pleasant environment for both VNRD and FRDs. They are made to understand the need and reasons why they donate for the needy and their relatives, treat them with respect and obtain their informed consent before blood donation. These donors are given all relevant information and, in particular, provided with TTI test results. The blood service ensures and assures donors of the confidentiality of all personal information they provide, notably those related to health and exposure to TTI risks. The service is obliged to blood

donors to ensure the notification of positive test results and the availability of appropriate counselling and referral.

They have the responsibility to provide the service with all relevant information to the best of their knowledge about health conditions that may pose risks for their health and about activities or behaviours that increase their risk for a TTI. They have the responsibility to self-defer from blood donation if they believe they are unsuitable to donate. They have right to withdraw from blood donation at any time during the procedure for any reason, including doubts as to their suitability as a blood donor, without any need to explain this decision.

Ethical considerations of approaching school children as donors

According to the National Blood Service, not all school children can be approached except they are 17 years of age and above. In SSA where VNRDs are mostly secondary school students, peer pressure is considerable and ethical so that in some schools, over 70% of students present themselves for blood donation[243]. This pressure is further increased when leaders of the schools (principals and teachers) lead by giving blood themselves.

Blood donor recruitment and cost-effectiveness

Several arguments against compensated donation have little basis in evidence and would lead to many of today's voluntary donors being designated as paid, because of the large range of incentives used to recruit and retain them. In SSA, most collected blood originates from accessible and cheaper replacement donors while recruiting and retaining volunteers requires considerable costs not all countries can afford. The TMU of KATH, Kumasi, and many local FM radio stations have developed partnership calling more than once a year for donation at the radio stations where music, entertainment, and token gifts are available.

It has been demonstrated that the use of a culturally and socially adapted environment to make the gift of blood a pleasurable and festive experience generated a new pool of blood donors spontaneously repeating donations. This program indicates that retaining Ghanaian blood donors is possible at little extra cost to the blood centre and that such an approach may represent a substantial help in the efforts of SSA to collect volunteer blood.

The best cost-effectiveness for voluntary donors as far as I know is a study in Zimbabwe in 2016, where a unit of blood was costed to be $118.42[16]. This involved recruitment, collection, testing, processing and storage/distribution. Although the supply was inadequate and inconsistent, there was lower TTI prevalence and good quality assurance.

Variably, the cost in collecting blood from a family replacement donor in Malawi in 2007 was found to be $16.00[244]. Although the supply was inadequate and higher levels of TTI, the supply was consistent. Factors affecting these relative costs are complex but are in part due to the cost of donor recruitment in centralized systems. In the family replacement system, the cost of donor recruitment is entirely borne by families of patients needing a blood transfusion.

3.3 Study collaborators

Collaborators in this study included the Transfusion Research Capacity (T-REC) project which was initiated from 2011 to 2015, funded by the European Union Seventh Framework Programme

(FP7/2007-2013) under Grant Agreement No. 266194. The T-REC project was coordinated by the Capacity Research Unit (CRU) at the LSTM with the National Blood Service, Zimbabwe and universities in Groningen and Copenhagen as partners, and the Rigshospitalet of Copenhagen (National University Hospital), Denmark. Other collaborators were, NBSG, TMU, Research and Development Unit (RDU) of KATH, KNUST, Kumasi, all in Ghana.

CHAPTER 4

Syphilis screening practices in blood transfusion facilities in Ghana

The work described in this chapter has been published by Elsevier Ltd on behalf of International Society for Infectious Diseases (appendix 1.1).

4.1 Introduction

The objective was to investigate syphilis testing practices of transfusion facilities in various parts of Ghana.

Early reports of the transfusion-related transmission of syphilis led to the World Health Organization (WHO) recommendations for syphilis testing of blood donors [245]. These recommendations have been questioned, since syphilis antibodies among blood donors can often be the result of previous infections or even unspecific reactions. Nevertheless, syphilis screening continues to be a requirement in many countries.

In Ghana, as in many other African countries, the purchase of blood bank reagents is poorly regulated, with local blood banks purchasing whatever reagents are available and affordable. Additionally, the reagent cost per test for syphilis testing in Ghana depends mainly on the bargaining power of the facility management system in the open market. This decentralized purchasing system may lead to increased costs of reagents, as well as failures in quality and consistency. In addition to decentralized reagent purchasing, the lack of written SOPs and effective TTI guidelines for donor care may hamper quality and care.

To achieve this objective 1, a survey was conducted among facilities across that screened blood donors. The survey compared current syphilis screening practices in Ghana with the

recommendations of the WHO and NBSG regarding the use of assays for screening blood donors for syphilis and the performance of the assays. The prevalence of syphilis antibodies in blood donors was also estimated. Additionally, the survey determined whether written SOPs or guidelines were in place for syphilis screening and whether donors with positive syphilis tests were referred for clinical follow-up.

4.2 Methods

4.2.1 Study Design

The study was designed to interview the in-charges of all health facilities undertaking blood transfusion testing in Ghana in order to get as close to a national survey as possible.

Out of the many different categories of hospitals in Ghana, a total of 149 health facilities across the country practice blood transfusion under the NBSG. Three of the facilities were teaching hospitals located in the Greater Accra, Ashanti, and Northern regions. Ghana has 10 administrative regions and each of them has a regional hospital which have less beds than the teaching hospitals. There are 58 district hospitals that are distributed across the country based on the level of development of the region, so some regions have more transfusion centres than others. There are also other health facilities that screen blood donors such as 36 mission hospitals, eight private hospitals, and seven clinics that are distributed across the country.

4.2.2 Facilities included in the survey

The NBSG in Ghana has a register of 149 blood transfusion facilities which screen blood for transfusion. An attempt was made to survey all of these laboratory heads by telephone interview and/or email questionnaire. However, for a few of the smaller, more remote facilities, a lack of accurate contact details meant that some could not be contacted and therefore did not participate in the survey. The respondents who were interviewed to represent the facilities were laboratory

technical heads (health professionals) with enough experience to release all available laboratory information. These respondents were interviewed between January 2014 and February 2015 about information on their January – December 2012 syphilis screening results. Because of limited resources and time, it was not possible to physically visit all the facilities in person.

4.2.3 Development of the interview questions for the telephone/email survey

The survey was developed based on information obtained from the NBSG about the syphilis testing practices in the various health/transfusion facilities in Ghana. The survey questions focused on the aims and objectives of the study. An introduction was included in the survey to explain to the respondents the importance of the study. The respondents were briefed that there were no wrong or no right answers and asked to provide honest opinions and answers, and they were assured of anonymity. The respondents then agreed to participate before they were interviewed. Each facility was asked questions (for telephone interviews) or sent the same questions by email (Appendix 4.1) pertaining to syphilis screening practices. Samples of the interview questions were pilot tested with nine transfusion facilities in Ashanti region. Corrections and modifications were made to clarify some of the questions and they were retested on two sites before being finalized for the interviews with the various facilities. The nine transfusion facilities were interviewed again using the revised questions and their responses, as well as all those from the other facilities, were included in the analyses.

4.2.4 Inclusion criteria

1. Transfusion facilities under the NBSG with laboratories testing for TTIs.
2. Transfusion facilities under the NBSG who gave their consent to participate in the study and who could be contacted.

3. Health professional available (biomedical laboratory scientists who were technical heads) who could be interviewed on behalf of the health facility.

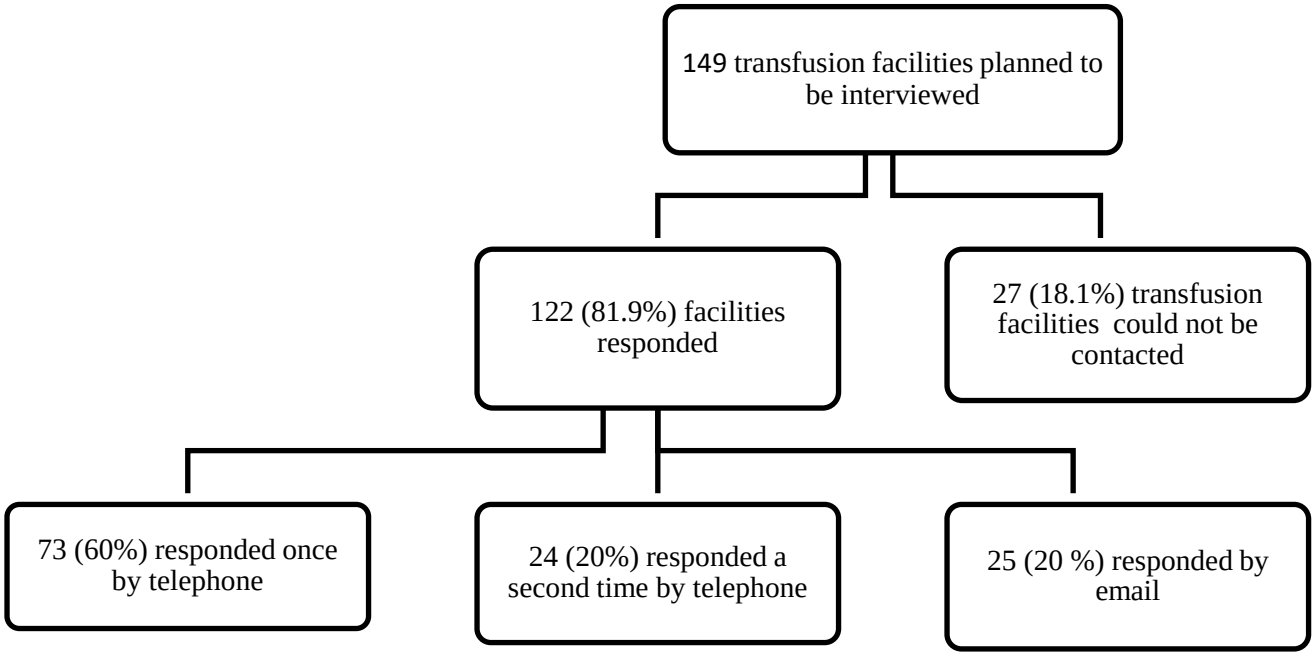
4.2.5 Training of research assistants

Majority of the interviews, e-mails and data entry were done by myself but because of the volume of work, two research assistants were recruited and trained to assist in the interviews, with e-mailing the questions to the facilities and with data entry. These research assistants were employees of KATH/TMU, Ghana so they were already familiar with blood donor screening processes. It was ensured that their involvement in this project did not disrupt their regular working activities.

4.2.6 Data collection

Contact numbers and e-mail addresses of the technical heads of facilities were obtained from the NBSG headquarters in Accra and other laboratory science colleagues in the various transfusion facilities in the country. Seventy-three of the 149 (60%) technical heads responded immediately by telephone, while 24 (20%) of them were interviewed twice before providing all of the information by telephone; 25 (20%) provided information through a semi-structured questionnaire by e-mail. The total number of non-respondents or facilities that could not be contacted, was 27 (18.1%); most of these were in remote areas with small catchment areas (figure 4.1).

Figure 4.1 A flow chart describing the participation of the transfusion facilities in the telephone/e-mail survey



4.2.7 Data Management

Data obtained after interviewing technical heads of the various transfusion facilities in the country were checked every day for completeness. Validation of the data entry was done at the end of every week by myself to make sure they were properly recorded from the data received from interviews and email sources.

4.2.8 Statistical analysis

Data from the interviews were collected using Epi Info version 3.5.3 (US Centre for Disease Control and Prevention, Atlanta, GA, USA). Data were transferred into an Excel spreadsheet, corrections were made by cleaning the data, and data were exported into Stata version 12.0 statistical software (StataCorp LP, College Station, Texas, USA) for analysis. Descriptive statistics of the variables were analysed and are presented in the form of tables and graphs. Proportions for

the various potential predictor variables (independent) and the outcome variables (dependent) were calculated and presented as percentages. Proportions were compared using chi-square (X^2) and t-test. Prevalence was estimated by calculating proportions and providing their respective confidence intervals (95% CI). Independent variables with p-values less than or equal to 0.05 were considered significant.

4.3 Results

4.3.1 Facilities and testing

Of a total of 149 health facilities known to be undertaking blood transfusion and screening for TTIs, 122 (81.9%) responded to the survey. In 2012, the total number of donations collected and screened for TTIs other than syphilis (i.e. HIV, HBV, and HCV) from the 122 transfusion facilities responding to the survey was 143,787 (table 4.1).

The total number of transfusion facilities not screening for syphilis was 64 (52%). When asked for the reasons, 49 facilities (77%) reported lack of funds to purchase reagents. Fourteen facilities (21%) reported that although syphilis screening is recommended, the refrigeration of blood units for more than 5 days, kill *T. pallidum*. One transfusion facility (2%) reported that screening for syphilis was not mandatory.

The total number of donations at the 58 (48%) transfusion facilities screening for syphilis was 91,386 units, of which 3,371 were syphilis antibody seroreactive, resulting in an estimated seroprevalence of 3.7% (95% CI 3.6–3.8). Of the facilities screening for syphilis, two of the three (67%) teaching hospitals screened for syphilis and contributed the highest percentage (40.4%) of the total donations (figure 4.1). Furthermore, 8 of the 10 (80%) regional hospitals screened for syphilis, but contributed only 17.7% (16 009/91 386) to the total donations, whilst 12 of the 36 (33%) mission hospitals screened for syphilis and contributed 15.4% (14,064/91,386) to the total

donations, as shown in table 4.1. Among the seven clinics, only three (43%) screened for syphilis and these contributed the least (1%) donations. However, the teaching hospitals reported the lowest syphilis rate of seroreactivity (3.2%), with the highest coming from the mission facilities (4.4%). Notably, almost half of the district hospitals did not test for syphilis.

4.3.2 Donor type and syphilis seroreactivity

The total number of donations screened for syphilis was 91,386 (63.6% of 143,787). The total number of voluntary donations screened for syphilis was 26,180 (28.6%, 95% CI 28.4–28.9), with 757 (2.9%) testing positive. Of the total of 65,206 (71.4%) family/replacement donations, 2,614 (4.0%) tested positive for syphilis (table 4.1). This indicates that the rate of syphilis seroreactivity from FD in its totality for this survey was significantly higher than the rate from VNRD ($p = 0.008$). However, there were differences in syphilis seroreactivity depending on the health facility in terms of VNRD and FD: while there was no difference between VNRD and FD in syphilis seroreactivity in the teaching facilities (table 4.1), there were differences in the other health facilities, where FD seroreactivity was significantly higher than VNRD seroreactivity, except at the mission facilities, where VNRD seroreactivity was significantly higher than FD seroreactivity. However, the data received from the transfusion facilities across the country did not indicate the sensitivity and specificity of the type of syphilis test used.

Table 4.1 Results of syphilis screening survey in Ghana – (January – December) 2012

Health facility type	Number of screening sites	Number of donations screened for TTIs other than syphilis	Centres screening for syphilis n (%)	Number of Donations (Proportions) screened for syphilis -2012	Av. Number of donations screened for syphilis per health facility per day (m)	Number and Proportion of donor types screened		Sero-reactive and estimated prevalence (%)		
						VNRD	FD	Total	VNRD	FD
Teaching	3	56951	2 (67)	36951 (64.9)	51	13390 (36.2)	23561 (63.8)	1176 (3.2)	424 (3.2)	752 (3.2)
Regional	10	19768	8 (80)	16009 (81.0)	6	5241 (32.7)	10768 (67.3)	578 (3.6)	74 * (1.4)	504* (4.9)
District	58	36650	30 (52)	20571 (56.1)	2	4305 (20.9)	16266 (79.1)	853 (4.1)	95* (2.2)	758* (4.7)
Clinic	7	2343	3 (43)	879 (37.5)	1	99 (11.3)	780 (88.7)	35 (4.0)	3* (3.0)	32* (4.1)
Private	8	4503	3 (38)	2913 (64.7)	3	383 (13.1)	2530 (86.9)	107 (3.7)	11* (2.9)	96* (3.8)
Mission	36	23572	12 (33)	14063 (59.2)	3	2762 (19.6)	11301 (80.4)	622 (4.4)	150* (5.4)	472* (4.2)
TOTAL/Average	**122**	**143787**	**58 (48)**	**91386 (63%)**	**4**	**26180 (28.6)**	**65206 (71.4)**	**3371 (3.7)**	**757* (2.9)**	**2614* (4.0)**

TTIs –transfusion-transmitted infections, VNRD-voluntary non-remunerated donors, FD-family donors, HIV Human Immunodeficiency Virus, HBV-Hepatitis B Virus, HCV-Hepatitis C Virus, Av.-Average, m-mean and * denotes statistically significant.

4.3.3 Types of assays used for syphilis testing

Of the total donations screened for syphilis in this survey, 28 565 (31.3% of 91 386) were tested using a recommended assay (TPHA; Fortress Diagnostics Limited, Antrim, UK). The non-recommended methods used as shown in table 4.2, were all syphilis rapid diagnostic tests (RDTs), with 60% (35 of the 58 transfusion facilities that test for syphilis) reporting ACON as the brand name (ACON Laboratories, Inc., San Diego, USA). Of the others, 11 of the 58 facilities (19%) reported First Response (Premier Medical Corporation Limited, Kachigam, India), seven of the 58 (12%) reported ABON (Abon Biopharm Company Limited, Hangzhou, China), three of the 58 (5%) reported Fortress (Fortress Diagnostics Limited, Antrim, UK), two of the 58 (3%) reported Wondfo (Guangzhou Wondfo Biotech, Guangzhou, China), and only one facility out of the 58 (1%) reported Determine (Allere Medical Company Limited, Matsuhidai, Japan) as RDT for

syphilis testing. From the survey, it was found that none of the facilities was using a second test after the initial RDT, to re-screen syphilis-reactive donations. Of the 58 transfusion facilities that test for syphilis, 47% validated their syphilis test kits before screening, while only 7% had written SOPs (table 4.2). The hospital management of 52 (89.7%) transfusion facilities purchased syphilis screening reagents on the open market (table 4.2). The variation in cost per test strip for syphilis screening varied 10-fold, from US$ 0.2 to US$ 2.0.

4.3.4 Follow-up of syphilis seroreactive donors

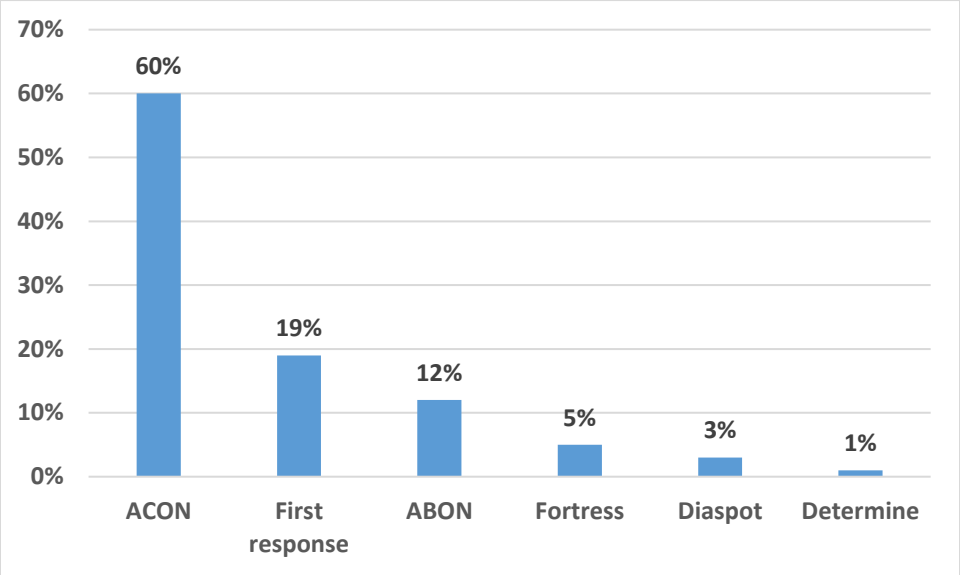
Of the 58 facilities that test for syphilis, 33 (56.9%) of them referred syphilis-reactive blood donors for clinical advice with 16 (16/58; 31%) facilities as the highest coming from the districts whilst only one each (1/58; 1.7%) being the lowest from both clinic and private facilities (table 4.2).

Table 4.2 Results of syphilis screening survey in Ghana – 2012

Health facility type	Number of centres that validate test kits n=58, (%)	Number of centres with written SOPs n=58, (%)	Number of centres that refer for clinical advice n=58, (%)	Number (%) of reagents purchased by Hospital Mgt n=58, (%)	Donations screened with recommended assays (TPHA) n=91386, (%)	Non-recommended assays (RDTs) used in the screening sites
Teaching	2 (3.4)	1 (1.7)	2 (3.4)	2 (3.4)	25726 (69.6)	Fortress
Regional	7 (12.1)	1 (1.7)	7 (12.1)	8 (13.8)	0	ACON, First Response and Determine
District	12 (20.7)	1 (1.7)	16 (31.0)	28 (48.3)	612 (3.8)	ACON, ABON, First Response and Wondfo
Clinic	0	0	1 (1.7)	1 (1.7)	165 (18.8)	ABON
Private	1 (1.7)	1 (1.7)	1 (1.7)	1 (1.7)	2062 (67.7)	Syphilis ultra-rapid test
Mission	5 (8.6)	0	8 (13.8)	12 (20.7)	0	ACON and First Response
Total	**27 (46.5)**	**4 (6.9)**	**33 (56.9)**	**52 (89.7)**	**28565 (31.3)**	

SOPs, standard operating procedures; TPHA, Treponema pallidum haemagglutination assay; RDTs, rapid diagnostic tests; n, total number. *Validate: prove the efficiency of a test kit.

Figure 4.2 The use of RDTs for testing in the facilities that test for syphilis – 2012 N=58



4.4 Discussion

This survey aimed to describe syphilis screening practices and seroprevalence of syphilis for blood donors in transfusion facilities in Ghana. The estimated national syphilis seroprevalence of 3.7% in this survey is similar to that found among healthy blood donors in Ghana and elsewhere in the region [7,246,247]. However, the prevalence varies from one facility to another and from a country to another. In such settings with these seroreactivity rates, the study also found poor quality of laboratory screening due to the lack of equipment, training personnel, reagents and standard procedures, and this highlights the need for systematic and better screening for syphilis to help ensure a safer blood supply.

The study found that about half of the studied facilities in Ghana were not screening blood donations for syphilis, which could lead to syphilis transmission through blood transfusion. Of

those facilities that were found to screen donated blood for syphilis, only a third used a recommended test. Among those facilities that were screening, half were not validating the kits, and of donors found to be syphilis-seropositive, more than a third was not referred for further clinical management.

The study additionally found that, there was significantly high syphilis sero-reactivity rates among FD compared with VNRD. Many parts of the world have also reported syphilis seroreactivity rates among FD similar to that found in the present study [111,248]. One of our findings (table 4.1) was that there was no difference in syphilis seroreactivity rates between VNRD and FD in teaching hospitals but there were in all other facility types. This could possibly be explained by a high proportion of VNRD in the teaching hospitals. Besides, infections in first-time VNRD are similar to FD since they are mostly first-time, non-repeat donors, so both groups could be considered to represent a single population with respect to infection rates. It is when a donor, either VNRD or FD, becomes a repeat donor that seroreactivity reduces. A study performed in one of the Ghanaian teaching hospitals indicated that the viral safety of replacement and first-time volunteer donors was similar, so they could be considered to constitute a single population of donors [42]. It went on to reveal no significant difference in prevalence of TTIs between first-time volunteer and replacement donors. From experience, the teaching hospitals have low repeat donations in volunteer donors where the majority of blood donors are either first-time volunteer or replacement donors, and this also happens in other SSA blood centres [249]. There is clear evidence that repeat donation brings a significant increase in blood safety and this could be equally applied to volunteer and to replacement donors [91].

Despite recommendations that all blood donors should be voluntary and non-remunerated, FD are common throughout SSA [250]. One reason for the higher rates of syphilis-positivity in FD is that

FD are older than VNRD and therefore have had a longer time to acquire syphilis antibodies. However, FD may be under pressure to donate blood when their relatives are admitted to hospital and in need of a blood transfusion, even when they know that they are potentially at risk of sexually transmitted diseases as a result of high-risk behaviours. They may be more likely to conceal a relevant medical history and the risky sexual behaviours that predispose them to infections and thus pose a threat to the safety of the blood supply. Despite this, family donations remain dominant in the African continent because family and community ties are often considerably stronger than in other types of society; making the gift of blood a natural contribution to relieve sufferers in hospitals [251]. Conversely, potential donors may be less willing to donate to someone not known to them. The WHO recommended that reliance on family donations should be phased out due to their association with an increased risk of TTIs [109]. The WHO states that blood from VNRDs who give blood out of altruism is the safest source of blood [252]. Establishing a panel of regular, voluntary, non-remunerated blood donors is therefore the most effective way of ensuring adequate ongoing supplies of safe blood.

There have been several policies and recommendations from the WHO that each transfusion service should develop written SOPs as guidelines covering all procedures in the testing of donated blood [253]. Written and followed SOPs are an integral part of a quality system, as they facilitate consistency in the performance of procedures in accordance with standards.

The survey demonstrated that only 6.9% of the facilities followed written SOPs, indicating poor quality systems where these should play a vital role in blood safety. The WHO has specified that consistency and reliability of performance in conformity with specified standards raises the quality of systems in promoting blood safety. Unfortunately, an earlier exercise carried out by MoH of Ghana, (which was reported in the Ghana National Blood Policy) to determine the status of the

blood services in regional and district health facilities in 2006, revealed that the quality assurance programme including SOPs that had been written and followed was under-developed and that the equipment at all sites was generally inadequate [25].

The present survey confirmed the existence of major problems within quality assurance systems and the supply of logistics services. Previously the NBSG had an external quality assessment (EQA) programme operating only at its headquarters. However, the NBSG checks internal quality assessment (IQA) processes at other blood banks elsewhere in the country by visiting the various facilities with quality audit and control samples. As indicated earlier, because the NBSG does not have control over the purchasing of reagents at individual health facilities, it becomes challenging to make recommendations on IQA.

The finding that 56.9% of facilities referred syphilis-reactive blood donors for clinical advice suggests that, at the other facilities, syphilis reactive donors remained untreated and potentially infectious and could be transmitting the disease to others. This represents a substantial public health risk.

The variation in costs for syphilis screening has significant implications, particularly in resource-poor settings in SSA. There is little published information on the variation in costs per test for syphilis, but reported costs in this study ranged from US$ 0.2 to US$ 2.0. In Ghana, the cost variation in syphilis test kits exists due to a lack of guidelines to indicate the effective and accepted test kits and their costs. As a result, many test kit types are available on the open market without proper validation and at different costs. For quality and consistency, the NBSG should be responsible for purchasing approved test kits before use in order to standardize the tests for syphilis screening of blood for transfusion. Screening for TTIs among blood donors can be a cost-effective approach to monitor the prevalence, distribution, and trends of the infections among blood donors.

However, cost of TTI testing is a major challenge to the provision of safe blood and blood products SSA. A report has indicated that, treponemal, non-treponemal, immunochromatographics assays for syphilis testing, are affordable and can significantly contribute to blood transfusion safety, as in most resource-poor countries [254]. The present survey did not indicate whether centralized purchasing would necessarily lead to lower prices, but it may help to reduce the cost variations and more importantly, would ensure proper validation.

The techniques used for syphilis screening are different from one country to another: the VDRL or RPR alone for some, and the VDRL and TPHA for others [252]. Tests and algorithms should be selected so that they correspond with the prevalence of the disease and match the technical expertise of the personnel and the availability of reagents and equipment [103]. The selection criteria for a screening strategy must include simple techniques, reliability, sustainability, and cost-effectiveness. Although they are not recommended for blood banks in Africa, rapid test techniques may be preferred because of their affordability, user-friendliness, the availability of test reagents, and good sensitivity and specificity; furthermore they do not require sophisticated laboratory materials [103]. The WHO recommends that each country should decide on the TTIs to be screened for as part of the blood screening programme and develop a screening strategy appropriate to its specific situation, influenced by the incidence and prevalence of infection, the capacity and infrastructure of the blood service, and the costs of screening [255]. The critical factor is the effective implementation of the strategy selected and the consistency of implementation within a well-managed quality system. The NBSG does recommend standardized syphilis screening of all donated blood, but this survey revealed that the guidelines were not generally being followed and serves as an example of the consequences when national guidelines are made without structures to enforce them and without the resources needed to implement them locally.

4.5 Study limitations

1. This survey was not able to reach all of the transfusion facilities in Ghana, especially those outside NBSG of which their workload was very minimal. Moreover, the facilities under the NBSG that were not able to provide data because they could not be reached were small villages in remote areas. As such, it is likely that the results provide close to a true reflection of the national situation.
2. The study relied on information provided by telephone and e-mail. Resource constraints meant that it was not possible to substantiate the findings first-hand. More so, the respondents interviewed might be scared of releasing true available laboratory information that could paint a bad picture for the facilities. Nevertheless, this was considered the best methodology with the resources available because some transfusion facilities are located in remote areas with challenging road access.
3. The estimated prevalence may not be a perfect reflection of the epidemiological situation in Ghana. This is because the donor population that was not screened could have had a higher or lower prevalence of syphilis than the screened population. For the population that was actually screened, variation in screening practices may have led to both under-reporting due to a lack of sensitivity or over-reporting due to poor specificity of the screening tests used.
4. None of the facilities were using a second test to re-test syphilis-reactive donations, for example a non-treponemal test to detect active infection. Therefore, it is difficult to estimate how many donations may have been infective, and how many patients receiving a blood transfusion are potentially at risk.

4.6 Conclusion

There is a relatively high prevalence of syphilis reactivity in the blood donor populations in Ghana, as elsewhere in sub-Saharan Africa. However, there is a low syphilis testing rate and a relatively high use of non-approved, non-validated test kits (RDTs) for syphilis screening, obtained at different costs, in Ghana. If these rapid tests are effectively validated and managed, they could be incorporated into the existing guidelines to enhance blood safety. However, the considerable mismatch between recommendations and actual practice for, or absence of, syphilis screening may compromise blood safety. Further studies on syphilis RDTs for blood donors are suggested, in order to improve their application in resource-poor settings.

CHAPTER 5

Improving the screening of blood donors with syphilis rapid diagnostic test (RDT) and rapid plasma reagin (RPR) in low- and middle-income countries (LMIC)

5.1 Introduction

5.1.1 Study background

The work described in this chapter has been published in *Transfusion Medicine Journal*.

1. A novel strategy for screening blood donors for syphilis at Komfo Anokye Teaching Hospital, Ghana - doi: 10.1111/tme.12279 (appendix 1.2).
2. Improving the screening of blood donors with syphilis rapid diagnostic test (RDT) and rapid plasma reagin (RPR) in low- and middle-income countries (LMIC) - doi: 10.1111/tme.12363 (appendix 1.3).

Objective 2: To determine the positive predictive value of the RDT that is most commonly used in KATH and Ghana for syphilis testing.

Objective 3: To estimate the proportion of blood donors in KATH, Kumasi who are really infectious through blood transfusion.

Syphilis screening is a big challenge in many low and middle-income countries (LMIC) that have a limited capacity for testing. High syphilis prevalence among healthy blood donors in Africa aggravates the problem in this region. Techniques for syphilis testing are very problematic and conventionally rely on a combination of non-treponemal and treponemal tests.

The non-treponemal antibody tests for donor screening include the VDRL [256] and the RPR [257]. The advantages are that these tests are inexpensive, fast, simple to perform and have acceptable sensitivity [258]. They are able to identify infected blood donors a few days before the treponemal test and thus are useful for acute infection. However, VDRL and RPR cannot be automated and

are therefore time-consuming if used for large-scale testing. Another major problem when using non-treponemal tests is the possibility of biological false positive reactions due to cross-reactivity with other conditions, such as viral infections, pregnancy, malignant neoplasms, autoimmune diseases and advanced age [51,166].

Treponemal tests for donor screening classically included the TPHA, TP-PA, FTA-ABS [259]. Treponemal tests typically remain positive even after treatment [257,260,261], implying that a donor previously diagnosed with syphilis who has been treated, or in whom the infection has resolved, cannot be distinguished from a new or untreated case of syphilis. However, newer, treponemal tests can be automated and therefore have low running costs and provide objective readings, making them useful for large blood centres.

5.1.2 Syphilis as a major concern

Syphilis is a major concern as a TTI in Ghana, including Kumasi, and as previously described in chapter four, the current guidelines for screening of syphilis in blood donors are in need of revision. This includes which methods may be applicable and affordable, as outlined in the following sections.

5.1.3 Syphilis RDT for Transfusion-transmitted infections (TTI)

RDTs for TTI screening are often preferred in LMIC as they are affordable, quick, require limited expertise and are therefore suitable for screening blood donors' pre-donation on mobile blood drives. Pre-donation screening of blood donors for TTI is the practice by which a prospective donor is tested at the donation site for the presence of one or more of the TTI agents by a rapid or quick method. Donation is deferred if the test is reactive for any of the TTI markers but on the other hand the donor is allowed to donate if the test is nonreactive for all available TTI markers [262]. Pre-donation testing of blood donors for TTIs is done in most developing countries because substantial

cost savings are made from resources, materials, and man-hours which would have been spent to obtain infected blood units which would later be discarded. Pre-donation screening where TTI seroprevalence is high is attractive as the costs of collection are avoided and the risk of mixing up screen positive and screen negative blood units' post collection in blood bank refrigerators is reduced. Pre-donation testing also allows the donor to be informed of any positive test on the spot and referred for confirmation and, if necessary, clinical follow up.

In developing countries and areas with limited resources, laboratory facilities are often unavailable for standard automated syphilis tests. Given the barriers to automated testing, many resource-limited countries are resorting to syphilis RDTs for TTI screening [101]. Although RDTs hold potential for increasing the safety of the region's blood supply compared to no testing at all, uncertainty surrounding the performance of some of the RDTs which are available has increased debate regarding their application to TTI screening [263-265]. Some rapid tests are highly sensitive and specific [22,166] but cannot differentiate between active and treated syphilis; others may give false positive reactions [199]. Irrespective of the advantages of these rapid tests, if they have a low positive predictive value (PPV) (i.e. a high false positive rate) and are used in blood banks, then many donors will be deferred when they carry no risk to the blood supply. Conversely, if the PPV is high (i.e. a low false positive rate), then few donors with no risk to the blood supply will be deferred. This is really important in LMIC settings where blood is often in critical short supply, coupled with a high burden of TTIs among donors and the general population.

At KATH, blood donors have been screened for HIV 1 and 2, HBV, and HCV by pre-donation RDT since 2001, and approximately 10% of donors are deferred [22]. There is currently no post-donation testing unless there is a power shortage onsite during the donor session. This is because when blood donations are carried out in schools and public places (public drives), the centrifuges

use electricity in spinning blood samples before the tests are carried out using serum. If there is power shortage, blood units are collected onsite without the screening of the donors. Screening is performed as soon as the blood units reach the blood bank in the hospital.

KATH was previously not testing for syphilis because of constrained resources and presumptions that transmission risk is low and refrigerated storage renders the causative organism inactive. However, a study carried out at the hospital in 2011 identified a case of transfusion-transmitted syphilis (TTS) in an 8-year old girl. In investigating this case it was found that 57% of donations were stored for less than 4 days before being transfused [6]. This is important to know because *T. pallidum* survives for few days at 4◦C, thereafter, it is considered killed if blood products are refrigerated for over 4 days [40].

As a consequence, the hospital transfusion committee (HTC) at KATH, whose role is defined earlier in chapter two of this thesis, recommended the introduction of syphilis screening. The introduction of syphilis testing with RDT demonstrated a seroreactivity rate of 7.0%, and this had a critical and negative effect on the blood supply for the hospital since all these donors had to be deferred.

5.1.4 Novel algorithm for syphilis testing at KATH

The TMU implemented an algorithm (figure 5.1) which consisted of syphilis testing by Fortress RDT before blood collection followed by rapid plasma reagin (RPR) - IMMUTREP RPR, Omega Diagnostics – Scotland, UK - testing of syphilis RDT reactive donors [44]. The presumption was that by deferring only donors who were RDT positive and also positive for RPR, only donors suspected of active syphilis were rejected. Donors with previously treated syphilis could continue to donate and their blood would be released for transfusion. One of the risks of this strategy was that by combining two different syphilis tests, the algorithm might still defer too many blood donors, due

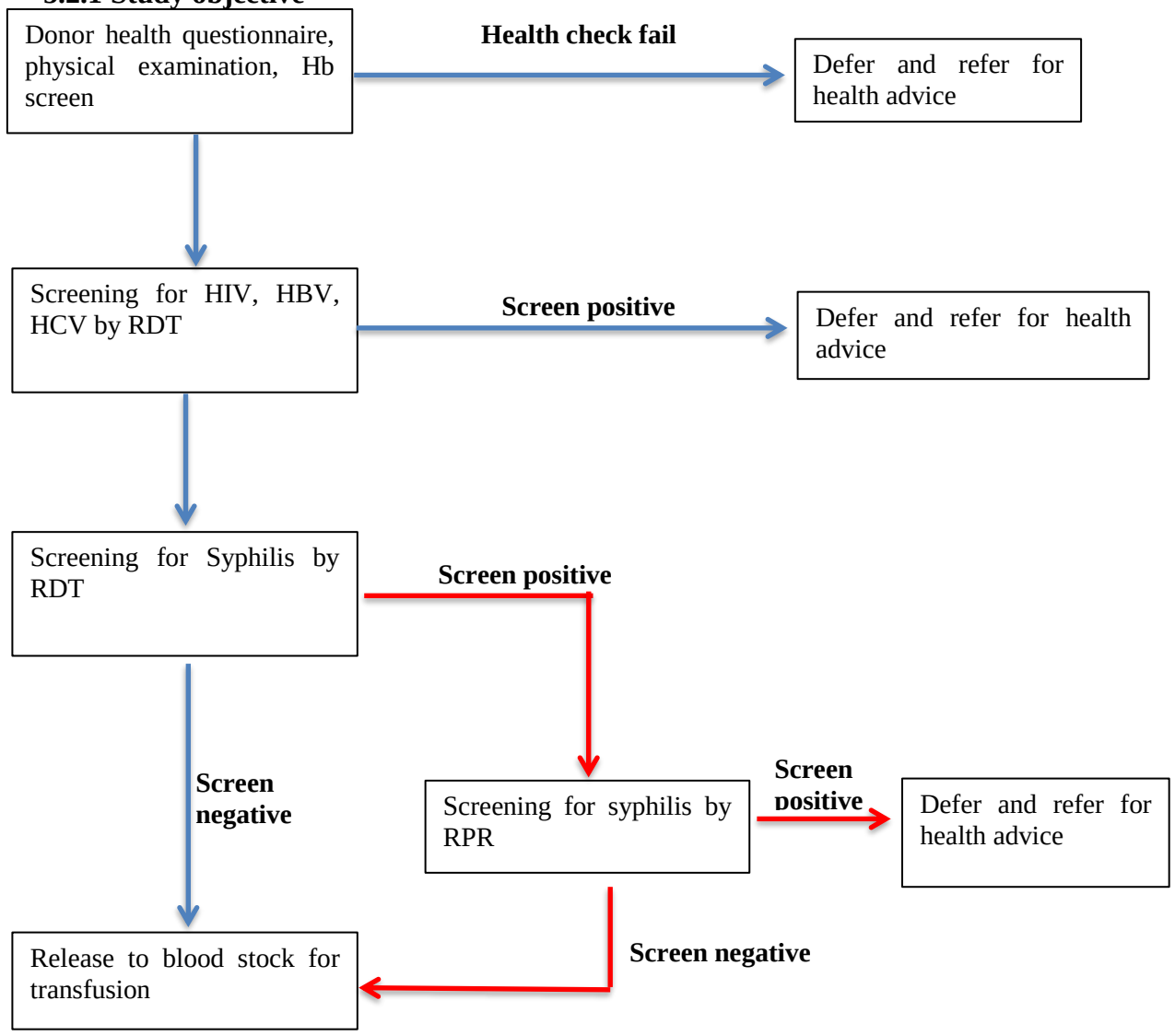
to false reactivity in the two tests. In that case, the algorithm would still unnecessarily reduce blood supply and it would expose donors to unneeded worries, stigma, and therapy.

Therefore, the following study was undertaken.

5.2 OBJECTIVE and METHODS

Figure 5.1 Algorithm for syphilis screening of blood donors, TMU-KATH (new pathway shown in red)

5.2.1 Study objective



The present study aimed to validate the positive predictive value (PPV) of the newly implemented algorithm by applying a gold standard retest algorithm combining two different automated anti-

TP immune assays and an immunoblot of syphilis RDT reactive donors to improve blood donor screening.

5.2.2 Study design

This was a descriptive cross-sectional study conducted in the TMU of KATH. The purpose of the study was to improve the screening of blood donors in TMU with the use of syphilis RDT and RPR by validating PPVs of both test kits. The study algorithm was to screen prospective blood donors initially tested with a treponemal RDT (Fortress® Diagnostics Limited – Antrim, UK) according to routine standard operational procedures. Blood donors who were initially syphilis RDT sero-reactive were selected for further testing. Sero-reactive samples from all consenting donors were further tested according to routine standard procedures with RPR (BD Macro-Vue^{TM} Card test, New Jersey, USA) at KATH/TMU laboratory to identify potential active syphilis infections.

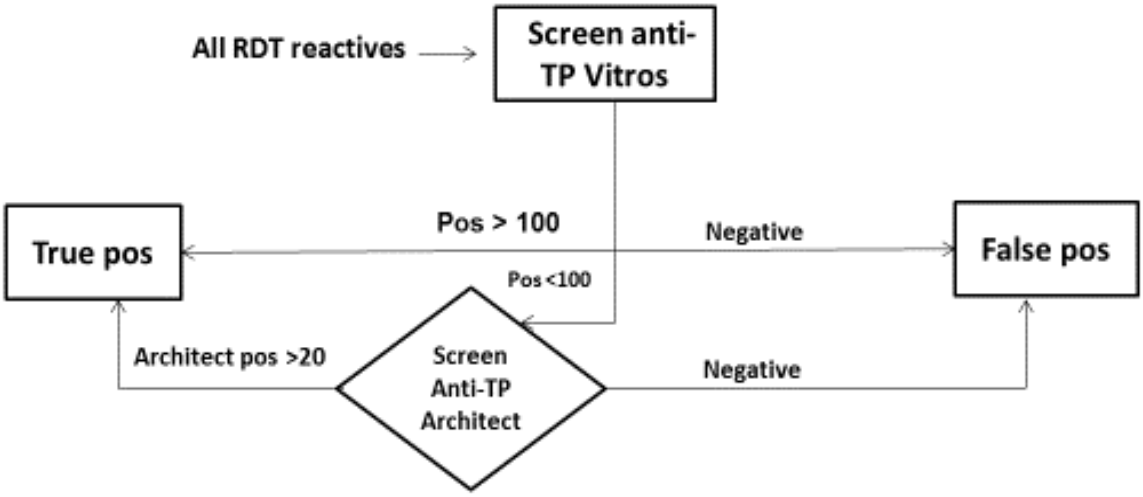
For gold standard confirmation of the Fortress® RDT, al1 RDT reactive samples were subsequently retested in an algorithm combining two automated treponemal immunoassays and a treponemal immunoblot. Initial retesting was performed by Vitros® syphilis *Treponema pallidum* antibody (TPA) chemiluminescence immunoassay using the Vitros ECi/ECiQ Immunodiagnostic Systems as described in chapter two, under treponemal automated (Enzyme immunoassay) of this thesis [181]. All the confirmatory testing was performed at the department of Clinical Immunology, Copenhagen university hospital, Denmark.

For samples where the cut-off value was equals to, or higher than, 100 (S/CO≥100) with Vitros TPA, the result was declared confirmed positive and the sample was not tested further (figure 5.2). All Vitros low positive samples (S/CO <100) were additionally tested with another qualitative anti-TP immunoassay Architect® Syphilis TP (Abbott Diagnostics, Wiesbaden, Germany) which

also detects antibodies binding to the recombinant TP antigens TpN15, TpN17 and TpN47 (figure 5.2).

Thus, a reactive Fortress® RDT sample was considered confirmed positive for specific anti-TP antibodies, if the Vitros syphilis TPA were highly reactive (S/CO ≥ 100) or if Vitros syphilis TPA was low reactive (1< S/CO <100) but the sample was also reactive in Architect (figure 5.2).

Figure 5.2 A flowchart of syphilis confirmatory testing



5.2.3 Sample size

The prevalence of syphilis antibodies within the Ghanaian blood donor population has been estimated to be between 3.0% and 8.0% [6,7]. For the purpose of power estimation, we assumed a prevalence of syphilis antibody around 4.0%. We aimed to determine the prevalence of syphilis antibodies among blood donors using Ortho Vitros® TPA, Architect syphilis TP assay, with a confidence limit of +/- 0.5%.

With this expected prevalence, we needed to test 18,000 potential blood donors to be able to arrive at a sample size of 640 syphilis reactive blood donors to obtain a confidence limit of +/- 0.5 %.

The specificity of the Fortress quick test is reported to be 99.6% (Fortress® Diagnostics Limited – Antrim, UK). With a false positive rate of 2.6 (640 X 0.4%) i.e. 3 false positive samples, the positive predictive value would be estimated to be between 97.4 and 99.9% [266].

5.2.4 Hospital training

Study assistants including nurses, phlebotomists and laboratory staff were recruited, briefed and trained for data/blood sample collection, labelling, and storage of samples for testing. Written SOPs were developed, reviewed and approved by study investigators.

5.2.5 Laboratory case definitions

All specimen collection and testing was conducted within the hospital, with donor care provided by the experienced nurse practitioners, laboratorians, and phlebotomists. Participants were prospective blood donors who had already been tested and were RDT sero-reactive for syphilis. A nurse assisted the participants in reading the consent forms or explained to them in one local dialect (Twi) before signature or thumbprint. Specimens were then taken from participants by a phlebotomist after they had agreed to be included in the study. Specimen collection was done by the healthcare professionals, and all screening and bio-data to be used for the analysis were collected from the participants and recorded on a pre-designed spreadsheet.

5.2.6 Study population

5.2.6.1 Syphilis RDT testing

The sampling frame was drawn from prospective blood donors, both VNRD and FD, who were willing to donate blood for KATH blood bank. These were blood donors aged between 17 and 59

years who had filled in their donor health questionnaire, and weighed over 50Kg, with blood pressure below 140/90 mm/Hg and Hb screened with Copper Sulphate solution above 13.0 g/dL.

Between a time period of February 2014 and January 2015, 16,016 prospective blood donors who came to donate blood for the KATH blood bank were initially tested for *T. pallidum* antibodies using plasma or serum as part of the routine pre-donation system. The syphilis screen was performed with a treponemal RDT (Fortress® Diagnostics Limited – Antrim, UK) according to routine SOPs to detect antibodies (IgG and IgM). These blood donors were from Kumasi and its environs. Approximately 30% were FD who came to the hospital premises to donate blood to replace that which had been given to their sick relatives, while 70% were VNRD mostly from second cycle schools, religious institutions, and public drives.

5.2.6.2 Principles of syphilis RDT test

The syphilis Fortress® Ultra RDT test strip with the use of whole blood/serum/plasma is a rapid chromatographic immunoassay for the qualitative detection of antibodies to *T. pallidum* in whole blood, serum or plasma in blood donor samples.

In the RDT test procedure, recombinant syphilis antigen is immobilized in the test region of the strip. After a donor specimen is added to the specimen pad, the specimen antibodies (IgG and IgM) react with the syphilis antigen coated particles applied to the specimen pad.

The mixture migrates chromatographically along the length of the test strip and interacts with the immobilized syphilis antigen. The double antigen test format can detect both IgG and IgM in the specimen. If the specimen contains TP antibodies, a red line will appear in the test region, indicating a reactive result (Figure 5.4).

Figure 5.3 Syphilis Fortress test kit for testing blood donors

Figure 5.4 A picture of syphilis RDT of a blood donor in the TMU, KATH, showing a nonreactive and reactive result

If the specimen does not contain TP antibodies, a red line will not appear in this region indicating a negative result. To serve as a procedural control, a purple line will always appear in the control line region which indicates that appropriate volume of specimen has been added.

5.2.6.3 Laboratory testing of blood donors

All the test strips, specimen from blood donors, and buffer were allowed to equilibrate to room temperature (15-30^0 C) prior to testing.

1. All test strips were removed from the sealed foil pouch and used as soon as possible.
2. Samples were centrifuged and serum or plasma was separated from the cells. Serum or plasma were used for the testing.
3. Two drops of serum or plasma (approximately 50µL) was transferred from a dropper onto the specimen pad of the test strip.
4. A drop of buffer (approximately 40 µL) was added to the specimen pad and results were available in 10 minutes. Results were interpreted within 30 minutes.

5.2.6.4 Interpretation of RDT results

1. **REACTIVE** – Two distinct lines appear. One line is in the control region (C) and the other line in the test region (T). The intensity of the red colour in the test region varies depending on the concentration of TP antibodies present in the specimen.
2. **NEGATIVE** – One purple line appears in the control region (C). No apparent red line appears in the test line.
3. **INVALID** – Control line fails to appear.

Sensitivity and specificity of the Fortress® RDT using serum or plasma are stated to be 99.7% and 99.6% respectively - for the qualitative detection of antibodies (IgG and IgM) to *T. pallidum* in serum or plasma.

5.2.7 Inclusion criteria

1. Blood donors who were eligible to donate blood according to KATH/national standards (weight >50kg, systolic blood pressure ≤ 140mmHg and age 17 to 60 years).
2. Reactive sample on syphilis RDT
3. Donor consented to participate in the study

5.2.8 Exclusion criteria

1. Blood donors who were reactive for syphilis but also reactive on tests for other TTIs.
2. Blood donors who were reactive but unable to provide a blood sample.
3. Consent not given to participating in the study.

5.2.9 Sample taking and storage

All blood donors who were declared initially syphilis seroreactive with Fortress RDT were asked to report to the TMU donor clinic head nurse for interpretation of their results and for advice.

These donors on arrival were offered to be participants of the study. The syphilis sero-reactive blood donors to be included in the study were informed about the study aims and objectives, and the possible benefits and any negative aspects. Concerning negative aspects, it was explained to them that, there was the possibility of feeling pain and bruises at the place when blood samples were taken. For the benefits, it was explained to them that there was the direct access to a medical doctor in the event that there was a true positivity and active infection at no extra expense.

After the explanation of the study and assessment of their understanding, participants were requested to sign or thumbprint an informed consent form (appendix 5.1) Upon their consent, 5 mL of whole blood was collected from 526 blood donors. After collection, the samples were centrifuged and serum was separated. To avoid repeated freezing and thawing of samples, each sample was aliquoted into 5 vials and labelled with the following purposes;

1. Vial 1 was labelled for RPR testing which was performed at the study site.
2. Vial 2 was labelled for *Treponema pallidum* haemagglutination assay (TPHA) also performed at study site as a local gold standard.
3. Vial 3 was labelled for confirmatory testing which was performed at Copenhagen with Vitros TPA and Architect.
4. Vial 4 was labelled for quality control testing which was performed at 4 different testing sites in the Kumasi transfusion facilities.
5. Vial 5 was labelled as a spare.

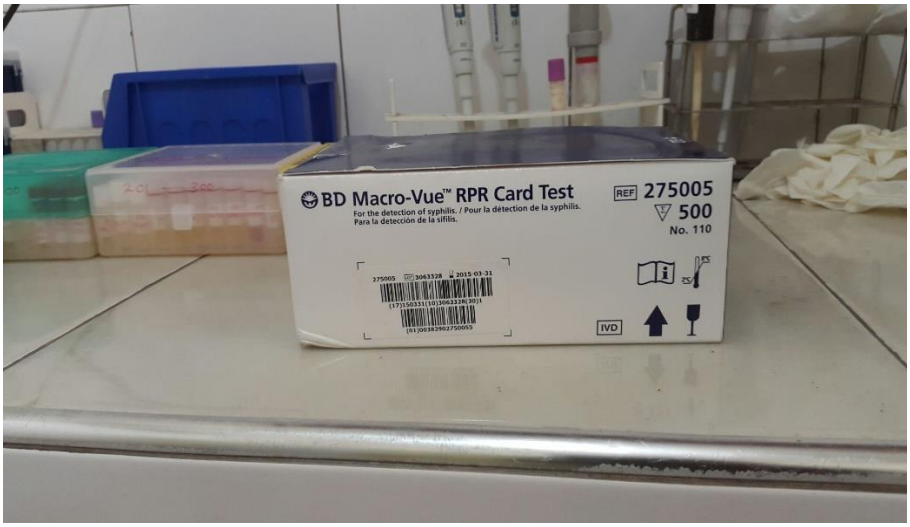
These samples were stored, deep-frozen (-20 to -70^0C) until further testing.

5.2.10 Potential Active syphilis test

5.2.10.1 RPR testing

The Macro-Vue™ RPR 18 mm circle card test (figure 5.5) is a nontreponemal testing procedure for the serologic detection of syphilis. This card test was originally developed for field use where testing could be performed without laboratory equipment [267].

Figure 5.5 An RPR Card test kit for testing initially reactive blood donor samples in the study



5.2.10.2 Principles of the RPR test

The RPR card antigen suspension is a carbon particle cardiolipin antigen (figure 5.5) which detects "reagin", an antibody-like substance present in serum or plasma of persons with syphilis, and occasionally in serum or plasma of persons with other acute or chronic conditions. The reagin binds to the test antigen, consisting of cardiolipin-lecithin-coated cholesterol particles, causing macroscopic flocculation. When a specimen contains an antibody, flocculation occurs with a co-agglutination of the carbon particles of the RPR card antigen, which appear as black clumps against

the white background of the plastic-coated card. In comparison, nonreactive specimens appear to have an even light-grey colour (fig 5.6).

5.2.10.3 Test procedure with 18 mm BD Macro-Vue™ RPR card test

As part of the study procedure, the labelled vial 1 for RPR for each of 526 consenting blood donors who was sero-reactive with the Fortress® RDT syphilis test were taken from the fridge and thawed. All these samples were then tested according to routine standard procedures with RPR (BD Macro-Vue™ Card test, New Jersey, USA) to identify potential active syphilis infections.

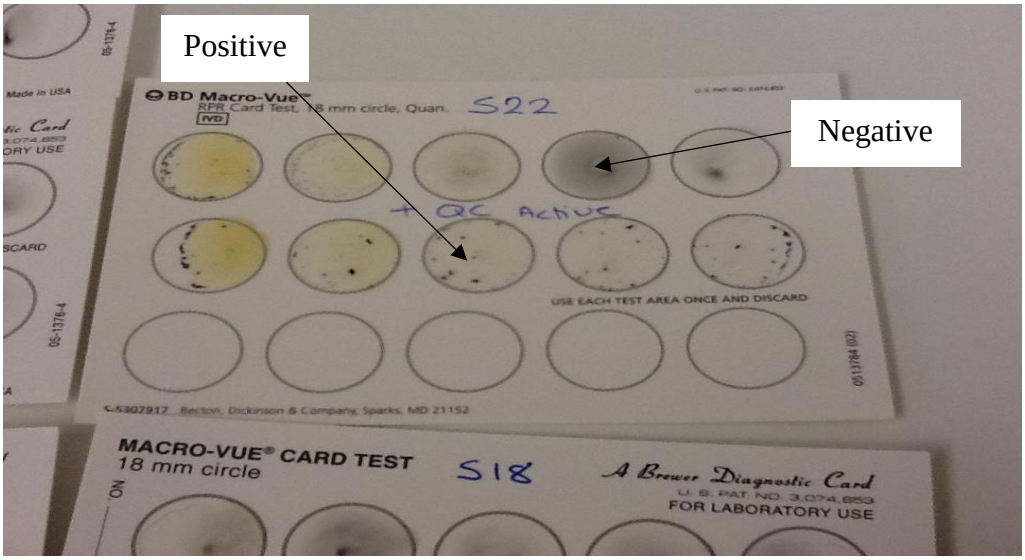
1. A serologic pipette was used to remove 0.05 mL of the specimen (serum or plasma) from the blood collecting tube and care was taken not to transfer cellular elements.
2. Specimen taken was placed onto the circle of the diagnostic test card.
3. Using a new dispenstirs device, each specimen was spread to fill the entire circle. Dispenstirs device was discarded after spreading. The procedure was repeated for each specimen tested.
4. The antigen dispensing bottle was gently shaken before use. Holding it in a vertical position, several drops were dispensed in dispensing bottle cap to make sure the needle passage was clear. One "free-falling" drop (20 G, yellow hub needle) was placed onto each test area. Mixing of antigen and specimen was accomplished by rotation.
5. The test card with mixed antigen and specimen was rotated for 8 min (± 30 s) under a humidifying cover, on a mechanical rotator at 100 ± 2 rpm.

Following rotation, to help differentiate nonreactive from minimally reactive results, a brief rotation and tilting of the card by hand (3 or 4 to-and-from motions) was made. Immediately, the card was read macroscopically in the "wet" state under a high-intensity incandescent lamp or strong daylight.

5.2.10.4 Reading and reporting the BD Macro-Vue™ RPR card test

As seen in the picture below (figure 5.6), individual reactions were evaluated in the "wet" state, under a high-intensity luminous lamp or strong sunshine. Immediately following rotation, cards were read and recorded as positive (i.e. black clumps) or negative (i.e. even light grey).

Figure 5.6 An RPR card test indicating positive and negative results



5.2.10.5 Clinical handling of RPR positive blood donors

Blood donors who were syphilis sero-reactive by RDT which indicated possible past or current infection, were not deferred. Rather, blood units were collected and then quarantined until an additional screening test in the form of RPR was performed. This non-treponemal syphilis test identified possible active infection and, therefore, a potential for transmission of syphilis to the recipient of the blood unit. Units that tested positive by RPR were discarded and the relevant donors were contacted and referred for further investigation and/or treatment. For transfusion purposes, the referred donors are not allowed to donate blood for a period of three years even if they undergo successful treatment (i.e. they are temporarily deferred). This is because syphilis is

fully treatable with antibiotics, usually by injection. After 3 years with evidence of treatment, these donors may be allowed to donate blood again [268].

Conversely, syphilis RDT sero-reactive units testing negative by RPR were released for transfusion.

5.2.11 Syphilis confirmatory testing

5.2.11.1 Vitros® Syphilis TPA assay

Vitros® Syphilis TPA chemiluminescence immunoassay using the Vitros ECi/ECiQ Immunodiagnostic Systems is a qualitative assay that detects total antibodies (IgG and IgM) to TP reacting with biotinylated and horseradish peroxidase (HRP)-labelled recombinant TP antigens TP15, TP17, TP47 and bound to streptavidin-coated wells. The assay was mainly validated in a western population with a specificity of 99.8% (CI 98.7-100%) and a sensitivity of 100% using Syphilis Mixed Titre Performance Panel PSS202 (BBI Diagnostics) and clinical samples from known syphilis treated patients of both Caucasian and African origin.

5.2.11.1.1 Principles of the Vitros® Syphilis TPA

An immunometric immunoassay technique was used which involves the reaction of IgG, IgM or IgA antibodies present in the sample with a biotinylated TP antigen and a horseradish peroxidase (HRP)-labelled TP antigen conjugate. The antibody-antigen complex is captured by streptavidin on the wells. Unbound materials are removed by washing. The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound conjugate catalyses the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The illuminating reaction detected from the bound HRP-conjugates

is directly proportional to the concentration of anti-TP antibodies, and high signal samples (signal at Cut-off (S/CO) >100) were considered confirmed positive.

5.2.11.2 ARCHITECT Syphilis TP

All Vitros low reactive samples (S/CO <100) were additionally tested with another qualitative anti-TP immunoassay Architect® Syphilis TP (Abbott Diagnostics, Wiesbaden, Germany) also detecting antibodies binding to the recombinant TP antigens TpN15, TpN17, and TpN47. This is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of antibody to TP in human serum/plasma as an aid to diagnose syphilis and as a screen, for donated blood and plasma to prevent transmission of TP to recipients of blood and blood products.

5.2.11.2.1 Principles of the ARCHITECT Syphilis TP

The Architect® Syphilis TP is a two-step immunoassay for the qualitative detection of antibody to TP in donor serum or plasma using CMIA technology with flexible assay protocols. In the first step, donor sample, microparticles coated with recombinant TP antigens (TpN15, TpN17, and TpN47) and assay diluent are combined. Anti-TP antibodies present in the donor sample bound to the TP coated microparticles. After washing, the anti-human IgG and IgM conjugate is added to the second step. Following another wash cycle, a pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units. The presence or absence of anti-TP antibodies in the donor sample is determined by the chemiluminescent signal in the reaction to the cut-off signal. The donor is considered reactive for anti-TP if the chemiluminescent signal in the sample is greater than or equal to the cut-off signal.

5.2.11.3 INNO-LIA Syphilis Score

As quality control (QC) measure, 78 out of 526 (15%) syphilis RDT sero-reactive samples randomly selected, were further tested with a line immunoassay (LIA) (Furijebio, Ghent, Belgium). These samples comprised of 24 Vitros high positives, 26 Vitros low positives and 28 Vitros negatives. This assay detects antibodies binding to the same recombinant TP antigens TpN15, TpN17 as well as to TpN47 and a synthetic peptide TmpA derived from *T. pallidum* proteins [187]. The INNO-LIA Syphilis kit used in this study is an easy methodology that makes use of colour-coded reagents and simple interpretation criteria. It shows a high degree of performance as a multi-parametric confirmation method. The assay was used as a QC measure to confirm the presence or absence of specific antibodies to *T. pallidum* antigens in a sub-set (15%) of initially-reactive blood donor sera and plasma.

5.2.11.3.1 Principle of the INNO-LIA

The line immunoassay was based on the enzyme immunoassay (EIA) principle. Three recombinant proteins (TpN15, TpN17, and TpN47) and one synthetic peptide (TmpA) are coated as distinct lines on a nylon strip with plastic backing. In addition to these syphilis antigens, four control lines are coated on each strip: background control lines, 3+ positive control which was also used as sample addition control line, 1+ positive control, and cut-off line.

The donor samples were incubated in a test trough together with the multiple antigen-coated test strips. Specific *T. pallidum* antibodies, if present in the sample, bound to the individual syphilis antigen lines on the strip. Afterwards, a goat anti-human IgG labelled with alkaline phosphatase was added. This was bound to any syphilis antigen/antibody complex previously formed. Incubation with a substrate produced a dark brown colour in proportion to the number of specific

antibodies present in the sample. Colour development was stopped with sulphuric acid after the substrate was added for 30 minutes.

5.2.11.3.2 Interpretation of the INNO-LIA results

A sample was considered NEGATIVE for *T. pallidum* antibodies if all syphilis antigen lines had a negative reactivity rating or if one syphilis antigen line scored ±.

A sample was considered POSITIVE for *T. pallidum* antibodies if two or more syphilis bands showed a reactivity of ± or higher.

A sample was considered INDETERMINATE for *T. pallidum* antibodies if one syphilis band showed a reactivity of 1+ or higher.

N/B The results were read by the person who performed the test and read secondly by another person in the laboratory blinded to the first reader's results. If both agreed, then the final results were recorded. If both do not agree, a third person is called to read as a tie breaker.

5.2.12 Data management and analysis

5.2.12.1 Data Management

Data obtained from the study participants were double-entered, given code numbers for referral purposes on the spreadsheet and checked every day at KATH study site. Validation of the data entry was done at the end of every week to make sure they were properly recorded from the clinical forms and donor testing books. Confirmatory data entry was done at Copenhagen Rigshospitalet and sent to Kumasi, the study site. Data recorded from the blood donor testing books in the

laboratory and computer spreadsheet (confirmatory data included) were compared and discrepancies resolved by referring back to the original clinical forms of the blood donors.

5.2.12.2 Data Analysis

Background data consisting of sex, age, number of donations, donor type and routine testing results were recorded on a spreadsheet. Data were then exported into STATA SE version 12 (STATACORP, College station, Texas USA) which was the statistical software used for the analysis. All string variables (e.g. reactive and nonreactive, voluntary and replacement, male and female etc.) were converted to numerical values (0 and 1) for easy analysis and interpretation. Descriptive statistics of the variables were presented in the form of tables, pie charts, and figures. Proportions for the various potential predictor variables (independent) and the outcome variable (dependent) were calculated as means and proportions. We estimated positive predictive values (PPV) by calculating proportions and providing their respective confidence intervals. Multi-variable logistic regression was performed on syphilis reactivity as an outcome variable. Age, sex and donor type were included as potential predictor variables (independent) with results presented as odd ratios and 95% Confidence Intervals. A p-value of <0.05 denoted a statistically significant difference in all statistical comparisons.

5.3 Results

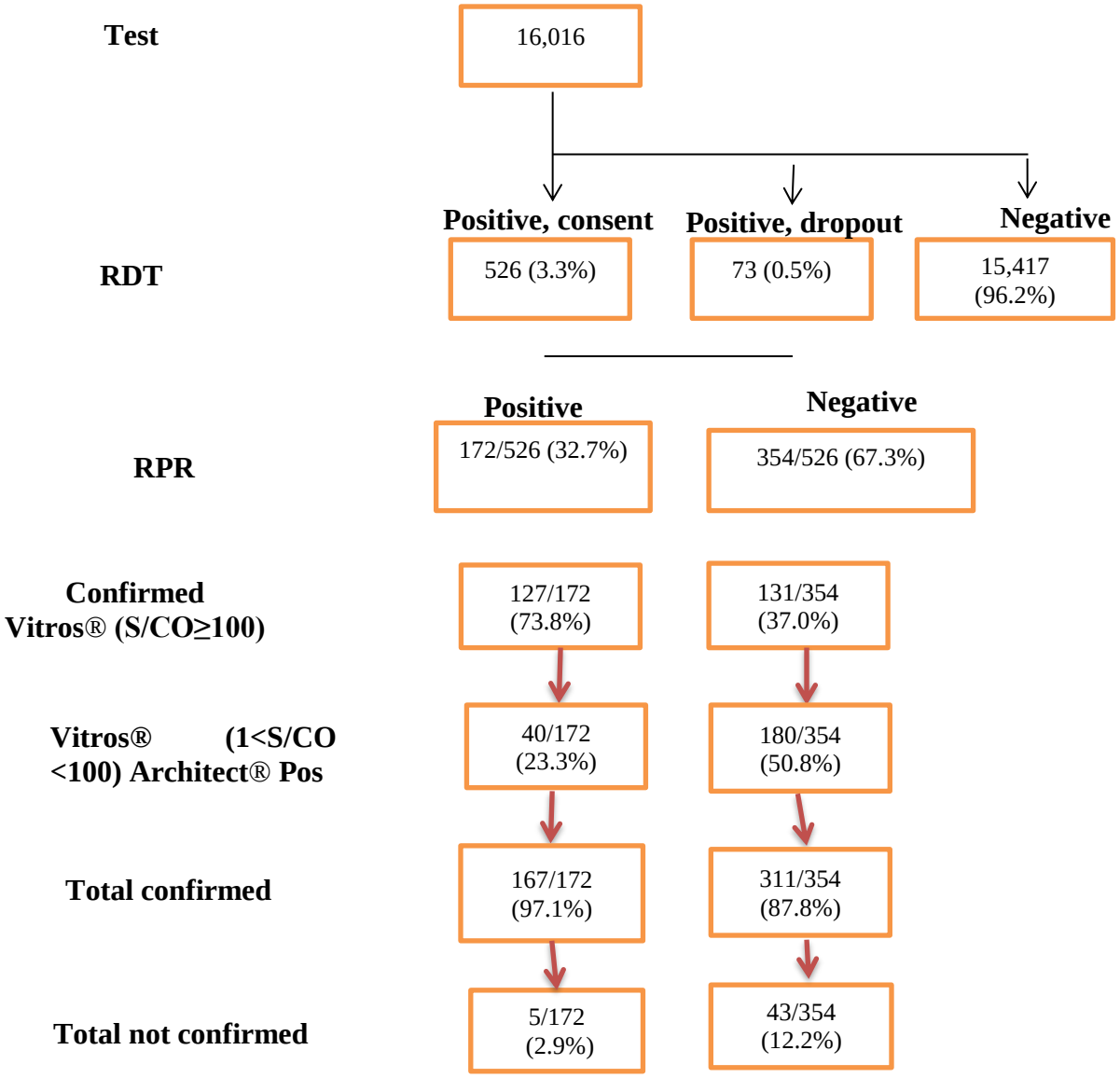
5.3.1 Syphilis prevalence of blood donors in the study population

599 out of study population of 16,016 prospective blood donors reacted to the syphilis Fortress® RDT test making an estimated sero-reactivity rate of 3.7% (95% [CI] 3.5 - 4.1). Of these, 526 (3.3%) syphilis seroreactive blood donors were included in the study of which 199 (37.8%) of them were VNRD (95% [CI] 33.8 - 42.1) and the rest were FD.

5.3.2 Exclusions in the study population due to co-infections

Seventy-three (12.2%) out of the 599 were blood donors who reacted with the Fortress syphilis test but were excluded from the study (figure 5.7). This is because 41 (6.8%) of them were co-infected with HBV, 15 (2.8%) were co-infected with HIV and seven (1.2%) were co-infected with HCV, while ten (1.7%) did not consent.

Figure 5.7 Algorithm for syphilis confirmatory testing of sero-reactive blood donors at KATH/TMU



RDT; - rapid diagnostic test, RPR; - rapid plasma reagin, S/CO-; sample over cut-off
N/B-Architect® was performed on specimen that were low reactive with Vitros® (1< S/CO <100)

5.3.3 Age of the study population

The blood donors tested were aged 16 to 59 years with a mean age of 25 (SD=9.1) compared with the sub-group of syphilis sero-reactive donors who showed a range of 17 to 53 years with a mean age of 31 years ($p<0.001$). Generally, the proportion of blood donors aged 16-25 years (59%) was

higher among syphilis RDT negative donors (figure 5.8) than among syphilis RDT positive donors aged 26-35 years (42%) (figure 5.9).

Figure 5.8 Age group distribution of the study population (blood donors)

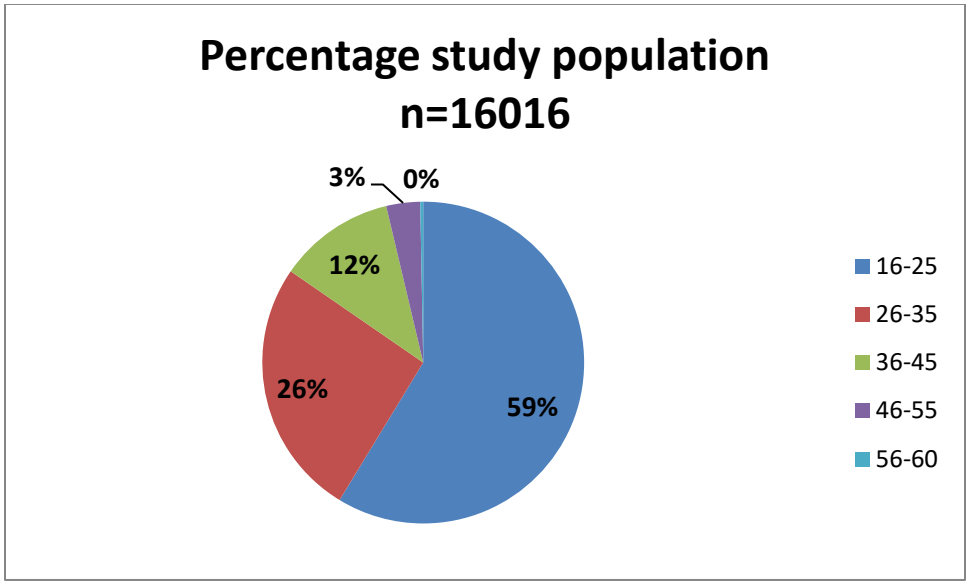
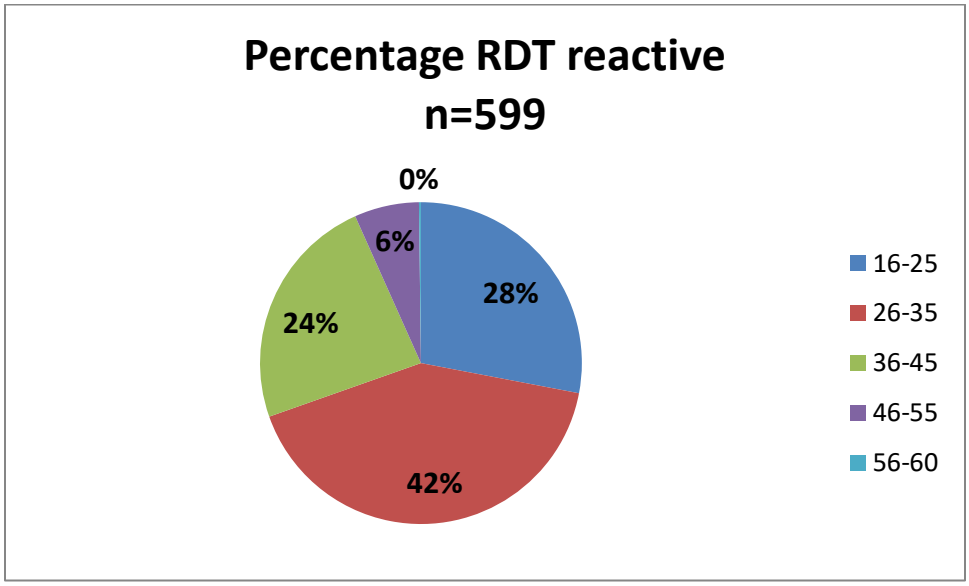


Figure 5.9 Age group distribution of the syphilis RDT positive blood donors



5.3.4 PPV of Syphilis Fortress RDT according to donor type

Of the total (16,016) blood donors tested, 10,218 (63.8%) were VNRD, of which 199 (1.95%) were syphilis sero-reactive by RDT (95% [CI] 1.73 - 2.28) as shown in table 5.2. Of the 5,798 FD tested, 327 were syphilis sero-reactive, which was significantly higher [5.64% (95% [CI] 5.08 - 6.26)] p<0.001 compared to VNRD.

Of the 526 RDT syphilis sero-reactive samples tested with Vitros®, 478 were reactive and confirmed by the algorithm (both Vitros and ARCHITECT TPA), making a PPV of 90.9% (table 5.1) and confirmed syphilis seroprevalence of 2.98% (95% [CI] 2.73 - 3.25). The proportion of sero-reactive FD (309/327, 94.5%) by RDT who were confirmed by Vitros TPA (table 5.2) was statistically significantly higher than that of confirmed sero-reactive VNRD (169/199, 84.9%), (p=0.001).

5.3.5 PPV of syphilis RDT and RPR test kits for sero-reactive blood donors

Of the 526 syphilis RDT reactive donor samples, 172 (32.7%) were RPR positive (95% [CI] 28.8 - 36.8). Out of these, 167 were confirmed with Vitros making a PPV of 97.1% (table 5.1). Thus, the PPV was higher among syphilis Fortress RDT and RPR reactives (97.1%) than in the total population of RDT reactives (90.9%) as shown in table 5.1. Conversely, the PPV was significantly higher among RPR reactive FD (99.1%) than among RPR reactive VNRD (93.3%) (p=0.001). More FD and more donors aged 26 or over were RPR positive and RPR positivity increased with age (table 5.2). Similarly, the PPV of combining RDT and RPR testing was highest among FD and among donors aged 26 or above (table 5.2). Out of the five RPR false positives, four were VNRD of whom three were aged between 16 and 25 years and one was aged between 26 and 35 years. The RPR false positive FD was aged between 26 and 35 years. 311 out of 354 (87.8%) RPR negative donors tested positive with Vitros®.

Table 5.1 Syphilis RDT and RPR sero-reactive blood donors

RDT reactive Blood donors	RPR		TOTAL (%)
	Positive (%)	Negative (%)	
Confirmed with Vitros/Architect	**167 (97.1)**	**311 (87.8)**	**478 (90.9)**
Unconfirmed with Vitros/Architect	**5 (2.9)**	**43 (12.2)**	**48 (9.1)**
TOTAL	**172 (32.7)**	**354 (67.3)**	**526**

RDT; -rapid diagnostic test, RPR; -rapid plasma reagin, %; -percentage

5.3.6 PPV of syphilis RDT according to donor age

Syphilis RDT sero-reactive donors showed a range of 17 to 53 years with a mean age of 31 years. The PPV of the Fortress RDT when testing VNRD ranged from 74.4% to 100% for the ages ranging between 17 and 55 years whilst that of FD ranges from 91.2% to 100% for the same age groups (table 5.2). Generally, the PPVs of the RDT for syphilis confirmed positive donors increased with age for both donor types. Although syphilis confirmed sero-prevalence of FD was 5.33%, there was higher prevalence as the age increases (table 5.2). The prevalence of syphilis confirmed VNRD was 1.65%, but there was a higher prevalence of 5.24% among the 36-45 age group.

Table 5.2 Syphilis RDT and RPR sero-reactive blood donors confirmed with Vitros® TP stratified according to age

Blood donors	Total RDT tested (%)	RDT + (%)	RDT+, Vitros®+ (%, PPV)	RDT+, RPR+ (%)	RDT+, RPR+, Vitros®+ (%, PPV)
VNRD	10218 (63.8)	199 (1.95)	169 (1.65, 84.9)	60 (0.59)	56 (0.55, 93.3)
Age group (yrs.)					
16-25	7525 (73.6)	78 (1.04)	58 (0.77, 74.4)	27 (0.36)	24 (0.32, 88.9)
26-35	1750 (17.1)	76 (4.34)	68 (3.89, 89,5)	23 (1.31)	22 (1.26, 95.7)
36-45	687 (6.7)	38 (5.53)	36 (5.24, 94.7)	9 (1.31)	9 (1.31, 100.0)
46-55	221 (2.2)	7 (3.17)	7 (3.17, 100.0)	1 (0.30)	1 (0.30, 100.0)
56-59	35 (0.4)	0	0	0	0
GENDER					
Females	3477 (34.0)	24 (0.69)	16 (0.46, 66.7)	6 (0.17)	6 (0.17, 100.0)
Males	6741 (66.0)	175 (2.60)	153 (2.27, 87.4)	54 (0.80)	50 (0.74, 92.6)
FD	5798 (36.2)	327 (5.64)	309 (5.33, 94.5)	112 (2.12)	111 (1.91, 99.1)
Age group (yrs.)					
16-25	1869 (32.2)	57 (3.05)	52 (2.78, 91.2)	24 (1.28)	23 (1.23, 95.8)
26-35	2405 (41.5)	148 (6.15)	139 (5.78, 93.9)	48 (2.00)	48 (2.00, 100.0)
36-45	1186 (20.4)	95 (8.01)	91(7.67, 95.8)	32 (2.70)	32 (2.70, 100.0)
46-55	321 (5.5)	27 (8.41)	27 (8.41, 100.0)	8 (2.50)	8 (2.50, 100.0)
56-59	18 (0.4)	0	0	0	0
GENDER					
Females	538 (9.3)	13 (2.42)	13 (2.42, 100.0)	4 (0.74)	4 (0.74, 100.0)
Males	5260 (90.7)	314 (5.97)	296 (5.63, 94.1)	108 (2.05)	107 (2.03, 99.1)
TOTAL	16016	526 (3.28)	478 (2.98, 90.9)	172 (1.07)	167 (1.04, 97.1)

RPR; - rapid plasma reagin, %; -percentage, VNRD; - voluntary non-remunerated blood donors, FD; - family donors, RDT; - rapid diagnostic test, +; - positive, -; negative, PPV; - positive predictive value.

5.3.7 The effect of age, sex and donor type on syphilis reactivity

By multivariable logistic regression, we showed a positive association between syphilis reactivity and the following parameters: increased age, male sex, and FD status (table 5.3). Syphilis sero-reactive effects of male sex and FD status were similar whether a positive endpoint was defined as RDT+, RDT+ Vitros®+, RDT+ RPR+ or RDT+ Vitros®+ RPR+ (table 5.3). The sero-reactive effect of age was weaker for endpoints including RPR reactivity (table 5.3). Male sex was a stronger predictor of syphilis reactivity than status as FD (Table 5.3). This was estimated from the odd ratios which were higher in males compared to FD.

Table 5.3 Multiple variable logistic prediction of syphilis reactivity

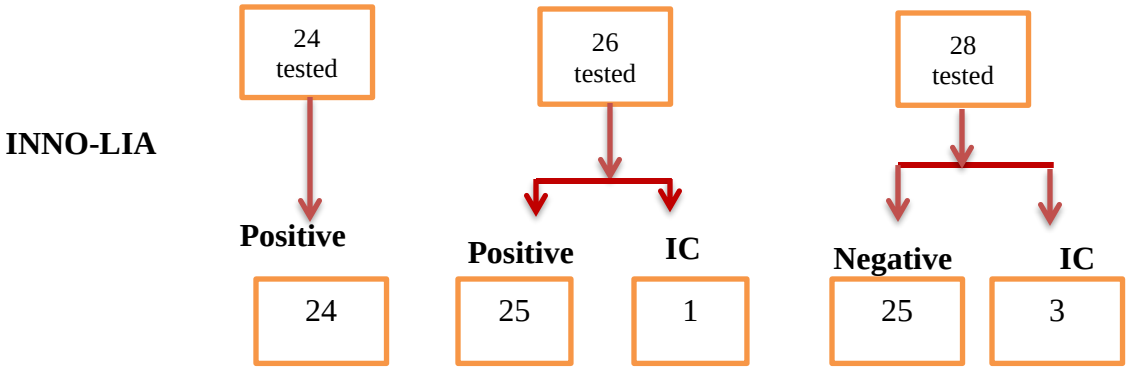
RDT + 526/16016 (3.28%)	**Odds Ratio**	**P-values**	**[95% Conf. Interval]**
Age (years)	1.04	<0.001	1.03 1.05
Gender (male)	2.99	<0.001	2.11 4.04
Donor type (FD)	1.92	<0.001	1.60 2.30
RDT+, Vitros®+ 478/16016 (2.98%)			
Age (years)	1.05	<0.001	1.04 1.06
Gender (males)	3.41	<0.001	2.29 5.08
Donor type (FD)	1.75	<0.001	1.44 2.14
RDT+, RPR+ 172/16016 (1.07%)			
Age (years)	1.03	<0.001	1.02 1.05
Gender (male)	3.22	<0.001	1.67 6.22
Donor type (FD)	2.04	<0.001	1.45 2.88
RDT+, RPR+, Vitros®+ 167/16016 (1.04%)			
Age (years)	1.03	<0.001	1.02 1.05
Gender (male)	2.72	<0.001	1.49 4.99
Donor type (FD)	2.06	<0.001	1.46 2.89

RPR; - rapid plasma reagin, %; -percentage, FD; - family donors, RDT; - rapid diagnostic test, +; - positive, PPV; - positive predictive value.

5.3.8 Samples tested with INNO-LIA as quality control

Twenty-eight (58%) out of 48 syphilis Fortress RDT samples which were negative with Vitros® test were tested with INNO-LIA (figure 5.10). Twenty-five of them were negative while the rest were inconclusive.

Figure 5.10 Quality control with INNO-LIA for syphilis testing of sero-reactive blood donors



Approximately 15 % of the samples tested with Vitros® were further tested with INNO-LIA as a quality control of the validation algorithm.
IC; - Inconclusive, S/CO-; sample over cut-off

All 24 (~9%) samples which were Vitros® high positives were confirmed with INNO-LIA and 25 of 26 with low reactivity with Vitros® were confirmed with LIA, and one was inconclusive although the Architect signal was positive.

5.3.9 Testing syphilis RDT reactive donors with TPHA as an alternative gold standard

Further tests were performed using a specific serological Chromatest *Treponema Pallidum* haemagglutination assay (TPHA, Linear Chemicals, Barcelona) on syphilis sero-reactive blood donor samples. This was because, in our previous studies [93], 31.3% of total donations in Ghana were tested for syphilis with TPHA as WHO recommended assay. It was based on this that if this assay is validated with good PPV, it can be used as a gold standard in our local settings.

We compared the performance of the RDT syphilis seroreactivity with TPHA to determine the predictive values of both. Of the total of 526 RDT positive blood donors, 501(95.2%) were positive with TPHA with syphilis donor reactivity of 3.13% (table 5.4). Out of these, 315 (with a sample reactivity of 5.43%) were from FD and 186 (with a sample reactivity of 1.82%) were from VNRD (table 5.4). A total 170 (33.9%) TPHA positives were confirmed with RPR signifying potential active/recent syphilis infection whereas two (8.0%) of the TPHA negatives were RPR positive. Additionally, all the FD who were TPHA negative were also negative with RPR. Significantly, of the 501 TPHA reactive blood donor samples tested, a total of 475 (sample reactivity of 2.97%) were confirmed with Ortho-Vitros having a PPV of 94.8%.

Table 5.4 Sample reactivity of TPHA with syphilis RDT and Ortho-Vitros

Blood donors	Total tested with RDT (%)	Total reactive with RDT + TPHA (%)	Total reactive with RDT+TPHA+ Ortho-Vitros® (%)	Total reactive with RDT+ Ortho-Vitros (%)
VNRD	10218 (63.8)	186 (1.82)	169 (1.65)	169 (1.65)
FRD	5798 (36.2)	315 (5.43)	306 (5.28)	309 (5.33)
TOTAL	16016	501 (3.13)	475 (2.97)	478 (2.98)

%; -percentage, VNRBD; -voluntary non-remunerated donors, FD; - family donors, TPHA; - *Treponema pallidum* Haemagglutination Assay, RDT, rapid diagnostic test, %; -Percentage.

5.4 Discussion

5.4.1 PPV of syphilis RDT

Of the 526 syphilis sero-reactive participants, there was RDT PPV of 90.9% and RPR PPV of 97.1% when confirmed with Vitros TPA. In effect, 172 (1.07%) out of 16,016 blood donors were suspected to have active syphilis and thus their blood units were discarded.

Syphilis infection in blood donors continues to pose a major threat in many developing countries including Ghana [7,93]. The Fortress syphilis RDT which was used for this study has a sensitivity and specificity of 99.7% and 99.6% respectively according to the manufacturer. When compared to the gold standard Vitros® TPA, the Fortress RDT had a PPV of 90.9%. Similar syphilis RDTs in other studies [269] have a PPV of 95.2% with sensitivity and specificity of 93.6% and 92.5% respectively. As PPV relies on both test specification and disease prevalence it is not surprising that other studies have shown PPVs of some RDTs to be both lower and higher than ours [270]. When we combined an RDT with the nonspecific RPR syphilis test, a much higher PPV was achieved (97.1%) compared to single RDT (90.9%). Thus, by combining both RDT and RPR, donors with confirmed previous or treated syphilis infections and donors with non-specific RDT reactions

could avoid deferral and they could therefore still contribute to the blood supply. Consequently, instead of discarding the 526/16,016 (3.28%) blood units which were syphilis positive by RDT over 11-month period, only 172 (1.07%) (i.e. those which were RPR positive and thus possibly actively infected) would be discarded and the rest (354/16,016=2.2%) of the blood units could be released for transfusion thereby contributing to improving blood supply.

The key concept underlying blood safety especially in Africa and LMIC is the balance between blood supply and blood safety in the context of an inadequate blood supply, high prevalence of TTI and limited resources. As stated earlier, if there is a low PPV (i.e. high false positive rate) then many donors will be deferred when they carry no risk to the blood supply. Furthermore, a screening test with a low PPV/high false positive rate has the potential to cause unnecessary harm to blood donors by creating unnecessary doubts and providing wrong information about their infection risks.

In Ghana, there can be potential saving in blood units each year if the NBSG could adopt this strategy of combining syphilis RDT with RPR. For example, the survey study in the chapter four of this thesis reported that, 3,371 out of 91,386 units were syphilis reactive. From this study, we can estimate that if 32.7% out of 3,371 (1,102 units) were suspected active syphilis, then, 2,269 units of blood could be released for transfusion to increase blood supply in the country.

5.4.2 The use of syphilis RDTs in resource-poor settings

Pre-donation screening for TTIs with syphilis RDTs is an approach that has been proposed for use in resource-poor, high-prevalence settings without access to a stable pool of low-risk donors [271]. One of the reasons behind pre-donation testing is that it reduces blood bag wastage and the associated costs of consumables in collecting blood from donors which is then not used because

of positive screening tests. One study in Ghana demonstrated savings of more than $11,000 in blood bags and testing costs over a 1-year period using pre-donation screening [22].

5.4.3 Syphilis prevalence in Kumasi blood donors

In this study, we found the prevalence of syphilis in Kumasi blood donor population (FD and VNRD) with the use of RDT to be 3.7% which is not different from previous studies in Ghana [6,7]. Like other studies, we found a higher rate of syphilis reactivity among FD than among VNRD. This was additionally confirmed with the use of Vitros (table 5.4) where the sero-reactivity among FD was significantly higher than that of VNRD (p=0.001). This was only partly explained by the higher age and more males among FD since FD status was an independent positive predictor of syphilis reactivity in logistic regression analysis. There is an ongoing initiative to have 100% VNRD in Ghana and elsewhere in Africa which if successful may reduce syphilis sero-reactivity. This is because a high proportion of voluntary donations are collected from students in high schools, though overall family donors remain dominant on the African continent. The positive association between age and syphilis reactivity is most likely caused by a longer period of sexual exposure in older donors. However, a cohort phenomenon with older donors being more exposed to yaws in childhood may also contribute. The data confirm that younger donors are safer than older donors with respect to syphilis prevalence whereas the highest safety both with regards to infection risk and blood supply lies in a system of repeat donations as the main source of blood for transfusion [42].

5.4.4 Syphilis seroreactivity and active syphilis in blood donors

Our data showed that a total of 167 (1%) of 16016 tested blood donors were confirmed syphilis RDT and RPR reactive. Our logistic regression data additionally indicated that for both RDT and RPR positive donors, there are independent effects of age, male sex and FD donor type on syphilis

seroreactivity. These data excluded 73 (0.5%) blood donors with other TTI co-infections, which, if included, would have increased the rates of confirmed syphilis RDT and RPR reactivity over the 11-month period of study. The study did not include relatively more or less women discarded because of other TTI than men.

Of the 16,016 prospective blood donors tested with syphilis Fortress® test, there was an estimated sero-prevalence of 3.7% (599/16016). The syphilis seroprevalence in this study (3.7%) was different from our previous study (7.0%) in the same study site [44] although the same Fortress test was used in both studies. This was possibly because those donors with positive syphilis screening tests did not turn up to donate again. Again, in our previous study from Ghana [44], we found that when the syphilis RDT seroreactive samples were tested with RPR, the reactivity rate was not the same with this present study. This considerable discrepancy was probably due to changes in RPR test kits. The RPR test kit used in the previous study, (IMMUTREP RPR, Omega Diagnostics – Scotland, UK) differed in sensitivity and specificity from the one used in this study (BD Macro-VueTM Card test – New Jersey, USA). This could have reduced the measured RPR prevalence. Additionally, testing errors on the part of the laboratory scientists in both testing procedures could contribute to the discrepancy. This could be explained that the RPR test kit used in the previous study will diagnose more donors of having suspected active infections which in public health wise, will have doubts in their minds although not confirmed.

Our data thus support the combined use of RDT and RPR to detect active syphilis in potential blood donors, which would enable more focused, deferral of potential active syphilis cases for treatment. These cases of suspected active syphilis can be identified with a minimal loss of donors as within the 11-month period of this study, the hospital was able to save 354 units of blood to save lives. Thus, choosing the combined syphilis RDT and RPR could be cost effective. In effect,

the use of RPR as a second test will not only increase blood availability, but, blood donors with suspected active syphilis will be identified and referred for treatment, and blood donors with positive RDT tests that are not confirmed by the RPR test will not be needlessly worried.

5.4.5 Syphilis automation and RDT in LMIC

Automated serologic testing platforms require proper training, reagent management, and laborious quality systems to ensure output reliability. The purchase of automated equipment for syphilis testing like Vitros ECi/ECiQ Immunodiagnostic Systems and ARCHITECT TPA which are able to provide effective and reliable results for blood donors for safe transfusion, may be very expensive and time-consuming and require staff training in LMIC. Even automated, highly sensitive, expensive and technically difficult laboratory screening methods - e.g. nucleic acid testing (NAT) - are not routinely available in resource-constrained settings [272]. In resource-restricted settings where syphilis prevalence is high, expensive automated equipment for testing may not be affordable. In practice, it is likely that LMICs will need to rely on RDTs and RPR test kits with relatively good sensitivities and specificities which are user-friendly, of good quality and efficacious. It is important to repeat this study in other resource-poor settings where syphilis prevalence is high. Apart from specificity, sensitivity and cost in considering RDT, another relevant aspect is the rapid availability of results (within about 15 minutes) from syphilis RDTs and RPR tests compared to automated machines.

The strength of this study is the description of a real-life performance with regards to the PPV of a newly suggested combined syphilis testing algorithm, combining an RDT and RPR for the identification of potential active syphilis. The algorithm used for gold standard confirmation was robust as it involved two different Treponema-specific tests used sequentially to confirm weak

and negative results. The INNO-LIA assay has been shown to provide highly reliable confirmatory diagnostic information of anti-TP antibodies as it was used as a quality control for the confirmation algorithm of anti-TP antibodies.

5.4.6 Limitations of the study

1. The assumption that RDT positive donors testing negative in RPR were without significant risk for transfusion was not tested by recipient look back or by molecular testing of donors.
2. The proportion of truly syphilis-reactive donors missed by the initial RDT (sensitivity and specificity) was not evaluated because of resource constraints.
3. We cannot assume infectivity among all confirmed RPR reactive donors. Additionally, infectivity among seroreactive blood donors who were positive with RPR was not determined.
4. By excluding donors with other TTI, the performance of the used tests when co-infected cannot be deducted from the present study.

5.5 Conclusion

In a blood bank system like the one in Kumasi, Ghana, with a relatively high prevalence of syphilis and where infrastructure to support formal laboratory testing is often lacking, syphilis screening with RDTs may provide a reasonable technology. We believe that screening blood donors for syphilis with RDT with proper validation in settings similar to ours can contribute to improving blood safety without jeopardizing the blood supply. The combination of both RDT and RPR reduces the loss of donors and increases the blood available for transfusion. The combined RDT and RPR testing has a satisfactory high PPV, meaning that unnecessary discarding of blood for transfusion and false syphilis diagnoses of donors are minimized. The high PPV of a combined

RDT and RPR algorithm suggests that further routine confirmation of a donor deferred with dual RDT and RPR reactivity is not needed. This adds to the robustness and cost efficiency of the suggested syphilis screening algorithm.

The hospital transfusion committee in the study area was instrumental in initiating the combined strategy of syphilis testing with RDT and RPR, showing the critical role, such committees can play in the implementation of evidence-based measures to improve blood safety and availability even when resources are limited.

CHAPTER 6

Performance of a syphilis RDT in selected transfusion facilities in Ghana.

6.1 Introduction

6.1.1 Syphilis RDTs

This chapter relates to objective 4, which is to determine the performance of a widely used syphilis RDT in selected transfusion facilities in Ghana.

In Ghana and many developing countries, syphilis testing is usually performed using rapid diagnostic tests (RDT) [19,93]. This technique is preferred over the conventional assays like EIA, TPHA, and RPR because RDTs are simple to perform, give quick responses, are affordable, are readily available in the market and do not require skilled labour [44]. Another advantage of the syphilis RDT is their flexibility in terms of using both whole blood and serum/plasma samples from subjects as stated in chapter two [192]. With an increasing population size and expansion of blood donor facilities to rural areas, the demand for these RDT kits has increased in Ghana and many developing countries.

It is the responsibility of the laboratory to ensure that test kits for the syphilis RDTs are of good quality and standard reactivity. The investigation of syphilis infection is important for both detection and exclusion of blood donors. As such, the poor effectiveness of less sensitive diagnostic kits could pose a significant risk to blood recipients. Similarly, low specificity can cause unneeded loss of donors and thus hamper blood supply. Although most RDTs do contain an internal quality control (QC) in the form of a control line in the strip or cassette, periodic external QC using laboratory-based tests is recommended.

The syphilis RDT method can be completed in 10–30 minutes depending on the manufacturer's instructions and can be used in rural and peri-urban areas for the screening of blood donors because of its user-friendliness. The first objective of this thesis about the survey screening practices in Ghana [93], reported the variation in cost per test strip for syphilis screening which varied 10-fold, from US$ 0.2 to US$ 2.0 which is generally considered to be affordable. However, the influx of various brands of these RDTs into the Ghanaian market without appropriate evaluation [273] is worrying and could pose a risk to the health of the entire country in terms of transfusion safety.

In Ghana, our study, as described in previous chapters, has shown that two-thirds of the facilities testing for syphilis are using RDTs which are not recommended by the WHO [93]. Some of the syphilis RDTs that are available in Ghana include; ACON, ABON, Determine, First response, Fortress, and Wondfo. Although a study we performed in 2016 (chapter 4 of this thesis) reported ACON as the predominant RDT used [93], this has recently been replaced by ABON (Abon Biopharm Company Limited, Hangzhou, China) as the RDT which is reportedly used by most of the facilities in Ghana. This has led to the need to develop a local QC panel for evaluating the performance of the ABON syphilis RDT. There is currently no commercially available panel for quality checks on syphilis RDTs.

This study was therefore undertaken to set up a QA process and to evaluate the performance of the ABON syphilis RDT kit used in selected blood transfusion facilities in Ghana. Fortress, another syphilis RDT in Ghana for the qualitative detection of antibodies (IgG and IgM) to *T. pallidum* in serum or plasma was used in the study site at KATH (sensitivity 99.7%), as a screening test for this ABON evaluation study [112] whilst Vitros TPA was used as the gold standard. The information provided from this study will be useful as a model for ensuring that reliable and reproducible

syphilis RDT results are obtained consistently within a laboratory and among different laboratories performing the same tests.

6.1.2 Type of study

This was a cross-sectional study conducted to set up a QA process to evaluate the performance of a syphilis RDT (ABON) used for screening blood donors and to determine the PPV, sensitivity and specificity of the test kit when used by transfusion facilities in Ghana.

6.1.3 Study area

This cross-sectional study was carried out at four different hospital facilities with laboratories for testing blood samples for TTIs. These facilities were located in Ashanti region of Ghana and were; Ankaase Mission Hospital, The University hospital (KNUST), Mampong Government hospital, and Gary Marvin Memorial hospital. These four facilities were chosen based on the criteria that they were:

1. Already using rapid tests to screen for syphilis – Our previous study [93] which forms part of this thesis, found that more than two-thirds of the facilities were using RDTs for syphilis testing.
2. From different districts within Ashanti region, Ghana and accessible within 2 hours traveling distance from the teaching hospital.
3. Tested more than 500 blood donors annually.

6.1.4 Description of the four study facilities

Ankaase Mission Hospital – is located in Ankaase town near the city of Kumasi in the Ashanti region of central Ghana. It is a general district hospital started in 1991 with assistance from the

Methodist Church Ghana and the mission society. Currently, it provides medical care for more than 10,000 inhabitants in the surrounding towns and villages with a capacity of 90 beds. The hospital has a pharmacy, X-ray and ultrasound, laboratory with blood transfusion capability, and endoscopy capabilities. The hospital tests roughly 4000 blood donors annually for TTIs (HIV, HBV, HCV, and syphilis) all with RDTs of whom 20% are from voluntary donations whilst the rest (80%) are from family/replacement donations. The hospital also offers obstetrics, general surgery, urology, and optometric service. An average of 150-200 outpatients is seen daily in the clinic. In addition, the hospital reaches out to the broader community through nutrition education, literacy classes, and care for persons living with HIV/AIDS. Preventive healthcare at Ankaase Methodist hospital is a high priority and guided by a health board instituted by the community. The public health unit which is staffed by the community, provides immunizations, general health education, health screens for mothers and babies, and particular interventions against Buruli ulcer, malaria, hepatitis B, and HIV infection.

***The University hospital (KNUST*)** - started as a dressing station in 1952 and has grown by additions and modifications into a full-fledged 100-bed hospital. The hospital currently caters for a population of over 200,000 made up of students, staff, and dependents, while the rest are from surrounding communities, including Ayigya, Bomso, Ayeduase, Kotei, and Boadi. It is the medical arm of the KNUST. It is located in the northwest part of Kumasi, Ashanti Region, playing the role of a district hospital. The University hospital offers services in the general medical care as well as specialist services. The facilities at the hospital comprise of medical records, a dispensary, and mini-laboratory among others. The hospital screens blood donors for HIV, HBV, and HCV but started testing for syphilis in 2013, all with RDTs. Roughly 3000 blood donors are being tested annually of which 70% are FD. The students' clinic (which is part of the university hospital to ease

congestion) has a stand–by ambulance and a power plant to provide emergency services. Work is progressing steadily on the construction of the first phase of an 800-bed teaching hospital for the KNUST in Kumasi.

Mampong Government hospital – was established in the year 1973, serving people from Mampong, Pru, Atebubu, Nkoransa and Offinso districts. The hospital is located in the town of Mampong and it is the only hospital serving the entire Mampong municipality (population: 88,625). Mampong is a 60 to 90-minute drive from KATH, one of the major tertiary referral centres in Ghana.

The hospital has 98 beds in the general wing and an additional 56 beds located one kilometre away in the maternity wing, however, the maternity wing effectively functions as a separate hospital. The emergency ward has 14 beds and there are a modern laboratory and theatre, a physiotherapy department, a back-up generator and a water system. The Mampong Government hospital operates on total family replacement blood donation where approximately 1000 prospective blood donors are tested annually with RDTs for all TTIs

Gary Marvin Memorial hospital - is a private maternity hospital in Kumasi, Ghana. This hospital is located in the township of Kotwi, on the outskirts of Kumasi road leading to Obuasi. It is the only referral hospital in the district of Atwima-Kwanwoma, which has a semi-rural population of 98,167 inhabitants.

The hospital which was first established in 2004 as a maternity home currently has 36 beds and an outpatient department. The average attendance is 3000 patients/month. Every month, an average of 60 babies are delivered, mostly by normal delivery but also by caesarean section. This centre was chosen particularly because syphilis congenitally affects 500,000 or more infants annually in

SSA [66], wherefore including a hospital with focus on deliveries would be important. They also perform various operations such as hysterectomies, emergency treatment for miscarriages and ectopic pregnancies, hernia repairs and laparotomies. Their in-patient facilities include a maternity ward, labour ward, paediatrics ward, surgical male and female wards, as well as special wards for single patient occupancy.

6.2 Methods

6.2.1 Study design

ABON, (Abon Biopharm Company Limited, Hangzhou, China) is a syphilis RDT kit commonly used by blood donation facilities to screen blood for syphilis in Ghana. ABON syphilis RDT was chosen for this evaluation study because it is the predominant RDT used by the facilities in Ghana [93]. The four selected facilities were supplied with identical ABON test kits which were purchased from a local supplier. The facilities were also supplied with 'already tested' (i.e. known syphilis status) samples (see 6.2.2). These samples were blindly tested by the facility laboratory scientists with the same conditions prevailing in all the facilities, and the results were recorded on spreadsheets and returned to the researcher.

6.2.2 Sample collection and preparation of a panel of samples of known syphilis status

526 syphilis initially reactive blood donor samples were collected from prospective blood donors who came to KATH to donate blood between February 2014 and January 2015 after they were informed of the study and consented. For details, please refer to Chapter 5. The samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes, centrifuged and plasma was separated from the whole blood and stored at −20°C. All these samples were tested with Vitros

syphilis TPA to verify their true positivity. The samples were again tested with RPR to detect suspected active infections which could infect transfusion recipients of the unit.

A QA panel was made comprising of 100 samples already tested with Fortress syphilis RDT. Of these, 70 were syphilis Fortress RDT sero-reactive and thirty were syphilis Fortress seronegative (figure 6.1).

Of the 70 samples tested with Fortress RDT and RPR, a confirmatory testing with Vitros assay was performed on them, and 60 were confirmed positive whilst 10 were negative (figure 6.1). For each sample, its degree of reactivity (negative, low positive, medium positive, strong positive) with Vitros was noted.

Multiple aliquots of the 100 samples were made so that each of the four study facilities was provided with the following; 20 strong Vitros positives, 20 medium Vitros positives, 20 low Vitros positives, 10 Vitros negatives and 30 known Fortress RDT negatives. These were sent as a blinded test panel so the receiving laboratories were unaware of the status of the samples. They were asked to test the 100 samples using the ABON RDT kits provided through the project.

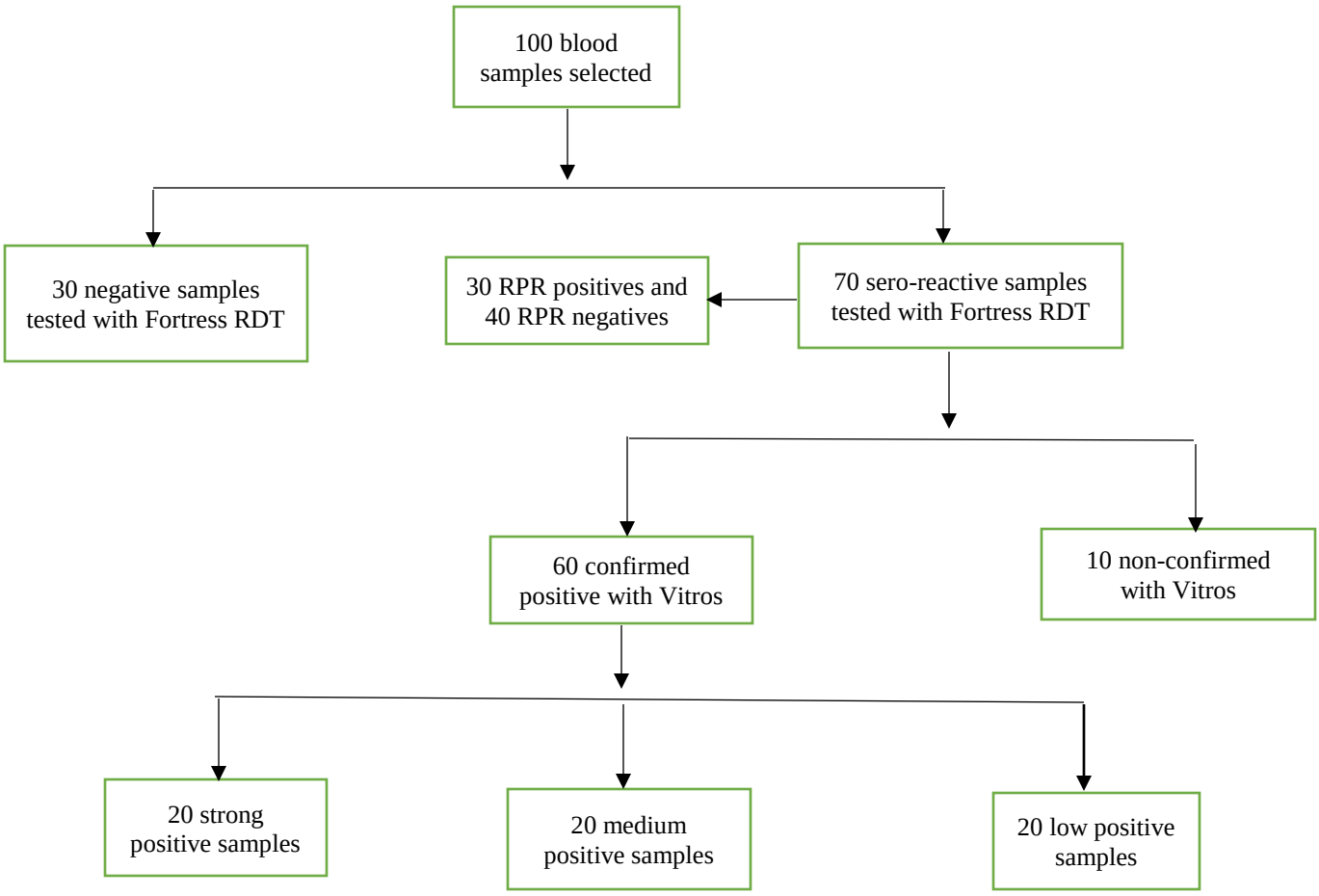


Figure 6.1: A flowchart of samples selected for the syphilis quality assurance study

6.3 Results

To maintain confidentiality, the facilities have been anonymised in this section. Of the 70 QC samples that were syphilis sero-reactive by Fortress RDT, overall 67 [(95.7%), 95% CI 88.1-98.5] of them were found to be positive by the 4 facilities using the ABON test (table 6.2). Of the 60 samples that were confirmed positive with Vitros TPA, 2 facilities had all 60 being positive with ABON while the other 2 facilities had 59 out of the 60 being positive (table 6.2). Of the 30 samples that were negative with Fortress, each of the four facilities found that 28 were confirmed negative with ABON while two were positive (table 6.2). Thus, the overall sensitivity for the ABON when

compared with Vitros as gold standard was 99.2% [(59.5/60) ×100]. The total number of negative samples was 40 whilst ABON detected 33 as negative samples. Thus, the overall specificity was 82.5% [(33/40) ×100] as seen in table 6.1.

Table 6.1 Sensitivity and specificity of the ABON test kit used in the selected facilities

		Vitros TPA		
ABON syphilis RDT	Results	Positive	Negative	Total
	Positive	59.5	7	66.5
	Negative	0.5	33	33.5
	Total	60	40	100

RDT, rapid diagnostic test; TPA, *Treponema pallidum* assay

Of the 30 RPR positive samples that were included in the study, 2 of the facilities had all the 30 being positive while each of the other 2 facilities had 29 being positive.

Table 6.2 Comparison of results from four facilities of blind testing of samples with ABON compared to Vitros and RPR results

	Fortress RDT		**Fortress+ tested with Vitros**				**RPR (N=70)**	
Facilities	Total found positive (N=70)	Negative (N=30)	High positive (N=20)	Medium positive (N=20)	Low positive (N=20)	Unconfirmed (N=10)	Positive (N=30)	Negative (N=40)
Facility 1	67/3	2/28	20	20	20	3/7	29/1	2/38
Facility 2	67/3	2/28	20	20	19/1	2/8	30	3/37
Facility 3	67/3	2/28	20	20	20	3/7	29/1	2/38
Facility 4	67/3	2/28	20	20	19/1	2/8	30	3/37
Average (%)	95.7	6.7	100	100	97.5	25	98.3	6.3

RPR, rapid plasma reagin; N, number; RDT, rapid diagnostic test

6.3.1 Facility 1

At this facility, all the 60 Vitros confirmed samples, whether strong, medium and low positives, were found to be positive (100%) with ABON (table 6.2). Of the 30 samples that were negative

with Fortress RDT, this facility found that 28 (93.3%) were negative with ABON. It was additionally found that 60 (89.6%) out of the 67 ABON positive samples were confirmed with Vitros (p=0.001) (table 6.2). At this facility, 29 of the 30 (96.7%) RPR positive samples were positive with ABON whilst 38 of the 40 (95%) RPR negative samples were positive with ABON (Table 6.2)

Table 6.3 Comparison of results from four facilities of blind testing of samples with ABON compared to Fortress RDT and Vitros results

Fortress RDT	Facility 1 (ABON)		Total	Facility 2 (ABON)		Total	Facility 3 (ABON)		Total	Facility 4 (ABON)		Total
	Positive	Negative		Positive	Negative		Positive	Negative		Positive	Negative	
Positive	67	3	70	67	3	70	67	3	70	67	3	70
Negative	2	28	30	2	28	30	2	28	30	2	28	30
Total	69	31	100	69	31	100	69	31	100	69	31	100
VTROS	60	10	70	60	10	70	60	10	70	60	10	70
P value			0.001			0.001			0.001			0.001

RDT, rapid diagnostic test

6.3.2 Facility 2

At facility 2, all the 40 Vitros confirmed samples which were made up of strong and medium positives were positive with ABON (table 6.2). However, this facility recorded 19 positive samples tested with ABON out of the 20 low positives from Vitros (table 6.2). Thus, of the 60 samples that were confirmed with Vitros, 59 (98.3%) were positive with ABON. In this facility, all the RPR positive samples were positive with ABON whereas 37 of the 40 RPR negative samples were positive with ABON (table 6.2). Of the 30 Fortress negative samples, 28 (93.3%) of them were negative with ABON.

6.3.3 Facility 3

At this facility, all the 60 Vitros confirmed samples, whether strong, medium and low positives, were found to be positive (100%) with ABON (table 6.2). However, seven of the 10 unconfirmed with Vitros were positive with ABON, making the total ABON positives to be 67 out of 70 of the Fortress RDT sero-reactive samples. Of the 30 RPR positive samples, 29 (96.7%) were positive with ABON whilst 38 (95%) of the 40 RPR negative samples were positive with ABON (table 6.2). Of the 30 Fortress RDT negative samples, two of them were positive with ABON making the total positives at this facility to be 69 out of 70 (98.6%) and total negatives to be 31 as seen in table 6.3.

6.3.4 Facility 4

At facility 4, all the 40 Vitros confirmed samples that were strong and medium positives, were found to be positive with ABON; for the low positive samples, 19 out of 20 were positive with ABON (table 6.2). With the 10 samples that were not confirmed with Vitros, eight of them (80%) were positive with ABON as seen in table 6.1. However, all the 30 RPR positive samples were all positive with ABON; but 37 of the 40 RPR negative samples were positive with ABON.

Of the 30 samples that were negative with both Fortress RDT, 28 (93.3%) of them were negative with ABON at this facility.

6.4 Discussion

Syphilis infection continues to pose a major threat in many developing countries including Ghana. Few studies have attempted to describe the prevalence of syphilis among blood donors in different parts of SSA including Ghana [4,6,274] and the majority of these estimates were based on the use of syphilis RDTs.

The sensitivity and specificity of the ABON RDT used in this study by the 4 facilities performing syphilis screening was found to be 99.2% and 82.5% respectively when compared with the gold standard (Vitros TPA). Overall, it was a satisfactory performance when compared with Fortress RDT which has a sensitivity of 99.7%, and is similar to syphilis RDT performance reported in other studies. When the WHO compared the performance of 8 rapid syphilis tests to a combined reference standard of TPHA/TPPA, they reported sensitivities of 84.5%–97.7% [197,198] which were similar to our findings.

In a survey of syphilis testing practices in Ghana in 2016 [93] which forms part of this thesis in chapter four, the prevalence of syphilis was found to be 3.7%. Two-thirds of the facilities involved in the study used RDTs for testing but the screening was neither repeated nor confirmed with a different test. An evaluation of pre-donation screening in Mozambique using RDTs also found syphilis prevalence to be 1.2% [8]. Their testing strategy was to use the RPR test BD Macro-Vue RPR and they confirmed positive samples with SERODIA®- TPPA which was considered as their reference diagnostic method [8].

Overall there was good consistency between the ABON RDT results and the confirmatory tests which indicated the efficaciousness of the test. There were a few small differences in results on the same samples depending on the test used (table 6.2). These discrepancies could be due to a number of reasons such as poor detection limit, and poor storage conditions by the suppliers, users, and during transportation. Most rapid syphilis test kit manufacturers specify storage temperatures between 4-30ºC [275]. If the facility temperature is above 30ºC, periodic quality control checks to ensure the ongoing validity of the tests is especially important. There are some facilities in Ghana with storage challenges especially inconsistent power supply where room temperatures can rise above 30ºC and even lack of storage facilities where refrigerators are not available for test kits. In

this case, facilities should make sure proper storage conditions are met (4-30ºC) so as not to affect kit performance.

Transport can also impact on the performance of test kits because of potential variability in the duration and temperature conditions. For example, test kits that are transported by road in Ghana and elsewhere may be exposed to temperatures that might exceed 40ºC, while others that are transported by air may be exposed to temperatures below –40ºC [276]. It is important that the packaging reasonably protects the kits from extreme conditions during transportation, since this can affect detection limits. This would result in blood recipients being at risk of receiving blood which is 'false negative' for syphilis testing and failure to identify donors who may have syphilis infection.

The use of only one type of serological RDT may be insufficient for syphilis diagnosis and blood donor testing since this study found evidence of false sero-positivity during confirmation tests using Vitros TPA as the gold standard (table 6.2) This indicates that the use of syphilis treponemal RDT alone cannot be recommended in the transfusion facilities as donors will be deferred unnecessarily, but it should be complemented with a second test to confirm the syphilis sero-reactive as well as potentially infectious blood donors. Also, not all the 30 RPR positive samples were positive with ABON which also indicate that in the facilities, recipients could be at risk of being transfused with potential active syphilis blood units. Both nontreponemal and treponemal serological tests should be carried out in all suspected donor cases to ensure that truly infected donors and potential active infections are identified.

Although quick, easy to perform and affordable, syphilis RDT kits with low sensitivity could miss some positive samples and the recipients could be at risk of being transfused with infected blood. Highly sensitive RDTs may be used as first-line screening kits but all reactive samples should be

confirmed using commercially available RPR kits to detect potential active infections. This is because the study showed a Vitros positive sample which was missed by the ABON kit but was RPR positive. Thus, for blood donors, it is very important to have highly sensitive test kits to reduce chances of infectious blood being transfused into patients.

This ABON syphilis test kit used in this study like other syphilis RDTs is ideal for use in outreach and hospital facilities where syphilis testing is being offered to potential blood donors. This is a treponemal test, and blood donors who have a known history of syphilis infection without treatment should be advised not to have this test as results will continue to be positive. They should instead have a routine serologic specimen drawn for the standard lab-based syphilis testing, tested with RPR to monitor treatment. If they are not on treatment, the results of the RPR test will indicate positivity, and as such, will be deferred again.

6.4.1 Establishment of external quality assessment systems for syphilis rapid testing

Quality systems are the organizational structure and resources needed to implement quality requirements and include management, training, standards, documentation, traceability, and evaluation [277]. Quality systems are important for all tests including syphilis testing to ensure the accuracy, consistency, and reliability of test results. In this study we set up and piloted a new external quality systems (EQA) scheme to evaluate the syphilis RDT kits and to promote proper QA processes. EQA, which is the process that allows testing conducted by a laboratory, testing site or individual user to be compared to that of a source outside the laboratory, needs to be established for syphilis testing across Ghana. If properly established, the EQA can provide objective data on the quality of delivered services, and can reflect the quality of testing of blood donor specimens [276,278].

As far as I am aware this is the first time a QA testing panel has been set up in Ghana for syphilis. In Accra, there is a public health reference laboratory (PHRL) in Korle-Bu teaching hospital which deals with HIV QA but not syphilis. The influx of syphilis rapid kits on the market should be adequately controlled and kits need to be evaluated by the standards authority to ensure that they are of high quality. There should be a monitoring and evaluation system from the Food and Drugs Authority (FDA) in Ghana to regulate the use of these test kits and for ongoing review of their performance. The WHO has evaluated some commercially available rapid treponemal tests for performance and reproducibility [275]. This evaluation includes test performance, ease of use, the condition of use, storage conditions, shelf life, and price. It may be possible to work with the Public Health and Reference Laboratory (PHRL) and the FDA in Ghana so that they can build on this syphilis testing panel for national use. Scaling up syphilis EQA across Ghana will also need a specifically designed in-service continuing education program for laboratory personnel, and should be instituted at the various regions in the country to refresh the continuing laboratory personnel in carrying out effective duties [277].

6.5 Study limitations

1. One major limitation of the study is the small sample size of our study facilities that performed the testing. This was due to logistic constraints.
2. Of the 40 negative samples for the study, 30 of them were not tested with the gold standard but were assumed to be negative by the gold standard. This is because the study looked at the PPV, and al1 RDT negative samples were not sent to be retested with Vitros® TPA at the Department of Clinical Immunology, Copenhagen university hospital, Denmark. More so, the fact that these samples were retested with TPHA as WHO recommendation and

found all to be negative was the justification for the assumption that they would be negative with Vitros TPA.

We therefore, recommend that future studies be performed by more facilities and that other brands of syphilis RDT kits should also be evaluated.

6.6 Conclusion

The detection of markers of syphilis infection in blood donors is important to avoid potentially transmitting syphilis to a recipient and for making appropriate referrals for donors for clinical review and possible treatment. However, the use of insufficiently sensitive diagnostic kits could pose a significant risk to both blood donors and blood recipients. This study evaluated the performance of the syphilis ABON RDT, which is widely used in Ghana and other developing countries. By comparing Vitros TPA as gold standard, the respective sensitivity and specificity of the ABON syphilis screening kit was found to be 99.2% and 82.5%. Its performance was found to be satisfactory because of its relatively good sensitivity and specificity when compared with other syphilis rapid tests. However, the use of only one type of serological RDT may be insufficient for syphilis diagnosis and blood donor testing. Thus, a confirmatory test or a second test, preferably nontreponemal, is required to confirm the sero-reactive samples [44,112]. This confirmatory/second test will help detect truly syphilis positive as well as potentially infectious blood donors.

Laboratories that are committed to the principles of quality assurance generate relevant, reliable, and cost-effective results. A lack of quality assurance systems, as well as poor storage and transport of test kits, can contribute to poor test performance and affect detection limits of the test kits if not followed properly. Periodic external QA using laboratory-based tests should be carried out on all RDTs including those used for syphilis, by blood transfusion facilities. In this study, we have demonstrated how such a system can operate in Ghana. Lastly, national organisations such as the

FDA needs to be engaged to monitor the influx and quality of these RDTs to ensure they are of good quality and standard reactivity for their effective use.

Lastly, I would also advice the T-REC, - an international consortium of academics and health practitioners working to strengthen the capacity of African researchers to do research on blood transfusion, - to foster collaboration between countries like Ghana, Zimbabwe and others in performing quality testing to enhance blood safety for transfusion.

CHAPTER 7

Recall of symptoms and treatment of syphilis and yaws by healthy blood donors screening positive for syphilis in Kumasi, Ghana

7.1 Introduction

7.1.1 Study background

The objective of this study (objective 5) is to determine the relative proportions of those with clinical histories attributable to yaws rather than syphilis in confirmed syphilis antibody positive donors.

Yaws is an often ignored non-venereal endemic treponematosis. It causes significant morbidity in patients but has a low mortality because treatment can be effected with the use of azithromycin which is a central component of the new World Health Organization (WHO) strategy to eradicate yaws.

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The clinical manifestations of yaws include multiple papillomas, non-tender ulcers, sores, plantar hyperkeratosis, and pigmentation of the palms and soles, followed by gummata in the last phase [14].

The site of infection in an individual depends upon the living habits and environmental conditions in the area where they live because even breaks in the skin caused by injuries, bites, or excoriations can precipitate this disease [279]. It particularly affects children aged 2–5 years living in poor socioeconomic conditions in certain rural, wet, and tropical areas of West and Central Africa,

South East Asia, and the South Pacific islands [280] ; these are areas where conditions of overcrowding, poor water supply, and lack of sanitation and hygiene prevail.

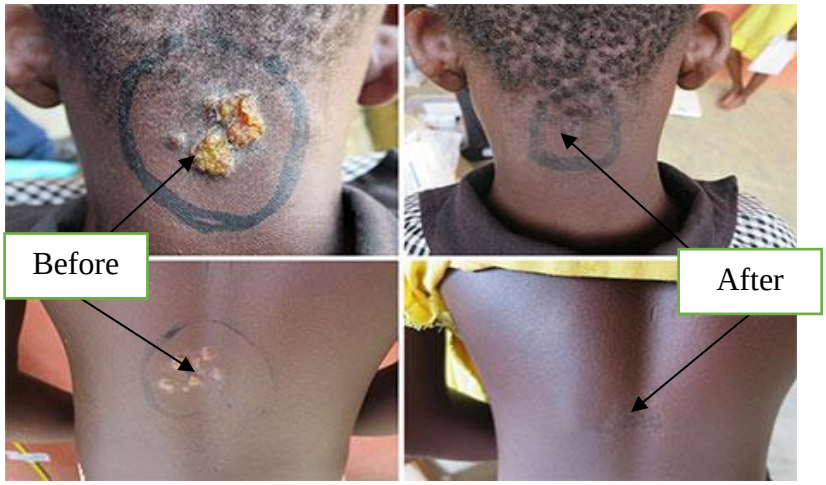

Figure 7.1 Yaws lesions before and after treatment with antibiotics

Favourable climate conditions such as humidity and a constant warm temperature appear to be especially important factors for yaws to flourish [15]. Presently, little is heard about this disease because it occurs in remote areas where health service coverage is very low.

Africa is the continent most affected by yaws based on the WHO estimates from the 1990s. India reported its last cases in 2003, while Indonesia and Timor-Leste still have some cases left. Papua New Guinea and two other Pacific island countries, Solomon Islands and Vanuatu have reported cases and there are some pockets in the Amazon region [281].

A major campaign in the 1950s and 1960s to eradicate yaws by means of community-wide treatment with long-acting, injectable penicillin reduced the number of cases of the disease by 95% from 50 million to 2.5 million worldwide; however, yaws was not eradicated [282]. The problem was nearly solved, but there were resurgences particularly in the 1970s and in 2006 [281]

According to the WHO 'Some of the issues and challenges that have hampered yaws eradication historically include the lack of an effective device for surveillance as well as case detection, limited political commitment and resources, limited capacity of general health staff to recognize and treat yaws, ensuring drug supply and logistics management, creating community awareness through appropriate advocacy campaigns and extending the services to remote and hard to reach areas [202].

The Eastern, Central and Western regions of Ghana are the most affected places but all the 10 regions and nearly 170 districts have reported yaws cases. In Ghana, a total of 28 000 cases were reported in 2008 and 25 000 in 2010 [202] (table 7.1). In the context of neglected tropical diseases, the WHO launched the global yaws elimination initiative in 2007 to address the persistence and resurgence of this disease. Again in 2012, the WHO launched a new initiative to eradicate yaws globally by 2020 using not only the new azithromycin-based treatment policy in 2012, but also, the initial mass treatment and biannual resurveys (the Morges strategy) [202]. This was based on the discovery that a single dose of oral azithromycin is at least as effective as injectable penicillin G benzathine [283].

Table 7.1 Annual (2002-2008) yaws case notification rates by region per 100,000 population

Regions	2002	2003	2004	2005	2006	2007	2008
Western	157	239	581	224	153	153	136
Central	628	501	665	172	211	194	136
Greater Accra	-	12	169	13	7	10	11
Eastern	931	516	393	141	228	415	619
Volta	67	360	-	584	88	283	33
Ashanti	79	71	134	55	55	47	50

Brong Ahafo	205	133	202	69	84	63	92
Northern	-	-	60	55	4	5	30
Upper East	24	5	7	4	2	7	34
Upper West	517	14	30	10	60	41	68
TOTAL	241	189	229	120	87	113	118

Source: Yaws situation in Ghana, 2008 Ghanahttp://www.who.int/yaws/resources/Yaws_Ghana_2008.pdf?ua=1

This strategy provided two simplified treatment policies:

a. Total community treatment (TCT): this was recommended for treatment of an entire endemic community, irrespective of the number of active clinical cases.
b. Total targeted treatment (TTT): this was recommended for treatment of all active clinical cases and their contacts (household, school, and playmates). This was used during repeat surveys or retreatment or in response to a localized outbreak and was also applicable for imported cases.

The purpose of these new policies was to ensure a more pragmatic, aggressive approach of dealing with cases and all contacts, so that transmission can be interrupted in a reasonably short time, leading to eradication. As oral drugs are easier to administer than injection penicillin, it is recommended, if practicable, to undertake large-scale treatment once a community is identified as endemic.

7.1.2 Yaws and syphilis relationship

The relationship of *T. pallidum* subsp. *pertenue*, the causative agent of yaws and *T. pallidum* subsp. *pallidum*, the causative agent of syphilis, has been the subject of much debate for more than 300 years [284]. There is today a progressively increasing recognition, continually supported by new

developments, that syphilis and yaws should be regarded as a closely related group of infections which, under different environmental influences, may develop into essentially similar or even identical clinical syndromes [15,285].

7.1.3 Similarities of yaws and syphilis

Since endemic syphilis and yaws are both non-venereal treponematoses they have a close similarity in their epidemiological pattern [15]. On one hand, both are prevalent in under-developed regions among rural populations with low economic and social standards and poor education and sanitation; on the other hand, with increasing industrialization, social development, and education both will progressively decline and eventually disappear as endemic diseases but may linger on as small foci in isolated inaccessible hamlets and villages. The greatest clinical similarities between yaws and syphilis are seen in the late gummata and ulcerating lesions which result from the affected state of the tissues. Both *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue* are non-cultivable and morphologically indistinguishable even by fluorescent or treponemal immobilization tests [144]. Early stages of yaws and syphilis bear some resemblances, but late lesions of yaws are believed to be restricted to skin, bones, and joints. Indeed, there is a proposed theory by several investigators that the *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue* have grew from a common ancestor but are now, in fact, different diseases [286,287].

In both yaws and syphilis, the amount of reagin - the substance in the blood which is responsible for a positive response to the Wassermann test - decreases with time and is lower in the latent stage in older persons than in younger people, corresponding to the onset of the infection in the childhood [288]. Because of these similarities, the measures for controlling endemic syphilis and analogous conditions such as yaws can follow the same or similar principles [289].

7.2 Methods

7.2.1 Study Design

This was a cross-sectional study conducted in the TMU of KATH and involved interviewing blood donors who were syphilis seropositive. In West Africa where yaws is prevalent, it is assumed that previous yaws infections may also contribute considerably to the high proportion of blood donors being screened positive for syphilis. Unfortunately, serological test for syphilis cannot distinguish blood donors who are infected with syphilis or yaws. Traditionally, individuals in whom seropositivity for syphilis correlated with clinical signs and symptoms of yaws were presumed to have yaws. The study therefore aimed to investigate whether donors who were syphilis seropositive could recall any signs or symptoms of yaws infection that might indicate a positive syphilis test could be due to cross reaction with yaws.

The study algorithm was to interview prospective blood donors initially tested with a treponemal RDT according to routine SOP, further tested with RPR to identify potential active syphilis infections, and finally confirmed with a gold standard algorithm combining two automated treponemal immunoassays [112].

7.2.2 Donor recruitment

Between February 2014 and January 2015, 16,016 blood donors were tested for syphilis as described in chapter 5. Out of these, 478 cases were confirmed with the Ortho-Vitros Syphilis TP test as the gold standard and based on their address on clinical forms, they were invited to be interviewed to determine clinical history after they were consented based on ethical approval.

7.2.3 Development of the interview questions on consented donors

An interview guide was developed to interview confirmed syphilis seroreactive blood donors to determine the past or present clinical manifestations of yaws and syphilis. We developed a working

definition of yaws which included the fact that a donor had signs or symptoms when they were below 15 years of age which included presence of current or past sores or skin ulcers, and skin lesions/bumps on the face, hands, feet or genitals.

The interview was conducted by two trained health professionals with syphilis positive blood donors. The questions covered the presence or absence of current or previous sores or skin ulcers, and skin lesions/bumps on the face, hands, feet or genitals. They were also asked about slow-healing sores and at what age they had experienced symptoms. Furthermore, they were asked about any treatment given at the time of these symptoms. A pilot study was performed on seven blood donors who were initially syphilis reactive which led to the revised and final version of the interview guide. Contact numbers, e-mail addresses and residential addresses for the confirmed blood donors were documented from the clinical forms in the donor clinic of TMU.

7.2.4 Interview training

Two research assistants who were employees of KATH/TMU and were already familiar with blood donor clinic processes, were recruited and trained to perform the interviews and telephone calls as well as data entry. It was ensured that their involvement in this project did not disrupt their regular working activities.

7.2.5 Inclusion Criteria

1. Blood donors who were eligible to donate blood according to KATH/national standards (weight >50kg, systolic blood pressure ≤ 140mmHg and age 18 to 60 years).
2. Blood donors who were sero-reactive with syphilis RDT, and confirmed with Ortho-Vitros Syphilis TP test as the gold standard.
3. Consented to be in the study.

7.2.6 Exclusion criteria

Blood donors who were reactive for syphilis but also reactive for either HIV, HBsAg or HCV.

7.2.7 Data collection

Confirmed syphilis sero-reactive blood donors included in the study were asked to report to the TMU donor clinic by telephone because their telephone numbers were recorded on the clinical questionnaire forms. They were informed about the study aims and objectives in the local dialect (Twi). Participants were informed of the possible benefits of participating in the study which included the direct access to a medical doctor in the event that there is a true positivity and active infection at no extra expense. After the explanation of the study and assessment of their understanding by a set of standard questions, participants were requested to sign or thumbprint an informed consent form (appendix 5.2)

Four hundred and seventy-one out of the 478 confirmed syphilis seroreactive blood donors who were consented, were interviewed using a structured interview guide (appendix 4.2) to determine the past or present clinical manifestations of yaws and syphilis (Figure 7.2). Seven of syphilis confirmed blood donors could not turn up for the interview because three of the seven lived far away and could not be reached while four of them did not consent for the study.

7.2.8 Data quality

Data obtained from the study participants were checked every day for completeness. Validation of the data entry was done at the end of every week to make sure they were properly recorded from the interviews.

7.2.9 Data Analysis

Background data were recorded into a spreadsheet consisting of sex, age, the number of donations, donor type and routine testing results. Data were then exported into STATA SE version 12 (STATACORP, College station, Texas USA) which was the statistical software used for the analysis. All string variables were converted to numerical values for easy analysis and interpretation. Descriptive statistics of the variables were analysed and presented in the form of tables, pie charts, and figures.

7.2.10 Differentiations

The causative agents of both yaws and syphilis cannot be successively cultivated in vitro, are indistinguishable by dark field microscopy, and cannot be differentiated by classical syphilis serological methods [290,291]. However, subspecies-specific genetic signatures permit molecular differentiation using methods that include PCR, restriction fragment length polymorphism (RFLP), and DNA sequencing. Besides, additional genetic differences have been recently observed by Izard *et al* [292] who compared the sequences of the cytoplasmic filament polypeptides (CfpA) of *T. pallidum* subsp. *pallidum* Nichols and *T. pallidum* subsp. *pertenue* Haiti B strain. Clearly, more extensive genetic studies are needed to solve the controversy that surrounds the relationship between yaws and syphilis.

Traditionally, yaws and syphilis could only be distinguished by epidemiological characteristics and clinical manifestations [218]. The clinical manifestations of yaws and syphilis differ in some respects, but these differences can be largely explained by different environmental influences and by the probable subsequent adoption of certain biological characteristics by the treponeme [285].

Because of their serological similarities, it has been suggested that the relatively high prevalence of syphilis may be due to the cross reaction with yaws [293]. Although there is no evidence that yaws

can be transmitted by blood transfusion [144], the cross-reactivity between yaws and syphilis leads to problems for blood services since yaws may cause false positive screening tests for syphilis. This results in unnecessary rejection of donors or discarding of blood units which reduce blood availability. One question we need to ask ourselves is that do the presence of circulating syphilis antibodies (positive screening test) in donor blood provide evidence for the risk of transmitting the disease to recipients or could they sometimes represent a previous yaws infection?

The objective of this study is to describe the recalled medical history, clinical manifestations, and treatment of yaws and syphilis by syphilis seroreactive blood donors in Kumasi, Ghana in an effort to see if there is any evidence of possible previous yaws infection in donors that test positive for syphilis.

7.3 Results

7.3.1 Study population

The age of the 471 syphilis seroreactive subjects interviewed ranged from 17 to 53 years (mean age 31 years, standard deviation 8.6 years). In the total donor population of 16,016, 25% (4015/16016) were female while only one percent of the females (41/4015) were syphilis seroreactive ($p<0.001$). In this study, there were fewer females (29/471; 6.2%) than males (442/471; 93.8%) ($p<0.006$).

Of the 471 respondents, 28 (5.9%) gave a past of skin lesions and sores (Figure 7.2). Four (14.3%) individuals out of the 28 donors with a history of skin lesions and sores – all male and RPR-positive – recalled a diagnosis of syphilis. These four donors had previously received penicillin treatment following their exposure to syphilis. All four men reported the appearance of lesions/bumps on the skin and slow-healing sores, but only one of them had had these symptoms before the age of 15

years. It could not be clarified whether this donor had had yaws or syphilis at this young age; he had received penicillin treatment.

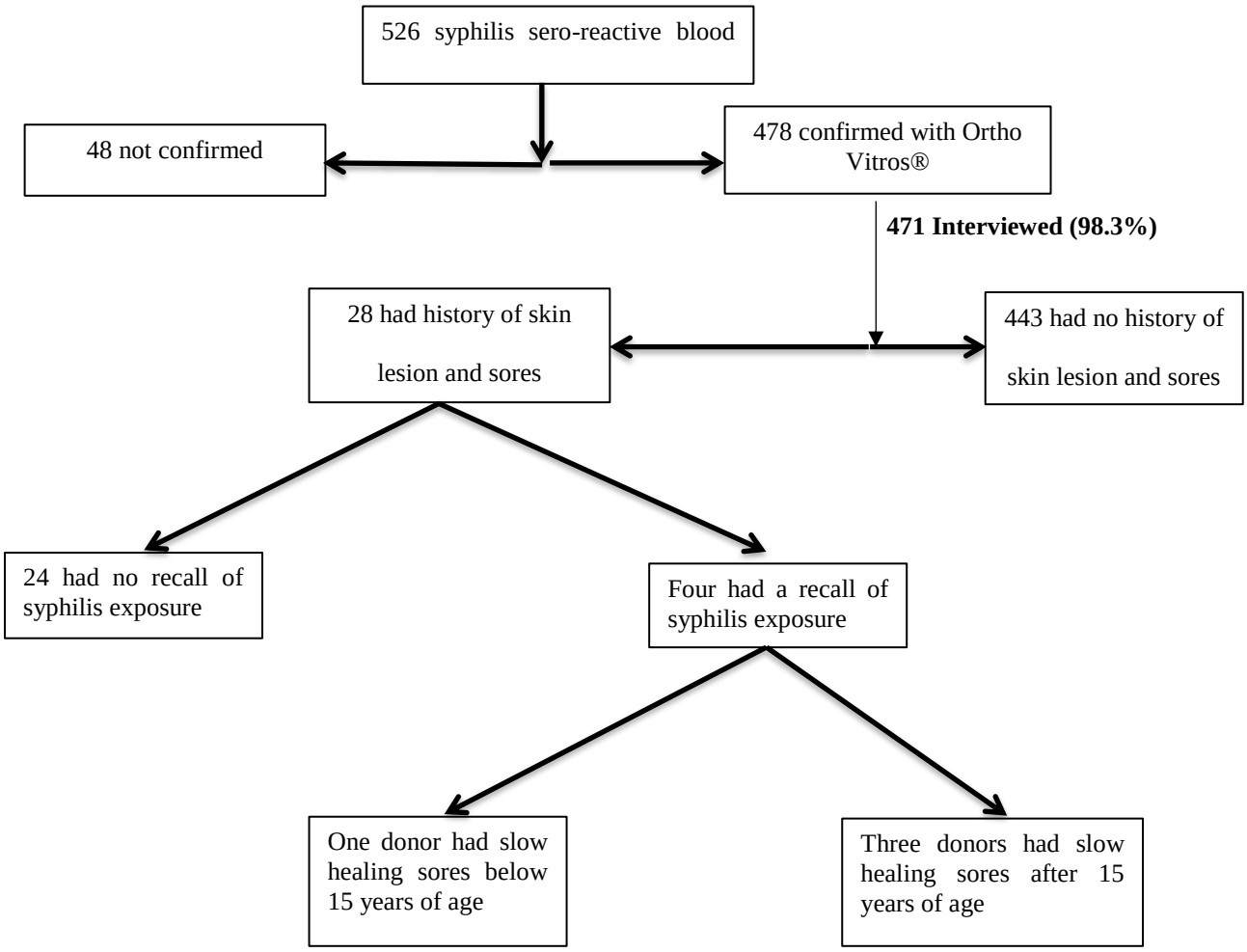

Figure 7.2 A flowchart of syphilis sero-reactive blood donors interviewed for clinical manifestations of yaws

7.4 Discussion

7.4.1 Clinical history of yaws among syphilis reactive blood donors

Although yaws is a bacterial infection closely related to syphilis, data from this study suggest that a clinical history of yaws is not frequent among blood donors who screen positive on syphilis

screening tests. Only a small proportion of confirmed sero-reactive donors had any recall of symptoms or treatment of yaws or syphilis, and only one of these had a slow healing sore below the age of 15 years. There was a poor correlation between the clinical history of yaws or syphilis infection and a positive screening test. Thus, the relative contribution of yaws and syphilis to frequent positive tests in endemic areas remains speculative. Traditionally, individuals in whom seropositivity for syphilis correlates with clinical signs and symptoms of yaws are presumed to have yaws. Children aged below 15 years are the most vulnerable to yaws infection [203] predominantly living in remote, rural communities in tropical countries [294].

7.4.2 Yaws diagnosis

There is no specific blood test for yaws, but because it is closely related to the bacterium that causes syphilis, the blood tests for syphilis are diagnostic in yaws as well. The clinical diagnosis can be confirmed by examining a sample from a skin lesion under a special type of microscope (darkfield examination).

The molecular methods to identify *T. pallidum* to the subspecies level has been previously reported [15,224,295] which involved the use of nested PCR combined with sequencing and/or RFLP of multiple treponemal targets. These methods are costly, laborious, and time-consuming although useful. Additionally, they cannot be suitable for rapid screening applications as well as screening blood donors. The addition of serological tests that detect both treponemal and non-treponemal antibodies greatly improves the odds of identifying suspected yaws cases.

Treatment of yaws is the same as syphilis where potent and cost-effective treatment with a single injection of long-acting Benzathine Penicillin is administered to infected people.

The present data suggest that adding clinical questioning to elucidate a possible clinical history of yaws or syphilis adds little further information to the ability of the current screening algorithm to differentiate between these two conditions as most syphilis seroreactive blood donors have no recall of yaws symptoms.

7.5 Study limitations

1. The study participants were interviewed after knowing that they had a positive test for syphilis. This represents a risk of recall bias, with reporting being influenced by the test results.
2. The study participants were not asked the precise location of the sores but given the low level of association between a history of sores and positivity of tests for syphilis or yaws, it is unlikely that this would be helpful in the differential diagnosis
3. There is furthermore a risk of misclassification bias, as many differential diagnoses exist for both syphilis and yaws.

7.6 Conclusion

A small proportion of confirmed seroreactive donors in this sample had any recall of symptoms or treatment for yaws or syphilis. These data suggest that clinical questioning may only add minimal further information to the current screening algorithm. The relative contribution of yaws and syphilis to frequent positive tests in endemic areas remains speculative.

CHAPTER 8

General conclusion and recommendations

8.1 Conclusion

Blood transfusion services and systems in developing countries are confronted with several challenges that threaten the availability, affordability, accessibility and safety of blood and blood components. Notable among these challenges is blood donor selection which comprises donor recruitment and donor testing. Syphilis is a very under-researched TTI yet it still poses a risk for recipients, particularly in poorer countries such as Ghana.

The objective of my study was to investigate syphilis testing practices in transfusion facilities in Ghana, to guide transfusion policies and practices, and advance transfusion medicine research in Ghana.

The inferences of this study and recommendations are presented objective by objective in the following chapter.

8.1.1 Objective 1: To investigate syphilis testing practices of transfusion facilities in Ghana.

8.1.1.1 Conclusion

This survey investigated syphilis screening practices and seroprevalence of syphilis in blood donors in 122 transfusion facilities in Ghana, resulting in an estimated seroprevalence of 3.7%. However, the teaching hospitals reported the lowest syphilis rate of seroreactivity (3.2%), with the highest resulting from the mission facilities (4.4%). the rate of syphilis seroreactivity from FD in its totality for this survey was significantly higher than the rate from VNRD.

This syphilis seroreactivity was relatively high in the blood donor populations in Ghana, and similar to elsewhere in SSA. However, there is a low syphilis testing rate as over a third of all donors were not screened for syphilis (63.6%, 91,386/143,787) during the period of survey. There was also a relatively high use of non-approved, non-validated test kits (RDTs) for syphilis screening, obtained at variable costs, in Ghana. If these rapid tests are effectively validated and managed, they could be incorporated into the existing guidelines to enhance blood safety. However, the substantial mismatch that existed between recommendations and actual practice for syphilis screening may compromise blood safety.

8.1.2 Objectives 2: To determine the positive predictive value of the RDT that is most commonly used in KATH and Ghana for syphilis testing.

8.1.2.1 Conclusion

Between a time period of February 2014 and January 2015, 16,016 prospective blood donors who came to donate blood for the KATH blood bank were tested with syphilis Fortress RDT, and 599 of these were syphilis seroreactive making an estimated sero-reactivity rate of 3.7%.

526 (3.3%) out of the 599 syphilis seroreactive blood donors were included in the study. Of these, 478 were confirmed by the algorithm (both Vitros and ARCHITECT TPA), making a PPV of 90.9%. Multivariate logistic regression showed a positive association between syphilis reactivity and age, sex, and donor type.

In a blood bank system like the one in Kumasi, Ghana, where syphilis prevalence is relatively high and resources are limited, syphilis screening with RDTs could provide a rational technology. We believe that screening blood donors for syphilis with RDT with proper validation in settings similar to ours can contribute to improving blood safety without threatening the blood supply.

The strength of this study is the description of a real-life performance with regards to the PPV of a newly suggested combined syphilis testing algorithm, combining an RDT and RPR for the identification of potential active syphilis.

8.1.3 Objective 3: To estimate the proportion of blood donors in KATH, Kumasi who are really infectious through blood transfusion.

8.1.3.1 Conclusion

Syphilis sero-reactive samples from 526 consenting donors were further tested according to routine standard procedures with RPR at KATH/TMU laboratory to identify potential active syphilis infections.

The RPR 18 mm circle card test, which is currently the most commonly used assay for donor screening, is a nontreponemal testing procedure for the serologic detection of suspected active syphilis. Of the 526 syphilis RDT reactive donor samples, 32.7% (172/526) were RPR positive. Out of these, 167 were confirmed with Vitros making a PPV of 97.1%. Thus, of the 478 confirmed Vitros positive samples, 34.9% (167/478) were suspected infectious. These blood units were removed from the refrigerator and discarded. Additionally, all the 167 blood donors who were RPR positive were recalled and referred to an internist for treatment. By using RPR as a second test, there are three benefits. Firstly, it reduces the number of blood units that are unnecessarily discarded due to false positive results. Secondly, blood donors who are identified as having suspected active syphilis can be referred for treatment. Finally, blood donors who are syphilis seroreactive but RPR negative (i.e. false positive) do not need to be notified thereby preventing unnecessary worry for them. Therefore, the combination of both RDT and RPR detects suspected active infections and reduces the loss of donors and consequently retaining more blood for transfusion. The combined RDT and RPR testing has a satisfactory high PPV so further routine

confirmation of a donor deferred with dual RDT and RPR reactivity is not needed. The hospital transfusion committee in the study area was instrumental in introducing the combined strategy of syphilis testing with RDT and RPR, showing the critical role, such committees can play in the implementation of evidence-based measures to improve blood safety and availability even when resources are limited.

8.1.4 Objective 4: To determine the performance of a predominant syphilis RDT in selected transfusion facilities in Ghana.

8.1.4.1 Conclusion

100 QC samples of which 60 were positive with Vitros and 40 were negative were sent to 4 selected transfusion facilities to be tested with syphilis ABON RDT for quality assessment. The testing of these samples with ABON gave overall sensitivity and specificity of 99.2% and 82.5% respectively when compared with the gold standard (Vitros TPA). The study has for the first time in Ghana, developed a panel of samples of known syphilis status and used them to evaluate the quality and performance of the ABON syphilis RDT. The use of such a quality system for syphilis testing is important for any laboratory or testing site to ensure accuracy, consistency, and reliability of test results which directly contributes to the safety of blood supply. There are now many different, and un-validated, syphilis test kits in Ghana and this study has shown that it is important to control and validate these kits since.

Transfusion facility laboratories have the responsibility to ensure that test kits that are routinely used for syphilis testing are of good quality and standard reactivity. The effectiveness of less sensitive diagnostic kits could pose a significant risk to both blood donors and blood recipients.

8.1.5 Objective 5: In confirmed syphilis antibody positive donors, determine the relative proportions of those with clinical histories attributable to yaws rather than syphilis.

8.1.5.1 Conclusion

We interviewed 471 prospective blood donors who were treponemal RDT seroreactive, positive with RPR, and finally confirmed with a gold standard algorithm combining two automated treponemal immunoassays and a treponemal immunoblot. 28 of the 471 respondents (5.9%) gave a past history of skin lesions and sores. Four of the 28 respondents recalled a diagnosis of syphilis, of which only one of them, RPR positive male, had symptoms of yaws before the age of 15 years, and had received penicillin treatment. There was a poor relationship between the clinical history of sores or syphilis infection and a positive screening test so it is probably not worthwhile using clinical questioning as a means of differentiating yaws from syphilis in donors who screen positive with syphilis markers.

8.2 Recommendations

1. Teaching hospitals in Ghana should perform syphilis screening using current generation equipment for testing (e.g., TPHA or T. pallidum IgG-specific EIA), and that the smaller facilities use validated RDTs for testing. The challenge might be the cost implications, but this must be balanced against cost-effectiveness and public health benefits.
2. NBSG should ensure that written SOPs for syphilis screening are developed and incorporated into the laboratory guidelines for screening blood donors.
3. Highly sensitive RDTs may be used as first-line screening kits but all reactive samples should be confirmed using commercially available RPR kits to detect potential active infections. This will reduce unnecessary discarding of blood units and enable all infected donors to be referred for clinical advice.

4. A national quality assessment system should be established to evaluate and regulate syphilis RDTs to ensure that they have adequate sensitivity and specificity for use in screening blood donors.

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Appendices

1. Publications

1.1

Sarkodie F, Hassall O, Owusu-Dabo E, Owusu-Ofori S, Bates I, Bygbjerg IC, Ansah JK, Ullum H: **Syphilis screening practices in blood transfusion facilities in Ghana**. *Int J Infect Dis* 2016, **43**:90-94.

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Syphilis screening practices in blood transfusion facilities in Ghana

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SUMMARY

Objectives: The primary objective of this study was to compare laboratory practices for screening blood donors for syphilis at blood transfusion facilities in Ghana with the recommendations of the World Health Organization and the National Blood Service, Ghana (NBSG). The prevalence of syphilis antibodies in blood donors in Ghana was also estimated.
Methods: Over an 11-month period, from February 2014 to January 2015, a semi-structured questionnaire was administered to 122 laboratory technical heads out of a total of 149 transfusion facilities in Ghana. The response rate was 81.9%.
Results: A total of 58 (48%) transfusion facilities tested donors for syphilis, with an estimated 3.7% seroprevalence (95% confidence interval 3.6–3.8%). A total of 62 782 out of 91 386 (68.7%) donations were tested with assays that are not recommended. The estimated syphilis seroprevalence in voluntary donations was 2.9%, compared to 4.0% in family donations ($p = 0.001$). Only 6.9% of the health facilities were using standard operating procedures (SOPs).
Conclusions: Despite international and national recommendations, more than half of the studied health facilities that provide blood transfusions in Ghana are not screening blood donations for syphilis. These data show a considerable mismatch between recommendations and practice, with serious consequences for blood safety and public health.

1. Introduction

Early reports of the transfusion-related transmission of syphilis led to the World Health Organization (WHO) recommendations for syphilis testing of blood donors.[1] These recommendations have been questioned, since many syphilis antibodies among blood donors are the result of previous infections or even unspecific reactions. Furthermore, *Treponema pallidum* does not withstand cold storage.[2] However, as not all blood components can be assumed to be kept cold for a sufficient amount of time, if at all, and as syphilis may also serve as a potential surrogate marker for high risk behaviour in relation to HIV infection, syphilis screening continues to be a requirement in many countries.

There have been several studies conducted in many African countries indicating a high prevalence of syphilis antibodies in healthy blood donors.[3] The WHO recommends several syphilis screening tests: the enzyme immunoassay (EIA) and *T. pallidum* haemagglutination assay (TPHA) as specific tests, or the Venereal Disease Reference Laboratory (VDRL) and rapid plasma reagin (RPR) as non-specific screening tests.[4] Following a documented case of transfusion-transmitted syphilis in Ghana in 2011,[5] it was recommended that syphilis testing for blood donors be implemented so that recipients of blood transfusions would not be at risk of contracting syphilis.

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In Sub-Saharan Africa, blood donations are collected from two main donor categories: voluntary non-remunerated donors (VNRD) and family (replacement) donors (FD). Family donors – who are individuals prompted to provide blood units to replace blood transfused to their relatives or friends[6] – remain dominant on the African continent as a response to difficulties in recruiting and attracting VNRD.[7] However in Ghana, as elsewhere, there is a higher proportion of syphilis seroreactive donations among FD possibly because they are generally older than VNRD and possibly because they are at higher sexual risk.[8]

Out of the many different categories of hospital in Ghana, a total of 149 health facilities across the country practice blood transfusion under the National Blood Service, Ghana (NBSG). Three of the facilities are teaching hospitals located in the Greater Accra, Ashanti, and Northern regions. Ghana has 10 administrative regions and each of them has a regional hospital with bed occupancy lower than the teaching hospitals. However, the 58 district hospitals are distributed unequally. The distribution of the district hospitals is based on the level of development of the region, so some regions have more transfusion centres than others. Likewise, the other health facilities such as the 36 mission hospitals, eight private hospitals, and seven clinics are distributed unequally.

In Ghana, as in many other African countries, the purchase of blood bank reagents is poorly regulated, with local blood banks purchasing whatever reagents are available and affordable. Additionally, the reagent cost per test for syphilis testing in Ghana depends mainly on the bargaining power of the facility management system in the open market. This decentralized purchasing system may lead to increased costs of reagents, as well as failures in quality and consistency. In addition to decentralized reagent purchasing, the lack of written standard operating procedures (SOPs) and effective transfusion-transmitted infection (TTI) guidelines for donor care may hamper quality and care. To ensure the safety, efficacy, and adequacy of blood and blood products for patients, the Ghana National Blood Policy, which was approved by the cabinet in 2006, states that all blood units collected must be tested prior to transfusion for TTIs including syphilis, using approved, well-controlled techniques and procedures and in accordance with WHO guidelines. Furthermore, the NBSG should be responsible for the purchasing of well-approved test kits before use.

This survey compared current syphilis screening practices in Ghana with the recommendations of the WHO and NBSG regarding the use of assays for screening blood donors and their performance. The prevalence of syphilis antibodies in blood donors was also estimated. Additionally, the survey determined whether written SOPs or guidelines were in place for syphilis screening and whether donors with positive syphilis tests were referred for clinical follow-up.

2. Materials and methods

It was intended to interview the laboratory technical heads of all 149 transfusion facilities in Ghana between January 2014 and February 2015 and to request their 2012 syphilis screening results. The survey was conducted using a semi-structured questionnaire administered by telephone call or e-mail. Contact numbers and e-mail addresses were obtained from the NBSG headquarters in Accra and other laboratory science colleagues in the various transfusion facilities in the country. Seventy-three (60%) of the technical heads responded immediately by telephone, while 24 (20%) of them were interviewed twice before providing all of the information by telephone; 25 (20%) provided information through a semi-structured questionnaire by e-mail. The total number of non-respondents was 27 (18.4%); most of these were in remote areas.

2.1. Statistical analysis

Data from the interviews were collected using Epi Info version 3.5.3 (US Centers for Disease Control and Prevention, Atlanta, GA, USA), transferred into an Excel spreadsheet, and exported into Stata version 12.0 statistical software (StataCorp LP, College Station, TX, USA) for analysis. Prevalence was estimated by calculating proportions and providing their respective confidence intervals (95% CI).

2.2. Ethics statement

Approval for this survey was obtained from the ethics committees of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana (CHRPE/AP/423/13) and the Liverpool School of Tropical Medicine, Liverpool, UK (18/02/2014). Furthermore, an introductory letter was sent to all of the respondents from the head of the NBSG, with the assurance of their anonymity in the use of their data.

3. Results

3.1. Facilities and testing

Of a total of 149 health facilities known to be undertaking blood transfusion, 122 (81.9%) responded to the inquiry. In 2012, the total number of donations collected and screened for TTIs (HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV)) other than syphilis from the 122 transfusion facilities responding to the survey was 143 787 (Table 1). From the questionnaire administered, it was found that none of the centres was using a second test to re-screen syphilis-reactive donations.

The total number of transfusion facilities not screening for syphilis was 64 (52%). When asked for the reasons, 49 facilities (77%) reported a lack of funds to purchase reagents. Fourteen facilities (21%) reported that although syphilis screening is recommended, the refrigeration of blood units for more than 5 days kills *T. pallidum*. One transfusion facility (2%) reported that screening for syphilis was not mandatory.

The total number of donations at the 58 (48%) transfusion facilities screening for syphilis was 91 386 units, of which 3371 were syphilis antibody seroreactive, resulting in an estimated seroprevalence of 3.7% (95% CI 3.6–3.8%). Of the facilities screening for syphilis, two of the three (67%) teaching hospitals screened for syphilis and contributed the highest percentage (40.4%) of the total donations. Furthermore, eight of the 10 (80%) regional hospitals screened for syphilis, but contributed only 17.7% (16 009/91 386) to the total donations, whilst 12 of the 36 (33%) mission hospitals screened for syphilis and contributed 15.4% (14 064/91 386) to the total donations, as shown in Table 1. Among the seven clinics, only three (43%) screened for syphilis and these contributed the least (1%) donations. However, the teaching hospitals reported the lowest syphilis rate of seroreactivity (3.2%), with the highest coming from the mission facilities (4.4%). Notably, almost half of the district hospitals did not test for syphilis.

3.2. Donor type and syphilis seroreactivity

The total number of donations screened for syphilis was 91 386 (63.6% of 143 787). The total number of voluntary donations screened for syphilis was 26 180 (28.6%, 95% CI 28.4–28.9%), with 757 (2.9%) testing positive. Of the total of 65 206 (71.4%) family/replacement donations, 2614 (4.0%) tested positive for syphilis

Table 1
Results of the syphilis screening survey in Ghana—January to December 2012

Health facility	Number of screening sites	Number of donations screened for TTIs other than syphilis	Centres screening for syphilis (%)	Number of donations (proportions) screened for syphilis –2012	Average number of donations screened per health facility per day	Number and proportion of donor types screened			Sero-active and estimated prevalence (%)	
						VNRD	FD	Total	VNRD	FD
Teaching	3	56931	2 (67)	36931 (64.9)	51	13 390 (36.2)	23 561 (63.8)	1176 (3.2)	424 (3.2)	752 (3.2)
Regional	10	19768	8 (80)	16 009 (81.0)	6	5241 (32.7)	10 768 (67.3)	578 (3.6)	74[a] (1.4)	504[a] (4.9)
District	58	36 660	30 (52)	20 571 (56.1)	2	4305 (20.9)	16 266 (79.1)	853 (4.1)	95[a] (2.2)	758[a] (4.7)
Clinic	7	2343	3 (43)	879 (37.5)	1	99 (11.3)	780 (88.7)	35 (4.0)	3[a] (3.0)	32[a] (4.1)
Private	8	4503	3 (38)	2913 (64.7)	3	383 (13.1)	2530 (86.9)	107 (3.7)	11[a] (2.9)	96[a] (3.8)
Mission	36	23572	12 (33)	14063 (59.2)	3	2762 (19.6)	11 301 (80.4)	622 (4.4)	130[a] (5.4)	472[a] (4.2)
Total/ average	122	143787	58 (48)	91386 (63.6)	4	26 180 (28.6)	65 206 (71.4)	3371 (3.7)	757[a] (2.9)	2614[a] (4.0)

TTIs, transfusion-transmitted infections; VNRD, voluntary non-remunerated donors; FD, family donors.
[a] Significant.

(Table 1). This indicates that the rate of syphilis seroreactivity from FD in its totality for this survey was significantly higher than the rate from VNRD ($p = 0.001$). However, there were differences in syphilis seroreactivity depending on the health facility in terms of VNRD and FD: while there was no difference between VNRD and FD in syphilis seroreactivity in the teaching facilities (Table 1), there were differences in the other health facilities, where FD seroreactivity was significantly higher than VNRD seroreactivity, except at the mission facilities, where VNRD seroreactivity was significantly higher than FD seroreactivity. However, the data received from the transfusion centres across the country did not indicate the sensitivity and specificity of the type of syphilis test used.

3.3. Recommended assays and other assays for syphilis testing, and operating them

Of the total donations screened for syphilis in this survey, 31.3% were tested using a recommended assay (TPHA; Fortress Diagnostics Limited, Antrim, UK). The non-recommended methods used were all rapid diagnostic tests (RDTs), with 60% reporting ACON as the brand name (ACON Laboratories, Inc., San Diego, USA). Of the others, 19% reported First Response (Premier Medical Corporation Limited, Kachigam, India), 12% ABON (Abon Biopharm Company Limited, Hangzhou, China), 5% Fortress (Fortress Diagnostics Limited, Antrim, UK), 3% Wondfo (Guangzhou Wondfo Biotech, Guangzhou, China), and 1% Determine (Allere Medical Company Limited, Matsuhidai, Japan).

Forty-seven percent of the transfusion facilities validated their syphilis test kits before screening, while only 7% had written SOPs (Table 2).

The hospital management of 52 (89.7%) transfusion facilities purchased syphilis screening reagents on the open market (Table 2). The variation in cost per test strip for syphilis screening varied 10-fold, from US$ 0.2 to US$ 2.0.

3.4. Follow-up of syphilis seroreactive donors

Only 33 (56.9%) facilities referred syphilis-reactive blood donors for clinical advice (Table 2).

4. Discussion

This survey aimed to describe syphilis screening practices and seroprevalence for blood donors in transfusion facilities in Ghana. The estimated national syphilis seroprevalence of 3.7% in this survey is similar to that found among healthy blood donors elsewhere in the region.[9–11] The high occurrence of syphilis has provoked a greatly heightened emphasis on safety, with significant implications in relation to complexity and cost. The study found that about half of the studied facilities in Ghana were not screening blood donations for syphilis, which could lead to syphilis transmission through blood transfusion. Of those facilities that were found to screen donated blood for syphilis, only a third used a recommended test. Among those facilities that were screening, half were not validating the kits, and of donors found to be syphilis-seropositive, more than a third were not referred for further clinical management.

Many parts of the world have reported syphilis seroreactivity rates among FDs similar to that found in the present study.[8,12,13] One reason for the high rates is that FDs are older and therefore have had a longer time to acquire syphilis antibodies. However, FDs may be under pressure to donate blood when their relatives are admitted to hospital and in need of a blood transfusion, even when they know that they are potentially at risk of sexually transmitted diseases as a result of high-risk behaviours. They may

Table 2
Results of the syphilis screening survey in Ghana – 2012

Health facilities	Number of centres that validated test kits[a] n = 58, (%)	Number of centres with written SOPs n = 58, (%)	Number of centres that referred for clinical advice n = 58, (%)	Proportion of reagents purchased by hospital management n = 58, (%)	Donations screened with recommended assays (TPHA) n = 91 386, (%)	Non-recommended assays (RDTs) used at the screening sites
Teaching	2 (3.4)	1 (1.7)	2 (3.4)	2 (3.4)	25 726 (69.6)	Fortress
Regional	7 (12.1)	1 (1.7)	7 (12.1)	8 (13.8)	0	ACON, First Response, and Determine
District	12 (20.7)	1 (1.7)	16 (31.0)	28 (48.3)	612 (3.8)	ACON, ABON, First Response, and Wondfo
Clinic	0	0	1 (1.7)	1 (1.7)	165 (18.8)	ABON
Private	1 (1.7)	1 (1.7)	1 (1.7)	1 (1.7)	2062 (67.7)	Syphilis ultra-rapid test
Mission	5 (8.6)	0	8 (13.8)	12 (20.7)	0	ACON and First Response
Total	27 (46.5)	4 (6.9)	33 (56.9)	52 (89.7)	28 565 (31.3)	

SOPs, standard operating procedures; TPHA, *Treponema pallidum* haemagglutination assay; RDTs, rapid diagnostic tests.
[a] Validate: prove the efficiency of a test kit.

be more likely to conceal a relevant medical history and the risky sexual behaviours that predispose them to infections and thus pose a threat to the safety of the blood supply. Despite this, family donations remain dominant in the African continent because family and community ties are often considerably stronger than in other types of society; making the gift of blood is a natural contribution to relieve sufferers in hospitals.[7] Additionally, potential donors may be less willing to donate to someone not known to them. The WHO states that blood from VNRDs who give blood out of altruism is the safest source of blood.[1]

The survey demonstrated that only 6.9% of the facilities followed written SOPs, indicating poor quality systems where these should play a vital role in blood safety. Written and followed SOPs are an integral part of a quality system, as they facilitate consistency in the performance of procedures in accordance with standards. There have been several recommendations from the WHO that each transfusion service should develop written SOPs as guidelines covering all procedures in the testing of donated blood.[14] The WHO has specified that consistency and reliability of performance in conformity with specified standards raises the quality of systems in promoting blood safety. Unfortunately, an earlier exercise carried out by the Ministry of Health (which was reported in the Ghana National Blood Policy) to determine the status of the blood services in regional and district health facilities in 2006, revealed that the quality assurance programme including SOPs that had been written and followed was under-developed and that the equipment at all sites was generally inadequate. The present survey confirmed the existence of major problems within quality assurance systems and the supply of logistics services.

Previously the NBSG had an external quality assessment (EQA) programme only at its headquarters. However the NBSG checks internal quality assessment (IQA) processes at other blood banks elsewhere in the country. As indicated earlier, because the NBSG does not have absolute control over the purchasing of reagents at individual health facilities, it becomes challenging to make recommendations on IQA.

The finding that 56.9% of facilities referred syphilis-reactive blood donors for clinical advice suggests that, at the other 43.1% of facilities, syphilis reactive donors remained untreated and potentially infectious and could be transmitting the disease to others. This represents a substantial public health failure.

The variation in costs for syphilis screening has significant cost implications, particularly in resource-poor settings in Sub-Saharan Africa. There is little published information on the variation in costs per test for syphilis, but reported costs range from US$ 0.3 to US$ 4.5.[15] In Ghana, the cost variation in syphilis test kits exists due to a lack of guidelines to indicate the effective and accepted test kits and their costs. As a result, many test kit types are available on the open market without proper validation and at different costs. For quality and consistency, the NBSG should be responsible for purchasing approved test kits before use in order to provide safe blood. The present survey did not indicate whether centralized purchasing would necessarily lead to lower prices, but it may help to reduce the cost variations and more importantly, would ensure proper validation.

The techniques used for syphilis screening are different from one country to another: the VDRL or RPR alone for some, and the VDRL and TPHA for others.[1] Tests and algorithms should be selected so that they correspond with the prevalence of the disease and match the technical expertise of the personnel and the availability of reagents and equipment.[16] The selection criteria for a screening strategy must include simple techniques, reliability, sustainability, and cost-effectiveness. Although they are not recommended for blood banks in Africa, rapid test techniques may be preferred because of their affordability, user-friendliness, the availability of test materials, and good sensitivity and specificity; furthermore they do not require sophisticated laboratory materials.[16]

The WHO recommends that each country should decide on the TTIs to be screened for as part of the blood screening programme and develop a screening strategy appropriate to its specific situation, influenced by the incidence and prevalence of infection, the capacity and infrastructure of the blood service, and the costs of screening.[17] The critical factor is the effective implementation of the strategy selected and the consistency of implementation within a well-managed quality system. The NBSG does recommend standardized syphilis screening of all donated blood, but this survey revealed that the guidelines were not generally being followed and serves as an example of the consequences when national guidelines are made without structures to enforce them and without the resources needed to implement them locally.

This survey was not able to reach all of the transfusion facilities in Ghana, but since the facilities that were omitted represented a very small proportion of the total number of donations screened for syphilis it is likely that the results provide a true reflection of the national situation. The study relied on information provided by telephone and e-mail. Resource constraints meant that it was not possible to substantiate the findings first-hand. Nevertheless, this was considered the best methodology with the resources available because some transfusion facilities are located in remote areas with challenging roads. The estimated prevalence may not be a perfect reflection of the epidemiological situation in Ghana. This is because the donor population that was not screened could have had a higher or lower prevalence of syphilis than the screened population. For the population that was actually screened, variation in screening practices may have led to both under-reporting due to a lack of

sensitivity or over-reporting due to poor specificity of the screening tests used.

From the questionnaire administered it was found that none of the centres was using a second test to re-test syphilis-reactive donations, for example a non-treponemal test to detect active infection. Therefore it is difficult to estimate how many donations may have been infective, and how many patients receiving a blood transfusion are potentially at risk. It is planned to examine this in a further study.

In conclusion, there is a relatively high prevalence of syphilis reactivity in the blood donor populations in Ghana, as elsewhere in Sub-Saharan Africa. However, there is a low syphilis testing rate and a relatively high use of non-approved, non-validated test kits (RDTs) for syphilis screening, obtained at different costs, in Ghana. If these rapid tests are effectively validated and managed, they could be incorporated into the existing guidelines to enhance blood safety. However the considerable mismatch between recommendations and actual practice for syphilis screening may compromise blood safety. Further studies on syphilis RDTs for blood donors are suggested, in order to improve their application in resource-poor settings.

In terms of recommendations, as shown in Table 1, screening with the TPHA or a *T. pallidum* IgG-specific ELISA would be more appropriate for the workload in teaching hospitals compared with the other health facilities. It is recommended that teaching hospitals perform syphilis screening using current generation equipment for testing (e.g., TPHA or *T. pallidum* IgG-specific EIA), and that the smaller facilities use validated RDTs for testing. The challenge might be the cost implications, but we must also think of cost-effectiveness as a public health issue. It is also recommended that the NBSG ensure that written SOPs are developed and incorporated into the laboratory guidelines for screening as part of strong quality systems in the health facilities across the country, and that all syphilis-reactive donors are referred for clinical advice.

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A novel strategy for screening blood donors for syphilis at Komfo Anokye Teaching Hospital, Ghana

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SUMMARY

Objective: To implement and describe a novel syphilis screening strategy for blood donors.

Background: The seroprevalence of syphilis in blood donors is often high in low- and middle-income countries (LMIC) although the proportion of infectious donations is probably low. Syphilis screening may not happen at all; or the use of non-specific screening tests, which have high false positive rates, results in many donations being discarded unnecessarily. This can have a critical effect on already inadequate blood supplies.

Materials and Methods: Blood donors were screened at the time of donation with an anti-treponemal rapid diagnostic test (RDT) and blood collected irrespective of the result. Units screening negative for syphilis, human immunodeficiency virus (HIV) and hepatitis B and C were released to stock. RDT screen-positive units were re-tested with rapid plasma reagin (RPR) – units testing negative were released to stock and test-positive units discarded.

Results: Of the 2213 blood donors, 182 (8·2%; 182/2213) screened positive by RDT. In addition, 38 out of these 182 (20·9%) were RPR positive on post-donation testing. Over 2 months there was a 79% reduction in blood units discarded due to a positive syphilis screen.

Conclusion: In other LMIC, this novel strategy can contribute to improving blood safety without jeopardising blood supply.

Key words: blood donation testing, blood safety, serological testing.

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BACKGROUND

Syphilis, caused by the spirochaete *Treponema pallidum*, is prevalent in sub-Saharan Africa (SSA). Of the estimated 12 million new cases a year, a quarter occur in Africa (WHO, 2007). Syphilis is predominantly spread by sexual contact but may be transmitted by blood transfusion (Gardella *et al.*, 2002). Seroprevalence rates for syphilis in blood donors in SSA range from 1·1 to 14·4%, and in Ghana from 4·7 to 13·5% (Owusu-Ofori *et al.*, 2011; Bisseye *et al.*, 2013; Noubiap *et al.*, 2013). Transfusion-transmitted syphilis (TTS) is uncommon and there have been only a few cases documented over the past few decades (Hook & Peeling, 2004; Brant *et al.*, 2007). The low frequency of TTS reported may be due to a number of factors including: low prevalence in blood donors; effective screening methods; the refrigeration of blood products and the frequent use of antibiotics in transfusion recipients. However an additional factor in LMIC where the seroprevalence of syphilis in blood donors is higher is poor or absent of haemovigilance systems which may result in under-reporting of TTS. This was highlighted by a recently reported case in Kumasi, Ghana (Owusu-Ofori *et al.*, 2011).

Although WHO recommends screening all blood for transfusion for syphilis (WHO, 2010), *T. pallidum* survives for only a few days at 4 °C and therefore is killed during refrigeration of blood products (Adeolu & Olufemi, 2011). In most high-income countries where the seroprevalence of syphilis in blood donors is much lower and TTS is very rare, syphilis screening is routine. In contrast, in low- and middle-income countries (LMIC) where the seroprevalence of syphilis in blood donors is higher and refrigerated storage times of donated blood shorter, syphilis screening is often not undertaken (WHO, 2011).

Reasons for not screening for syphilis in LMIC are constrained resources and a presumption that transmission risk is low. Serological screening methods currently available are unable to determine infectious blood units. In addition they may not differentiate past, present or treated infections. Healthy blood donors thus may be declared falsely positive by the

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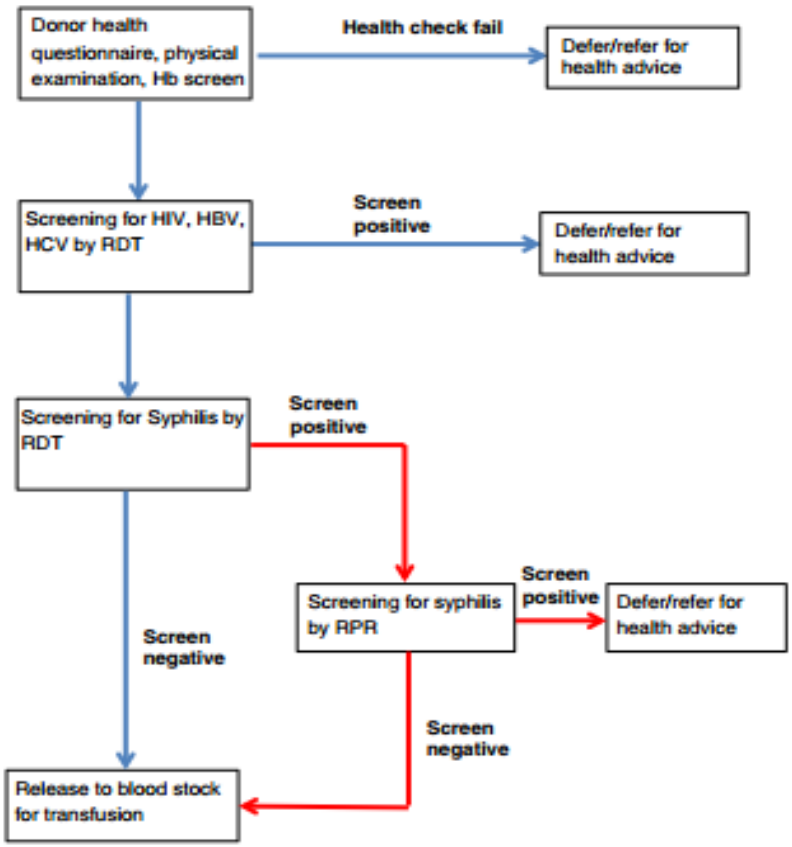

Fig. 1. Algorithm for syphilis screening of blood donors, Transfusion Medicine Unit, Komfo Anokye Teaching Hospital (new pathway shown in red).

serological screening tests for syphilis (Ratnam, 2005). Therefore, where seroreactivity rates are high, large numbers of uninfectious blood donations may be discarded unnecessarily. In LMIC, where blood for transfusion is in short supply, this can be critical and endanger lives.

The Transfusion Medicine Unit (TMU) at Komfo Anokye Teaching Hospital (KATH) in Kumasi manages about 18 000 blood donations a year. A high proportion of blood donors are secondary or tertiary students and blood shortages can occur during vacations and examination periods. Blood donors are screened for human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) by pre-donation rapid diagnostic test (RDT) and approximately 10% of donors are deferred (Owusu-Ofori *et al.*, 2005). There is currently no post-donation testing. RDT for TTI screening are often preferred in LMIC as they are cheap, quick, require limited expertise and are therefore suitable for screening blood donors pre-donation on mobile blood drives. Pre-donation screening where TTI seroprevalence is high is attractive as the costs of collection are avoided and the chances of mixing up screen positive and screen negative units post collection are reduced.

Until recently there was no screening for syphilis at TMU-KATH. However, a recent study at the hospital identified a case of TTS in an 8-year-old girl and that 57% of donations were stored for less than 4 days before being transfused (Owusu-Ofori *et al.*, 2011). As a consequence, the hospital transfusion committee recommended the introduction of syphilis screening. A cost analysis by the TMU supported pre-donation screening with a RDT over post-donation screening methods.

MATERIALS AND METHODS

In July 2014, pre-donation screening of blood donors for syphilis was introduced with an anti-treponemal RDT (Fortress Quick Test, Fortress Diagnostics, Antrim). This demonstrated a seroreactivity rate of 7·0% and the overall deferral rate for all transfusion-transmitted infections (TTI) increased to 16·5%. This had a critical and unsustainable effect on the blood supply for the hospital. The TMU therefore instituted a novel and pragmatic syphilis screening algorithm to improve blood safety but also to protect the blood supply.

In the new screening algorithm (Fig. 1) donors who screen positive for syphilis by RDT, which indicates possible past or current infection, are not deferred. Rather, blood units are collected and then quarantined until an additional screening test – rapid plasma reagin (RPR; IMMUTREP RPR, Omega Diagnostics, UK) – is performed. This non-treponemal syphilis test identifies possible active infection and, therefore, potential for transmission. Units testing positive by RPR are discarded and the donors contacted for referral for further investigation and/or treatment. Conversely, RDT positive units testing negative by RPR are released for transfusion.

RESULTS

In August and September 2014, 2455 blood donors presented for blood donation. Of these, 1959 (88·5%) were male, 1080 (48·8%) were voluntary donors and 1642 (74·2%) were first-time donors. Of the 2455 blood donors, 242 (9·9%) were deferred at pre-donation screening for HIV (2·6%; 64/2455), HBV (6·3%; 156/2455) and HCV (0·9%; 22/2455). After screening for HIV, HBV and HCV, 2213 donors underwent pre-donation screening for syphilis by RDT. Of these, 182 (8·2%; 182/2213) screened positive by RDT and 29 of these screened positive on subsequent RPR testing. Nine RDT positive donations were discarded in error before RPR testing. Thus, of 182 syphilis RDT positive blood donations 144 were RPR negative, considered uninfectious and released for transfusion; and 38 were discarded, an overall rate of 1·5% (38/2455). The number of units discarded due to a positive syphilis screen was therefore reduced by 79% (144/182).

Rates of syphilis RDT positivity were higher in family replacement donors (10·1%; 114/1133) compared with voluntary donors (6·3%; 68/1080) (chi-squared; $P = 0{\cdot}01$), and in male donors (8·8%; 172/1959) compared with female donors (3·9%; 10/254) ($P = 0{\cdot}008$). First-time donors had a greater RDT seroreactivity rate for syphilis (8·9%; 146/1642) than repeat donors (6·3%; 36/571) but the difference in this sample is not statistically significant ($P = 0{\cdot}053$).

DISCUSSION

The novel blood donor syphilis screening strategy described here resulted in a saving of 144 donations over a 2-month period and has several potential advantages in our setting: the risk of TTS is reduced compared with the status quo of no screening; first-line testing by RDT is cheap and can be incorporated into the existing pre-donation screening panel; the second-line RPR test, which requires laboratory expertise and resources, is only performed on a minority of samples; and the negative impact of syphilis screening on the blood supply is reduced.

The hospital transfusion committee was instrumental in initiating the new strategy, showing the critical role such committees can play in the implementation of evidence-based measures to improve blood safety and availability even when resources are limited (Opare-Sem *et al.*, 2014).

It is important to stress we do not know the false negative rate of this screening strategy or its residual risk and this requires further study. Furthermore, robust systems are necessary for the effective quarantining of RDT positive donations pending second-line testing. We anticipate that as the new screening strategy beds in errors resulting in units being discarded before second-line testing will be eliminated. We emphasise that although this novel strategy may be relevant for LMIC with limited resources, it is not necessarily appropriate for high-income countries with different donor characteristics, syphilis prevalence and resource constraints.

CONCLUSION

We believe that in settings similar to ours the novel strategy described here for screening blood donors for syphilis can contribute to improving blood safety without jeopardising the blood supply.

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Transfusion Medicine | ORIGINAL ARTICLE

Improving the screening of blood donors with syphilis rapid diagnostic test (RDT) and rapid plasma reagin (RPR) in low- and middle-income countries (LMIC)

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SUMMARY

Background: Syphilis testing conventionally relies on a combination of non-treponemal and treponemal tests. The primary objective of this study was to describe the positive predictive value (PPV) of a screening algorithm in a combination of a treponemal rapid diagnostic test (RDT) and rapid plasma reagin (RPR) test at Komfo Anokye Teaching Hospital (KATH), Ghana.

Materials and Methods: From February 2014 to January 2015, 5 mL of venous blood samples were taken from 16 016 blood donors and tested with a treponemal RDT; 5 mL of venous blood was taken from 526 consenting initial syphilis sero-reactive blood donors. These RDT reactive samples were confirmed with an algorithm, applying the Vitros®/Abbott-Architect® algorithm as gold standard.

Results: A total of 478 of 526 RDT reactive donors were confirmed positive for syphilis, making a PPV of 90·9%. Of the 172 (32·7%) donors who were also RPR positive, 167 were confirmed, resulting in a PPV of 97·1%. The PPV of the combined RDT and RPR (suspected active syphilis) testing algorithm was highest among donors at an enhanced risk of syphilis, family/replacement donors (99·9%), and among voluntary donors above 25 years (98·6%).

Discussion: Screening of blood donors by combining syphilis RDT and RPR with relatively good PPV may provide a reasonable technology for LMIC that has a limited capacity for testing and can contribute to the improvement of blood safety with a minimal loss of donors.

Key words: blood donors, blood safety, public health, rapid diagnostic test, rapid plasma reagin.

Syphilis screening is a big challenge in many low- and middle-income countries (LMIC) that have a limited capacity for testing. High syphilis prevalence among healthy blood donors in Africa aggravates the problem in this region. Techniques for syphilis testing are very problematic and conventionally rely on a combination of non-treponemal and treponemal tests. The non-treponemal antibody tests for donor screening include the Venereal Disease Research Laboratory (VDRL) (Harris *et al.*, 1948) and the rapid plasma reagin (RPR) (Larsen *et al.*, 1998). The advantages are that these tests are inexpensive, fast, simple to perform and more sensitive (Montoya *et al.*, 2006). They are able to identify the contaminated blood donors a few days before the treponemal test and thus are useful for acute infection. Moreover, quantifiable titers can establish a baseline to evaluate treatment response (Sena *et al.*, 2010a) as they usually revert to negative after successful treatment (Larsen, 1989; Romanowski *et al.*, 1991; Larsen *et al.*, 1998). However, VDRL and RPR cannot be automated and are therefore time-consuming if used for large-scale testing. In addition, results may be difficult to interpret, and this requires the sufficient training of health personnel to ensure correct testing and interpretation. Another major problem when using non-treponemal tests is the possibility of biological false positive reactions due to cross-reactivity with molecules in other conditions, such as viral infections, pregnancy, malignant neoplasms, autoimmune diseases and advanced age (Larsen *et al.*, 1995; Sena *et al.*, 2010a).

Treponemal tests for donor screening classically included the *Treponema pallidum* haemagglutination assay (TPHA), the

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T. pallidum passive particle agglutination (TP-PA) assay and the fluorescent treponemal antibody absorption (FTA-ABS) (Hunter *et al.*, 1964). However, newer, automated treponemal tests have reduced running costs and provide objective readings, making them useful for large blood centres. Treponemal tests typically remain positive even after treatment (Schroeter *et al.*, 1973; Gerber *et al.*, 1997; Larsen *et al.*, 1998), implying that a donor previously diagnosed for syphilis cannot be distinguished from a new or untreated case of syphilis.

In developing countries and areas with limited resources, laboratory facilities are often unavailable for standard automated syphilis tests. Given the barriers to automated testing, many resource-limited countries are resorting to syphilis rapid diagnostic tests (RDTs) for transfusion-transmitted infection (TTI) screening (Kaur & Kaur, 2015). Although quality RDTs have the potential to increase the safety of the region's blood supply, uncertainty surrounding the performance of some RDTs in the field has increased debate regarding their application to TTI screening (Scheiblauer *et al.*, 2006; Laperche & Francophone African Group for Research in Blood, 2013; Mbanya, 2013).

Some rapid tests are highly sensitive and specific (Owusu-Ofori *et al.*, 2005; Sena *et al.*, 2010a) but cannot differentiate between active and treated syphilis; others may give false positive reactions (van Dommelen *et al.*, 2008). Most rapid tests detect IgM, IgG and IgA antibodies and involve immunochromatographic strips in which one or multiple *T. pallidum* recombinant antigens are applied to nitrocellulose strips as a capture reagent. Irrespective of the advantages of these rapid tests, if they have a low positive predictive value (PPV) – high false positive rate – and are used in blood banks, then apparently, many donors will be deferred when they carry no risk to the blood supply. Conversely, if the PPV is high (low false positive rate), then few donors with no risk to the blood supply will be deferred. This is really important in LMIC settings where blood supplies are critical, coupled with a high burden of TTIs.

The Transfusion Medicine Unit (TMU) of the Komfo Anokye Teaching Hospital (KATH, Kumasi, Ghana) has been practicing pre-donation screening with viral RDTs for blood donors since 2000 (Owusu-Ofori *et al.*, 2005; Allain *et al.*, 2010). KATH operates on two types of blood donors, namely, voluntary non-remunerated blood donors (VNRBD), constituting 70% of blood collection, and family replacement donors (FRD), constituting 30% of blood collection for over a decade now. The decision to introduce syphilis screening as part of pre-donation screening was decided by the Hospital Transfusion Committee in 2012. However, a loss of blood for transfusion of 7% by syphilis RDT reactive donors was a threat to blood supply, and an algorithm that combined robustness, safety and minimal discard rates was needed. KATH implemented an algorithm consisting of syphilis testing by RDT before blood collection followed by RPR (IMMUTREP RPR, Omega Diagnostics – Scotland, UK) testing of syphilis RDT reactive donors (Sarkodie *et al.*, 2016a,2016b). The presumption was that by discarding only .donors who were also positive for RPR, only donors at risk of active syphilis were rejected, whereas donors with previous well-treated syphilis could continue to donate, and their blood would be released for transfusion. One of the risks is that although combining two different syphilis tests, the algorithm might still defer too many donors due to false reactivity in the two tests. In that case, the algorithm would still compromise blood supply, and it would expose donors to unneeded worries, stigma and therapy.

In order to study this aspect, the present study validates the PPV of the implemented algorithm by applying a gold standard retest algorithm combining two different automated anti-TP immune assays of 526 syphilis RDT reactive donors in improving blood donor screening.

MATERIALS AND METHODS

We conducted a descriptive cross-sectional study between February 2014 and January 2015. A total of 16 016 blood donors were initially tested according to routine standard operational procedures with a treponemal RDT (Fortress® Diagnostics Limited – Antrim, UK). Sensitivity and specificity of the Fortress RDT are stated to be 99·7 and 99·6%, respectively, for the qualitative detection of antibodies (IgG and IgM) to *T. pallidum* in serum or plasma. A total of 5 mL of venous blood was taken from 526 consenting initial Fortress RDT syphilis sero-reactive blood donors. All these samples were further tested according to routine standard procedures with RPR (BD Macro-Vue™ Card test, Branchburg, New Jersey, USA) to identify potential active syphilis infections.

For gold standard confirmation of the Fortress RDT, all 526 RDT reactive samples were subsequently retested in an algorithm combining two automated treponemal immunoassays and a treponemal immunoblot. Initial retesting was performed by the Vitros® Syphilis *Treponema Pallidum* Antibody (TPA) chemiluminescence immunoassay using the Vitros ECi/ECiQ Immunodiagnostic Systems described elsewhere (Gonzalez *et al.*, 2015). Briefly, the Vitros Syphilis TPA assay is a qualitative assay that detects total antibodies (IgG and IgM) to *Treponema pallidum* (TP) reacting with biotinylated and horseradish peroxidase (HRP)-labelled recombinant TP antigens TP15, TP17, TP47 and is bound to streptavidin-coated wells. The illuminating reaction detected from the bound HRP conjugates is directly proportional to the concentration of anti-TP antibodies, and high signal samples [signal at Cutoff (S/CO) >100] were considered confirmed (Fig. 1). The assay was mainly validated in a western population (data not shown), with a specificity of 99·8% (CI 98·7–100%) and a sensitivity of 100% using Syphilis Mixed Titer Performance Panel PSS202 (BBI Diagnostics, Bridgend, UK) and clinical samples from known syphilis-treated patients of both Caucasian and African origin.

All Vitros low reactive samples (S/CO <100) were additionally tested with another qualitative anti-TP immunoassay Architect® Syphilis TP (Abbott Diagnostics, Abbott Park, IL, USA), which also detects antibodies binding to the recombinant TP antigens TpN15, TpN17 and TpN47 (Fig. 1). A reactive

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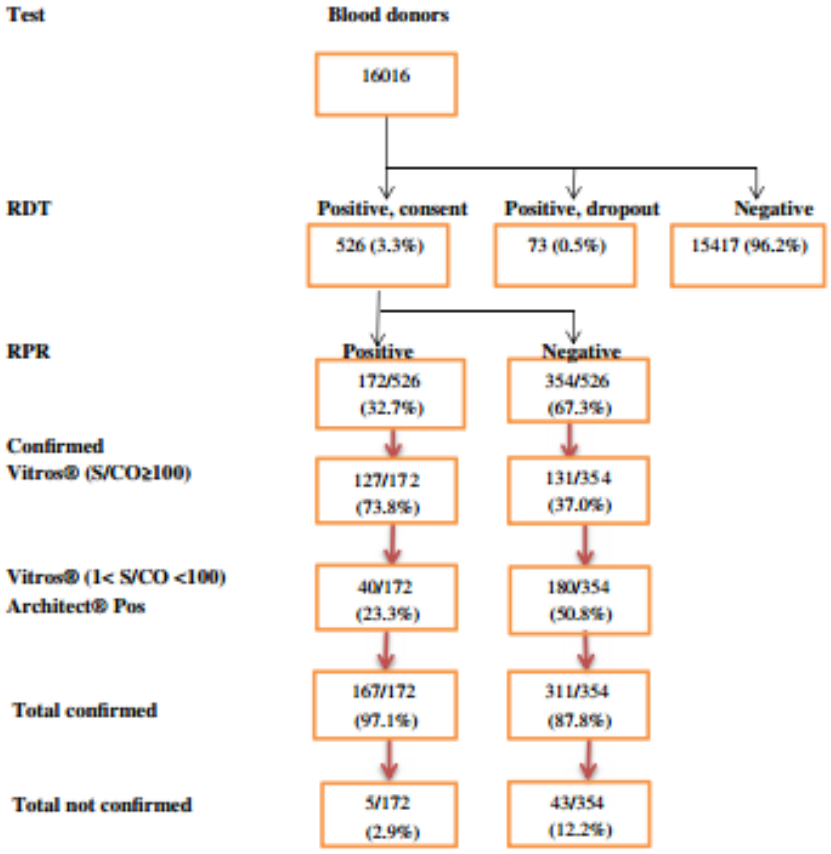


RDT; - rapid diagnostic test, RPR; - rapid plasma reagin, S/CO-; sample over cut-off
N/B-Architect® was performed on specimen that were low reactive with Vitros® (1< S/CO <100)

Fig. 1. Algorithm for syphilis confirmatory testing of sero-reactive blood donors, Transfusion Medicine Unit, Komfo Anokye Teaching Hospital.

Fortress RDT sample was considered confirmed positive for specific anti-TP antibodies if the Vitros Syphilis TPA was highly reactive (S/CO > 100) or if Vitros Syphilis TPA was low reactive (1 < S/CO <100) and Architect Syphilis TP reactive.

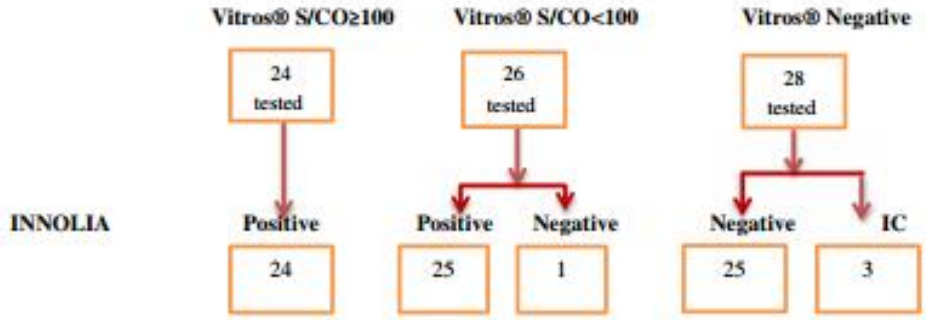
As a quality control measure, 78 of 526 syphilis RDT sero-reactive samples were further tested in a line immunoassay (LIA) (Furijebio, Ghent, Belgium) (Fig. 2). The LIA detects individuals binding to the same recombinant TP antigens TpN15, TpN17 as well as to TpN47 and a synthetic peptide TmpA derived from *T. pallidum* proteins (Ebel *et al.*, 2000).

Syphilis sero-reactive donors were informed of the study and signed an informed consent form according to the study protocol, which was approved by the Ethics Committees of Kwame Nkrumah University of Science and Technology (KNUST) Kumasi, Ghana (CHRPE/AP/423/13) and Liverpool School of Tropical Medicine, UK (18/02/2014).

Background data were recorded onto a spreadsheet consisting of gender, age, number of donations, donor type and routine testing results. Data were then exported to STATA (STATACORP, Texas, version 12·0) for analysis. We estimated PPVs by calculating proportions and providing their respective confidence intervals. Multivariable logistic regression was performed on syphilis reactivity as an outcome. Age, gender and donor type were included as independent variables, with results presented as odd ratios and 95% confidence intervals. A *P*-value of <0·05 denoted a statistically significant difference in all statistical comparisons.

RESULTS

A total of 599 out of 16 016 blood donors reacted to the syphilis Fortress test, making an estimated sero-prevalence of 3·7% (95% CI 3·5–4·1%). A total of 73 (12·2%) blood donors who reacted with the Fortress syphilis test were excluded from the study (Fig. 1) because 41 (6·8%) of them were co-infected with hepatitis B virus (HBV), 15 (2·8%) were co-infected with HIV and 7 (1·2%) were co-infected with hepatitis B virus (HCV), while 10 (1·7%) did not consent. Thus, 526 (3·3%) syphilis sero-reactive blood donors were included in the study, of whom 199 (37·8%)



Approximately 15 % of the samples tested with Vitros® were further tested with INNO-LIA as a quality control of the validation algorithm.
IC; - Inconclusive, S/CO-; sample over cut-off

Fig. 2. Quality control with INNOLIA for syphilis testing of sero-reactive blood donors, Transfusion Medicine Unit, Komfo Anokye Teaching Hospital.

Table 1. Syphilis RDT sero-reactive blood donors

	RPR		
RDT-reactive blood donors	Positive (%)	Negative (%)	Total (%)
Confirmed	167 (97·1)	311 (87·8)	478 (90·9)
Unconfirmed	5 (2·9)	43 (12·2)	48 (9·1)
Total	172 (32·7)	354 (67·3)	526

were VNRBD (95% CI 33·8–42·1%). Generally, blood donors tested were aged 16–59 years, with a mean age of 25 (SD = 9·1), compared with syphilis sero-reactive donors, who showed an age range of 17–53 years, with a mean age of 31 years ($P < 0·001$).

PPV of syphilis RDT reactive samples according to donor type

Of the 526 RDT syphilis sero-reactive samples tested with Vitros, 478 were reactive and confirmed by the algorithm, making a PPV of 90·9% (Table 1). Similarly, the proportion or PPV of sero-reactive FRD (309/327, 94·5%) that was confirmed (Table 2) was statistically significantly higher than that of confirmed sero-reactive VNRBD (169/199, 84·9%) ($P = 0·001$), although there was 100% PPV in some age groups. Of the total blood donors tested, 10 218 (63·8%) were VNRBD, of whom 199 (1·95%) were syphilis sero-reactive (95% CI 1·73–2·28%) as shown in Table 2. Of the 5798 FRD tested, 327 were syphilis sero-reactive, which was significantly higher [5·64% (95% CI, 5·08–6·26%)] $P < 0·001$ compared with VNRBD.

PPV of syphilis RDT reactive samples according to donor age

Syphilis RDT sero-reactive donors showed a range of 17–53 years, with a mean age of 31 years. The PPV of the VNRBD ranges from 74·4 to 100% for the ages ranging between 17 and 55 years, whilst that of FRD ranges from 91·2 to 100% for the same age difference (Table 2). Generally, the PPVs of the syphilis-confirmed reactive donors increase with age for all donor types except in VNRBD, where those aged between 46 and 55 years have a relatively lower prevalence (3·17%) but higher PPV (100%). Although syphilis-confirmed sero-prevalence of FRD was 5·33%, there was higher prevalence as the age increased. Similarly, the prevalence of syphilis-confirmed VNRBD was 1·65%, although there was a higher prevalence of 5·24% in the 36–45 age category.

PPV of syphilis RDT and RPR reactive samples

Of the 526 syphilis RDT reactive samples, 172 (32·7%) were RPR positive (95% CI 28·8–36·8%). Out of these, 167 were confirmed, making a PPV of 97·1% (Table 1). Thus, the PPV was higher among RDT and RPR reactives (97·1%) than in the total population of RDT reactives (90·9%) as shown in Table 1. Conversely, the PPV was higher among FRD (99·1%) than in VNRBD (93·3%). More FRD and more donors aged 26 and over were RPR positive and increased with age as the PPV increased (Table 2). Similarly, the PPV of RDT and RPR dual-reactive donors was the highest among FRD and among donors aged 26 and above (Table 2). Out of the five RPR false positives, four were VNRBD, out of which three were aged between 16 and 25 years, and one was aged between 26 and 35 years, while the other one (FRD) was between 26 and 35 years. Additionally, 311 of 354 (87·8%) RPR-negative donors tested positive with Vitros.

The effect of age, gender and donor type on syphilis reactivity

By multivariable logistic regression, we showed a positive association between syphilis reactivity and all included explanatory

Table 2. Syphilis RDT and RPR sero-reactive blood donors confirmed with Vitros TP, stratified according to age

Blood donors	Total RDT tested (%)	RDT + (%)	RDT+, Vitros+ (%, PPV)	RDT+, RPR+ (%)	RDT+, RPR+, Vitros+ (%, PPV)
VNRBD	10218 (63·8)	199 (1·95)	169 (1·65, 84·9)	60 (0·59)	56 (0·55, 93·3)
Age group (years)					
≤25	7525 (73·6)	78 (1·04)	58 (0·77, 74·4)	27 (0·36)	24 (0·32, 88·9)
26–35	1750 (17·1)	76 (4·34)	68 (3·89, 89·5)	23 (1·31)	22 (1·26, 95·7)
36–45	687 (6·7)	38 (5·53)	36 (5·24, 94·7)	9 (1·31)	9 (1·31, 100·0)
46–55	221 (2·2)	7 (3·17)	7 (3·17, 100·0)	1 (0·30)	1 (0·30, 100·0)
>56	35 (0·4)	0	0	0	0
Gender					
Female	3477 (34·0)	24 (0·69)	16 (0·46, 66·7)	6 (0·17)	6 (0·17, 100·0)
Male	6741 (66·0)	175 (2·60)	153 (2·27, 87·4)	54 (0·80)	50 (0·74, 92·6)
FRD	5798 (36·2)	327 (5·64)	309 (5·33, 94·5)	112 (2·12)	111 (1·91, 99·1)
Age group (years)					
≤25	1869 (32·2)	57 (3·05)	52 (2·78, 91·2)	24 (1·28)	23 (1·23, 95·8)
26–35	2405 (41·5)	148 (6·15)	139 (5·78, 93·9)	48 (2·00)	48 (2·00, 100·0)
36–45	1186 (20·4)	95 (8·01)	91(7·67, 95·8)	32 (2·70)	32 (2·70, 100·0)
46–55	321 (5·5)	27 (8·41)	27 (8·41, 100·0)	8 (2·50)	8 (2·50, 100·0)
>56	18 (0·4)	0	0	0	0
Gender					
Female	538 (9·3)	13 (2·42)	13 (2·42, 100·0)	4 (0·74)	4 (0·74, 100·0)
Male	5260 (90·7)	314 (5·97)	296 (5·63, 94·1)	108 (2·05)	107 (99·1)
Total	16016	526 (3·28)	478 (2·98, 90·9)	172 (1·07)	167 (1·04, 97·1)

+, positive.

parameters: increased age, male gender and status as a FRD (Table 3). The effects of male gender and FRD status were similar whether a positive end-point was defined as RDT+, RDT+ Vitros+, RDT+ RPR+ or RDT+ Vitros+ RPR+ (Table 3). The effect of age was weaker for end-points including RPR reactivity (Table 3). The male gender was a stronger predictor of syphilis reactivity than status as an FRD (Table 3).

Samples tested with INNO-LIA as quality control

Approximately 58% (28) of the samples that were negative according to the Vitros test were tested with INNO-LIA. A total of 25 of them were negative, while the rest (3) were inconclusive. All 24 (~9%) samples that were Vitros-high reactives were confirmed with INNO-LIA, and 25 of 26 low with Vitros were confirmed with LIA, and one was inconclusive.

DISCUSSION

Syphilis infection in blood donors continues to pose a major threat in many developing countries, including Ghana (Adjei *et al.*, 2003; Sarkodie *et al.*, 2016a,2016b). The Fortress syphilis RDT that was used for this study based on its performance characteristics has a sensitivity and specificity of 99·7 and 99·6%, respectively, according to the manufacturer. When compared to the gold standard in single testing, it gave a PPV of 90·9%. Similar syphilis RDTs in other studies gave a PPV of 95·2%, with sensitivity and specificity of 93·6 and 92·5%, respectively (Sato *et al.*, 2003). As PPV relies on both test specification and disease prevalence, it is not surprising that other studies have shown PPVs of some RDTs to be both lower and higher than this value (Pruett *et al.*, 2015). When combining a RDT with a non-specific syphilis test, in this case RPR, a much higher PPV was achieved (97·1%). Thus, by combining the two tests, both donors with confirmed but inactive TP infections and donors with unspecific RDT reactions could avoid deferral, and they could therefore still contribute to the blood supply. The key concept underlying blood safety, especially in LMIC, is the balance between blood supply and blood safety in the context of a poor blood supply, high prevalence of TTI compared and limited resources. As stated earlier, if there is a low PPV (high false positive rate), then many donors will be deferred when they carry no risk to the blood supply. Furthermore, a screening test with a low PPV/high false positive rate has the potential to cause unnecessary harm to blood donors because of fears and wrong information.

The use of syphilis RDTs in resource-poor settings

Pre-donation screening for TTIs with syphilis RDTs is a strategy that has been proposed for use in resource-poor, high-prevalence settings without access to a stable pool of low-risk donors (Salawu & Murainah, 2006). One of the reasons behind this was to reduce blood bag wastage and associated costs of consumables in collecting blood from donors, which was not used because of positive screening tests. One study in Ghana demonstrated savings of more than $11,000 in blood bags and testing costs over a 1-year period using pre-donation screening (Owusu-Ofori *et al.*, 2005a,2005b).

Table 3. Multiple variable logistic prediction of syphilis reactivity

RDT + 526/16016 (3·28%)	Odds ratio	*P*-values	95% CI
Age (years)	1·04	<0·001	1·03 – 1·05
Gender (male)	2·99	<0·001	2·11 – 4·04
Donor type (FRD)	1·92	<0·001	1·60 – 2·30
RDT+, Vitros®+ 478/16016 (2·98%)			
Age (years)	1·05	<0·001	1·04 – 1·06
Gender (male)	3·41	<0·001	2·29 – 5·08
Donor type (FRD)	1·75	<0·001	1·44 – 2·14
RDT+, RPR+ 172/16016 (1·07%)			
Age (years)	1·03	<0·001	1·02 – 1·05
Gender (male)	3·22	<0·001	1·67 – 6·22
Donor type (FRD)	2·04	<0·001	1·45 – 2·88
RDT+, RPR+, Vitros®+ 167/16016 (1·04%)			
Age (years)	1·03	<0·001	1·02 – 1·05
Gender (male)	2·72	<0·001	1·49 – 4·99
Donor type (FRD)	2·06	<0·001	1·46 – 2·89

Syphilis prevalence in Kumasi blood donors

In this study, we found the prevalence of syphilis in Kumasi blood donor population with the use of RDT to be 3·7%, which is not different from previous studies in Ghana (Adjei *et al.*, 2003, 2006; Owusu-Ofori *et al.*, 2011). Like other studies, we found a higher rate of syphilis reactivity among FRD than among VNRBD. This was only partly explained through higher age and more males among FRD as FRD status was an independent positive predictor of syphilis reactivity in a logistic regression analysis. There is an ongoing struggle to have 100% VNRBD in Ghana and elsewhere in Africa, which, if successful, may reduce syphilis sero-reactivity. Despite this, family donations remain dominant on the African continent. The association between age and syphilis reactivity is most likely caused by a longer period of sexual exposure. However, a cohort phenomenon with older donors being more exposed to yaws in childhood may also contribute. However, the data confirm that younger first-time donors are safer than older donors, whereas the highest safety both with regards to infection risk and blood supply lies in a system of repeat donations as the main source of blood for transfusion (Allain *et al.*, 2010).

Syphilis sero-reactivity and active syphilis in blood donors

Our data suggest that a total of 167 or 1% of tested blood donors were confirmed syphilis RDT and RPR reactive. Our logistic regression data additionally indicate that there are independent effects of age, male gender and FRD donor type on syphilis sero-reactivity. When considering RPR reactivity, the effect of age was smaller, indicating that higher age is a stronger prediction of previous syphilis infections than of recent infections. In our previous published article (Sarkodie *et al.*, 2016a,2016b), the decrease in reactive samples from RDT to RPR is approximately six times compared to this study, which is only approximately three times. This considerable discrepancy is probably due to changes in test kits. The RPR test kit used in the previous article (IMMUTREP RPR, Omega Diagnostics – Scotland, UK) differs in sensitivity and specificity from the one used in this study (BD Macro-VueTM Card test). Additionally, testing errors on the part of the laboratory scientists in both testing procedures could contribute to the discrepancy. As a lot of blood is transfused without storage, this may constitute a significant risk of syphilis transmission through transfusion as previously reported syphilis (Owusu-Ofori *et al.*, 2011). Our data thus support the combined use of RDT and RPR to detect active syphilis of potential blood donors, which would enable more focused deferral of potential active syphilis cases for treatment. These cases of suspected active syphilis can be identified with a minimal loss of donors. It is important to repeat this study in other resource-poor settings where syphilis prevalence is high.

Strengths and limitations

The strength of this study is the description of a real-life performance with regards to the PPV of a newly suggested combined syphilis testing algorithm, combining an RDT and RPR for the identification of potential active syphilis. The algorithm used for gold standard confirmation was robust as it involved three different Treponema-specific tests used sequentially to confirm weak and negative results. The INNO-LIA assay has been shown to provide highly reliable confirmatory diagnostic information of anti-TP antibodies (Ebel *et al.*, 2000) and was furthermore used as a quality control for the confirmation algorithm of anti-TP antibodies.

Three major limitations need to be mentioned. Firstly, the assumption that RDT positive donors testing negative in RPR were without significant risk for transfusion was neither tested by recipient look back or by molecular testing of donors. Secondly, the proportion of truly syphilis-reactive donors missed by the initial RDT was not evaluated because of resource constraints.

Finally, we cannot assume infectivity among all confirmed RPR reactive donors.

CONCLUSION

In a blood bank system like the one in Kumasi, Ghana, with a relatively high prevalence of syphilis and where infrastructure to support formal laboratory testing is often lacking, syphilis screening with RDTs may provide a reasonable technology. The combination of both RDT and RPR reduces the loss of donors and blood for transfusion. The combined RDT and RPR testing has a satisfactory high PPV, meaning that unneeded loss of blood for transfusion and false syphilis diagnoses of donors are minimised. The high PPV of a combined RDT and RPR algorithm suggests that further routine confirmation of a donor deferred with dual RDT and RPR reactivity is not needed. This adds to the robustness and cost efficiency of the suggested TP screening algorithm.

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Francis Sarkodie designed the study, performed the research, analysed the data and wrote the manuscript. Oliver Hassall contributed to the design, data analysis and manuscript writing. Ellis Owusu-Dabo contributed to the design, data analysis and manuscript writing. Shirley Owusu-Ofori contributed to the design and manuscript writing. Imelda Bates contributed to the design, data analysis and manuscript writing. Ib C. Bygbjerg contributed to the design, data analysis and manuscript writing. Alex Owusu-Ofori contributed to the design and manuscript writing. Lene Holm Harritshøj contributed to the confirmatory testing, analysis and manuscript writing. Henrik Ullum contributed to the design, confirmatory testing, analysis and manuscript writing.

CONFLICT OF INTEREST

The authors have no competing interests.

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1.4

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Short Communication

Recall of symptoms and treatment of syphilis and yaws by healthy blood donors screening positive for syphilis in Kumasi, Ghana

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SUMMARY

Objective: To describe the recalled medical history, clinical manifestations, and treatment of yaws and syphilis by syphilis seroreactive blood donors in Kumasi, Ghana.
Methods: Of the blood donors at Komfo Anokye Teaching Hospital, Kumasi, Ghana tested with the syphilis rapid diagnostic test (RDT) and later by rapid plasma reagin (RPR) test, 526 were seroreactive. Four hundred and seventy-one (89.5%) of these subjects were confirmed with the Ortho-Vitros Syphilis TP test as the gold standard and were interviewed to determine past or present clinical manifestations of yaws and syphilis.
Results: Of the 471 respondent donors, 28 (5.9%) gave a history of skin lesions and sores; four (14.3%) of these subjects, who were all male and RPR-positive, recalled a diagnosis of syphilis. All four reported having had skin lesions/bumps with slow-healing sores, but only one of them had had these symptoms before the age of 15 years.
Conclusions: A small proportion of confirmed seroreactive donors in this sample had any recall of symptoms or treatment for yaws or syphilis. These data suggest that clinical questioning adds little further information to the current screening algorithm. The relative contribution of yaws and syphilis to frequent positive tests in endemic areas remains speculative.

1. Introduction

Yaws is a neglected non-venereal endemic treponematosis caused by the bacterium *Treponema pallidum* subspecies *pertenue*.[1] It is spread by direct skin-to-skin contact and predominantly affects children less than 15 years of age living in poor socio-economic conditions in certain rural, wet, and tropical areas.[2] In Ghana, a total of 28 000 cases were reported in 2008 and 25 000 in 2010. In 2012, the World Health Organization (WHO) launched a new initiative to eradicate yaws globally by 2020 using the Morges strategy.[3] The clinical manifestations of yaws include multiple papillomas, non-tender ulcers, sores, plantar hyperkeratosis, and pigmentation of the palms and soles, followed by gummata in the last stage.[1]

Syphilis is a sexually transmitted disease caused by *Treponema pallidum* subspecies *pallidum*. It can also be transmitted via blood transfusion, although the actual risk is low.[4] Syphilis starts with a primary lesion (chancre – usually on the genitals), followed by a polymorphic rash and lymphadenopathy. This is followed by the occurrence of a generalized condition with parenchymal, systemic, and mucocutaneous manifestations.[5] The end result may include dementia, gummata, blindness, paralysis, or death.

Usually yaws and syphilis can only be distinguished by epidemiological characteristics and clinical manifestations, as the commonly used antibody tests cannot discriminate one disease from the other.[6]

This paper reports on the recalled history of clinical manifestations of yaws and syphilis by syphilis seroreactive blood donors in Kumasi, Ghana.

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2. Methods

Of the total of 16 016 blood donors tested with a treponemal Fortress rapid test (Fortress Diagnostics Ltd, Antrim, UK) for *T. pallidum* in serum or plasma antibodies (IgG and IgM), 526 (3.3%) were seroreactive for syphilis. These subjects were further tested with the rapid plasma reagin test (RPR) (BD Macro-Vue Card test; BD, Franklin Lakes, New Jersey, USA) to detect potential active infections. Out of these, 478 cases were confirmed with the Ortho-Vitros Syphilis TP test as the gold standard. Four hundred and seventy-one of these confirmed syphilis seroreactive blood donors were interviewed to determine past or present clinical manifestations of yaws and syphilis (response rate of 98.5%) (Figure 1). Subjects were interviewed by a laboratory scientist using a semi-structured questionnaire in the local dialect for the presence or absence of current or previous sores or skin ulcers, and skin lesions/bumps on the face, hands, feet, or genitals. They were also asked about slow-healing sores and at what age they had experienced symptoms. Furthermore, they were asked about any treatment given at the time of these symptoms.

Data were recorded on an Excel spreadsheet and exported into Stata version 12.0 software (StataCorp, TX, USA) for analysis. Approval for this study was obtained from the ethics committees of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, and the Liverpool School of Tropical Medicine, Liverpool, UK.

3. Results

The age of confirmed syphilis seroreactive subjects ranged from 17 to 53 years (mean age 31 years, standard deviation 8.6 years). There were fewer females (29/471; 6.2%) than males (442/471; 93.8%). Of the 471 respondents, 28 (5.9%) gave a history of skin lesions and sores (Figure 1). Four (14.3%) individuals out of the 28 donors with a history of skin lesions and sores – all male and RPR-positive – recalled a diagnosis of syphilis. These four donors had previously received penicillin treatment during their exposure to syphilis. Additionally, the four donors with a recall of syphilis diagnosis reported the appearance of lesions/bumps on the skin and slow-healing sores, but only one of them had had these symptoms before the age of 15 years. It could not be clarified whether this donor had had yaws or syphilis at this young age, although he had been treated.

4. Discussion

The data presented here suggest that a clinical history of yaws is not frequent among syphilis-positive blood donors. However, syphilis symptoms were also not reported frequently. Children aged below 15 years are the most vulnerable to yaws infection.[7] Only a small proportion of confirmed seroreactive donors had any recall of symptoms or treatment of yaws or syphilis. Thus the relative contribution of yaws and syphilis to frequent positive tests in endemic areas remains speculative. The present authors have previously suggested combined specific and non-specific syphilis testing to identify potential infectious donors.[8] The present data suggest that clinical questioning adds little further information to this screening algorithm. As a limitation, donors were interviewed after knowing that they had a positive test for syphilis. This represents a risk of recall bias, with reporting being influenced by the test results. There is furthermore a risk of misclassification bias, as many differential diagnoses exist for both syphilis and yaws.

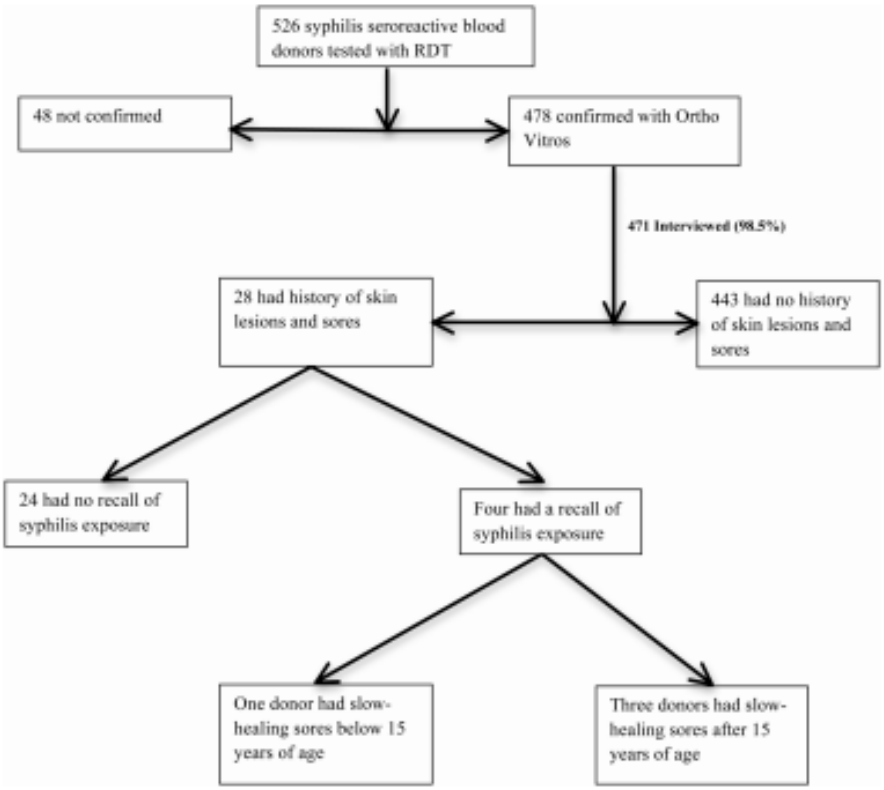

Figure 1. Flowchart of syphilis seroreactive blood donors interviewed for clinical manifestations of yaws.

However, despite these limitations, the conclusion that clinical questioning adds little further information when investigating syphilis seropositive blood donors in areas where both treponematoses exist seems solid.

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Conflict of interest: None of the authors declare any conflict of interest regarding this article.

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2. Abstracts and Posters

2.1

Abstract accepted at the 23rd Regional Congress of the ISBT, held in Amsterdam, The Netherlands, from June 2 – 5, 2013.for ISBT 2013

SYPHILIS TESTING AND ITS CROSS REACTIONS IN GHANAIAN BLOOD DONORS

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Background Information

Syphilis is a major public health problem worldwide but the prevalence of syphilis in Ghanaian blood donors vary. The continuing occurrence of syphilis illustrates that our control efforts still need to be improved. Published studies from Ghana show syphilis seroprevalence of between 7.5% and 13.5%. However, Accra Area Blood Centre, Korle Bu in Ghana has a prevalence rate of 1.23%, 2.89% and 4.86% among blood donors according to the 2009, 2010 and 2011 performance review respectively. Not all transfusion centers in Ghana are testing for syphilis in donated blood though it is mandatory. WHO recommends *Treponema pallidum* haemagglutination assay (TPHA) and Enzyme immuno assay (EIA) as screening tests that are specific serological markers of Treponema pallidum infections. However, majority of the centers use rapid tests for syphilis testing with higher risk of cross reactivity. Furthermore, all syphilis tests also detect antibodies against Treponema pallidum ssp. pertenue causing yaws. Syphilis and yaws can only be distinguished by epidemiological characteristics, clinical manifestations and several genetic signatures of the corresponding causative agents.

Objective: The objective of this study is to determine the prevalence of syphilis, its cross reactions and the potential infectivity of the true positives in the Ghanaian blood donor population.

Methodology: The study has been designed as a multicenter cross-sectional study which will involve a survey of the used methodologies and testing of their actual performance.

1. Survey data on syphilis testing will be collected from all identified testing sites in the country by telephone interviews using semi-structured questionnaires.
2. Within a period of 6 months, 5ml of serum shall be collected from 500 reactive blood donors tested with TPHA at Komfo Anokye Teaching Hospital, Kumasi. Of these reactive samples, hundred shall be aliquoted and sent to four different testing sites to be blindly tested by different personnel with TPHA.
3. After the collection of the 500 initial reactive blood donors tested with TPHA, Abbott Architect syphilis TP assay which is chemiluminescent microparticle immunoassay(CMIA) for the qualitative detection of antibody to Treponema pallidum in human serum and plasma together with RPR testing will be used to identify true Treponema pallidum Ab. positivity and active infections respectively.
4. Truly infected blood donors with Treponema pallidum Ab. shall be recalled for interview to identify histories of clinical manifestations of syphilis and yaws. This information will be useful in distinguishing yaws from Syphilis.

Preliminary Data/Results

A total of 22 syphilis initially reactive blood samples tested with *Treponema pallidum* Heamagglutination Assay (TPHA) in Accra Area Blood Centre (AABC), Ghana were sent to Copenhagen University Hospital, Rigshospitalet Copenhagen, Denmark for further testing. After further testing with Architect TP, Vitros TPA, Inno-Lia™ and RPR Card test, the results showed that 82% (18 out of 22) were true positives and 23% (5 out of 22) were reactive with RPR, signifying potentially active infectivity. (A table result is below). In conclusion, TPHA should be continued because of its good reactivity as the first line, and possibly repeated with RPR to reduce transmission of syphilis via blood transfusion.

Expected Results

It is expected that at the end of the study, the National prevalence of syphilis in the Ghanaian blood donors shall be known. The costs and performance of different testing regimes, the rate of false positives, the rate of active infections and the contribution of yaws would be further identified.

Table.1

EVALUATION OF SYPHILIS REAGENTS WITH SAMPLES FROM ACCRA AREA BLOOD CENTRE (AABC) - GHANA

ASSAY TYPE	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22
TPHA Treponemal	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
ARCHITECT TP Treponemal	√ 1.1	√ 11.6	√ 3.45	√ 22.7	√ 9.9	√ 1.7	√ 5.2	√ 28.6	√ 10.0	√ 1.5	√ 37.0	√ 17.1	**Neg**	√ 1.3	√ 1.4	√ 2.2	√ 31.0	√ 2.2	√	√	√	√
VITROS TPA Treponemal	**Neg**	√	√	√	√	√	√	√	√	**Neg**	√	√	**Neg**	**Neg**	**Neg**	√	√	√	√	√	√	√
INNO-LIA™ Treponemal	IND	√	√	√	√	√	√	√	√	**Neg**	√	√	**Neg**	**Neg**	√	√	√	√	√	√	√	√
RPR Card test Macro-Vue Non - treponemal	**Neg**	**Neg**	**Neg**	**Neg**	**Neg**	**Neg**	**Neg**	√	**Neg**	**Neg**	√	√	**Neg**	**Neg**	√	**Neg**	√	**Neg**	**Neg**	**Neg**	**Neg**	√ **1:16**

NB – Reactive in Anti- treponemal test signifies Active or Previous infection

Reactive in non-treponemal test (RPR) signifies presumptive evidence of current infection, inadequately treated infection, persistent infection or reinfection.

√ - Reactive

2.2

Poster presented at the 23rd Regional Congress of the ISBT, held in Amsterdam, The Netherlands, from June 2 – 5, 2013.for ISBT 2013

SYPHILIS TESTING AND ITS CROSS REACTIONS IN GHANAIAN BLOOD DONORS

T-REC
Building Research Capacity of Blood Transfusion Services in Africa

Francis Sarkodie[1], Ib C. Bygbjerg[2], Jorgen Skov Jensen[3], Oliver Hassall[4], Imelda Bates[5], Alex Owusu-Ofori[1], Justina K. Ansah[5], Shirley Owusu-Ofori[1], Henrik Ullum[2].

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[5]National Blood Service, Ghana.

Background Information
Syphilis is a major public health problem worldwide but not all blood centers or countries test blood donors for syphilis. Similarly, not all transfusion centers in Ghana are testing for syphilis in donated blood though it is mandatory.
Published studies from Ghana show syphilis seroprevalence between 7.5% and 13.5%. In Accra Area Blood Centre, prevalence rate of 1.23%, 2.89% and 4.86% were found among blood donors in 2009, 2010 and 2011 performance reviews respectively. WHO recommends *Treponema pallidum* haemagglutination assay (TPHA) and Enzyme immuno assay (EIA) as screening tests that are specific serological markers *of Treponema pallidum* infections. However, many centers including Ghana use rapid tests for syphilis with higher risk of cross reactivity.

Objective:
To determine the prevalence of syphilis, its cross reactions , and the potential infectivity of 'true' positives in the Ghanaian blood donor population.

Methodology:
Within a period of 6 months, 5ml of serum is being collected from 500 reactive blood donors tested with TPHA at Komfo Anokye Teaching Hospital, Kumasi. All reactive blood donors are further being tested with TPHA, Abbott Architect syphilis TP assay, a chemiluminescent microparticle immunoassay(CMIA) for the qualitative detection of antibody to *T. pallidum* in human serum and plasma, together with RPR testing to identify true *T. pallidum* Ab. Positivity and active infections respectively.

Preliminary Data/Results:
A total of 22 syphilis initially reactive blood samples tested with *T. pallidum* (TPHA) in Accra Area Blood Centre, Ghana were sent to Copenhagen University Hospital, Rigshospitalet Denmark for further testing with Architect TP, Vitros TPA, Inno-Lia™ and RPR Card test. The results showed that 82% (18 out of 22) were true (Inno-Lia™) positives and 27% (5 out of 22) were reactive with RPR, signifying potentially active infectivity.

Fig.1 Syphilis Reactivity with various test kits

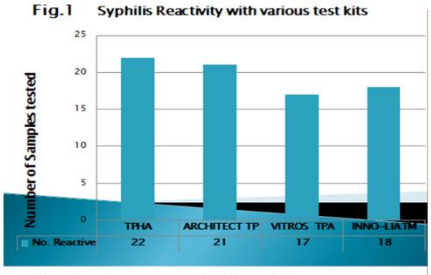
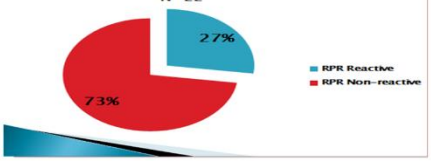


Fig. 2 Samples tested with Rapid Plasma Reagin (RPR) N=22



Conclusion:
The preliminary results show good accordance with TPHA testing and automated T. *pallidum* antibody testing.
A substantial proportion were RPR positive indicating potential active infections of importance to transfusion safety.

www.t-rec.eu

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 266194

2.3

Abstract accepted for Oral Presentation at the 7th AfSBT congress held in collaboration with Zimbabwe Medical Association (ZiMA) and Zimbabwe Quality Assurance Programme (ZINQA) for 30 July – 02 August 2014, Victoria Falls, Zimbabwe.

A NEW SCREENING STRATEGY FOR SYPHILIS AT KOMFO ANOKYE TEACHING HOSPITAL (KATH) IN KUMASI, GHANA

Francis Sarkodie[1, 2], Oliver Hassall[2], Ellis Owusu-Dabo[3], Shirley Owusu-Ofori[1], Imelda Bates[2], Ib C. Bygbjerg[4], Henrik Ullum[4,5].

[1] Komfo Anokye Teaching Hospital, Kumasi, Ghana [2]Liverpool School of Tropical Medicine, UK, [3]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana [4]University of Copenhagen, Denmark. [5]Department of Clinical Immunology, Copenhagen University Hospital Denmark

Introduction: Until 2013 the Transfusion Medicine Unit (TMU) at KATH did not screen blood for syphilis before transfusion because of evidence that refrigerated storage of blood prevents transmission. However, recent data showed that 57% of blood transfused at KATH had been stored for less than four days, and a case of transfusion-transmitted syphilis was identified (Owusu-Ofori, 2011). As a consequence, pre-donation screening for syphilis using a treponemal rapid diagnostic test (RDT) was introduced. This demonstrated a syphilis sero-reactivity rate of 7%, resulting in a high donor deferral rate and a serious negative impact on the blood supply. As syphilis sero-reactivity by RDT does not necessarily correspond to infectivity, a new screening algorithm was implemented: prospective blood donors continue to be screened with a treponemal test for past or current infection with RDT at the time of donation; blood is collected irrespective of these results and units screening positive quarantined in a separate fridge; these units are then screened with a second, non-treponemal test (Rapid Plasma Reagin, RPR) for current or recent infection; RPR positive donations are presumed to have a higher infection risk and are therefore discarded; RPR negative donations are released for further processing and transfusion. Donors who were found to have a positive RPR are offered treatment and deferred for 12 months.

Aims and Objective: The objective of this study is to report results of the implemented strategy for syphilis testing.

Methods: Syphilis screening using the RDT (Fortress Quick Test, Fortress Diagnostics-UK) and RPR (IMMUTREP RPR, Omega Diagnostics-Scotland, UK) was done according to the manufacturers' instructions and TMU-KATH standard laboratory procedures. Background data including donor type, age and gender were also recorded as part of standard blood donation practice at TMU-KATH.

Results: From July to September 2013, 4062 blood donations were screened of which 79.6% (3233/4062) were from male donors. Of these, 312 screened positive by RDT giving a sero-reactivity rate of 7.7% (95% CI 0.069 to 0.085). Of these 52 (18.5%) were positive by RPR, presumed potentially infective and the donation was consequently discarded. Thus, 260 units which screened positive by RDT and negative by RPR were released for transfusion.

Discussion and Conclusion: These data suggest that a reverse algorithm applying treponemal RDT as first line testing followed by a non-treponemal (RPR) test as second line may serve as a feasible screening strategy for syphilis. RDTs have several advantages, including user-friendliness, affordability and availability in resource limited settings. As the majority of syphilis RDT positive donors were negative in RPR testing, the suggested strategy may be useful in balancing the risk of transfusion-transmitted syphilis with protection of the blood supply.

3. Ethics Approvals

3.1 Ethics approval from LSTM, UK

LSTM
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Pembroke Place,
Liverpool, L3 5QA, UK
Tel: +44 (0)151 705 3100
Fax: +44 (0)151 705 3370
www.liv.ac.uk/lstm

Francis Sarkodie

Liverpool School of Tropical Medicine

Pembroke Place

Liverpool

L3 5QA

Wednesday, 19 February 2014

Dear Mr Sarkodie,

Re. Research Protocol (13.26RS) Syphilis Testing and its Cross Reactions in Ghanaian Blood Donors

Thank you for your correspondence of the 18/02/2014 providing the necessary in-country ethical approval documents required for this study. The protocol now has formal ethical approval from the Chair of LSTM Research Ethics Committee.

The approval is for a fixed period of three years and will therefore expire on 18/02/2017. The committee may suspend or withdraw ethical approval at any time if appropriate.

Approval is conditional upon:

• Continued adherence to all in-country ethical requirements.

• Notification of all amendments to the protocol for approval before implementation.

• Notification of when the project actually starts.

• Provision of an annual update to the Committee. Failure to do so could result in suspension of the study without further notice.

• Reporting of new information relevant to patient safety to the Committee

• Provision of Data Monitoring Committee reports (if applicable) to the Committee

Failure to comply with these requirements is a breach of the LSTM Research Code of Conduct and will result in withdrawal of approval and may lead to disciplinary action. The Committee would also like to receive copies of the final report once the study is completed.

Please quote your Ethics Reference number with all correspondence.

Yours sincerely

Dr Angela Obasi,

Chair, LSTM Research Ethics Committee

3.2 Ethics approval from CHRPE, SMS/KNUST, Ghana

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL
COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS

Our Ref: CHRPE/AP/423/13 — 15th November, 2013.

Mr. Francis Sarkodie
Transfusion Medicine Unit
Komfo Anokye Teaching Hospital
Post Office Box 1934
KUMASI.

Dear Sir

LETTER OF APPROVAL

Protocol Title *"Syphilis Testing and Cross Reaction for Syphilis in Ghanaian Blood Donors."*

Proposed Site: *Transfusion Medicine Unit- Komfo Anokye Teaching Hospital.*

Sponsor: *European Union.*

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- A notification letter of 15th May, 2013 from the Komfo Anokye Teaching Hospital (study site) indicating approval for the conduct of the study in the Hospital.
- A completed CHRPE Application Form.
- Participant Information Leaflet and Consent Form.
- Research Proposal.
- Questionnaire

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, renewable annually thereafter. The Committee may however, suspend or withdraw ethical approval at anytime if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Thank you Sir, for your application.

Yours faithfully,

Rev. Prof. John Appiah-Poku.
Honorary Secretary
For: CHAIRMAN

Room 7 Block J, School of Medical Sciences, KNUST, University Post Office, Kumasi, Ghana
Phone: +233 3220 63248 Mobile: +233 20 5453785 Email: chrpe.knust.kath@gmail.com / chrpe@knust.edu.gh

3.3 Ethics approval from NBSG

NATIONAL BLOOD TRANSFUSION SERVICE

Republic of Ghana

Post Office Box KB 78
Korle-Bu, Accra

Our Ref: NBTS/T-REC-107

Your Ref:.....

Date: 17TH SEPTEMBER, 2013

TO WHOM IT MAY CONCERN

LETTER OF INTRODUCTION – MR. FRANCIS SARKODIE

This letter serves to introduce to you, Mr. Francis Sarkodie, a Deputy Chief Biomedical Scientist at the Transfusion Medicine Unit (TMU) of the Komfo Anokye Teaching Hospital.

Mr. Francis Sarkodie is also an off-site PhD student with the Liverpool School of Tropical Medicine in the United Kingdom under the European Commission sponsored T-REC Project. The T-REC project aims to build research capacity in Blood Transfusion in Africa by coordinating and supporting training for a critical mass of researchers. In Ghana, the T-REC is working in collaboration with the National Blood Service, Ghana.

His PhD work is on Syphilis testing and their cross reactions in Ghanaian blood donors, and as part of his research work, he is required to collect data on syphilis testing across the country's Blood Centres and Hospital Blood Banks.

I would be very grateful if you kindly offer him the needed assistance with regards to data collection in your outfit.

Thank you in advance while counting on your kind co-operation.

Yours faithfully

DR. JUSTINA KORDAI ANSAH
THE DIRECTOR, NATIONAL BLOOD SERVICE
PRINCIPAL INVESTIGATOR (TI), T-REC GHANA.

Tel: 0302 - 666429 | 681281 - 2 | 676443 | **Fax:** 0302 - 678039

4. Data Collection Tools

4.1 Semi-structured questionnaire

QUESTIONNAIRE FOR BLOOD DONATION TESTING SITES

TOPIC: A survey of syphilis testing in transfusion facilities in Ghana.

N/B Please tick the box where applicable and fill in the space where you need to fill.

	Name of Testing Site			
	Date			
			Yes	No
1.	Is blood testing for transfusion transmissible infections done on all blood donations at this site before transfusion?		☐	☐
	- If no, please give approximate percentages of tested donations			%
2.	What types of blood testing are done routinely before blood is transfused?		Anti-HIV Ab	☐
			HBsAg	☐
			Anti-HCV	☐
			Anti-Syphilis	☐
			HIV Ag+Ab	☐
	- If syphilis is not included, indicate reason:(You can tick more than one)			
		- Syphilis testing is not mandatory according to WHO:		☐
		- Syphilis testing is not necessary because of low risk of infection from blood products:		☐
		- Lack of funds:		☐
		- Other:		
			Yes	No
3.	Has testing for syphilis ever been done at your site before blood transfusion?		☐	☐
	- If yes, why was the testing for syphilis stopped? (You can tick more than one)			
		- Syphilis testing is not mandatory according to WHO:		☐
		- Syphilis testing is not necessary because of low risk of infection from blood products:		☐
		- Lack of funds:		☐
		- Other:		

4.	How long have you been testing for syphilis in terms of years?					Years
				Yes		No
5.	Do you consider syphilis testing mandatory?			☐		☐
	- If no, why is the site performing the test?					
6.	What is the blood donor type at your site? - please give approximate percentages					
		Type:		Percentages:		
		Voluntary	☐		%	
		Replacement	☐		%	
		Walk - in	☐		%	
		Other	☐		%	

7.	In the years 2010 to 2012:	2010	2011	2012
	- How many blood donations were tested for syphilis?			
	- How many were syphilis reactive?			
	- How many voluntary blood donations were tested for syphilis?			
	- How many voluntary blood donations was syphilis reactive?			
	- How many family replacement blood donations were tested for syphilis?			
	- How many family replacement blood donations was syphilis reactive?			
	- How many walk-in blood donations were tested for syphilis?			
	- How many walk-in blood donations was syphilis reactive?			
	- What type(s) of trade name for HIV testing did your site use?			
		2010	2011	2012

	- What type(s) of trade name for HCV testing did your site use?				
	- What type(s) of trade name for HBV testing did your site use?				

		Yes	No
8.	Have you had co-infection of syphilis with other viral markers between 2010 and 2012?	☐	☐

			2010	2011	2012
	- If yes, indicate the total positive numbers:	Syphilis and HIV:			
		Syphilis and HBV:			
		Syphilis and HCV:			
		Syphilis, HBV and HIV:			
		Syphilis, HCV and HIV:			
		Syphilis, HCV and HBV:			
		Syphilis, HCV, HBV and HIV:			

9.	What type of reagent is used in your site for initial syphilis testing and who are the manufacturers?	☐	VDRL:	
		☐	RPR:	
		☐	TPHA:	
		☐	TPPA:	
		☐	Others:	

		Singles	Duplicates
10.	Do you test for syphilis in singles or in duplicates?	☐	☐

		Yes	No
11.	Do you repeat all positive results in your testing site?	☐	☐

	- If yes what reagent is used in the repeat testing and who are the manufactures?		☐	VDRL:	
			☐	RPR:	
			☐	TPHA:	
			☐	TPPA:	
			☐	Others:	

		Yes	No
12.	Is there any algorithm governing testing in your site i.e. type of test, singles or duplicate, repeat etc?	☐	☐
	- If yes, what is the algorithm?		

13.	What is the reagent cost per test for the various syphilis reagents you have been using?			VDRL:	
				RPR:	
				TPHA:	
				TPPA:	
				Others:	

14.	What is the average resources used to perform syphilis testing per day?	
	Number of employees perform syphilis testing per day:	
	Number of hours used on syphilis testing per day:	
15.	How is the reagent purchased?	Hospital management ☐

		Donation from NBTS Headquarters	☐
		Donations from external institutions	☐
		Others (specify):	
16.	How many test strips/reagents does the facility/site purchase for syphilis testing in a year?		
		Yes	No
17.	Did your site validate these test strips?	☐	☐
	- If yes, how many test strips are used in the validation exercise?		
		Yes	No
18.	After the testing do you invite donors whose test results are reactive to come back?	☐	☐
	- If yes, what percentage of such donors comes back for management?		
		Yes	No
19.	For syphilis reactive blood donors, does your site refer them to a clinician/clinical service?	☐	☐
	- If yes, please state to whom / where:		
	- Do you get any feedback from the clinician/clinical service to know whether it is true syphilis?	Yes ☐	No ☐
		Yes	No
20.	For donors who reacted to syphilis, would you consider other potentially causes of reactivity?	☐	☐
	- If yes, please state what other possible causes you consider:		

THANK YOU VERY MUCH

4.2 Interview guide/checklist for yaws infection

CHECKLIST FOR YAWS INFECTION

Please notice, that the information given here will be kept strictly confidentially, and not be available for others!

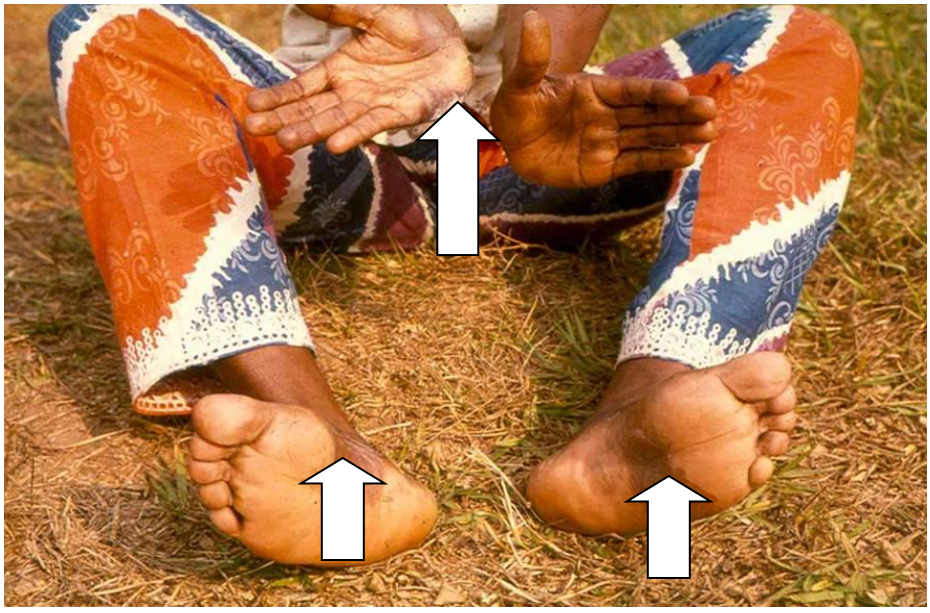
1. Have you ever been exposed to yaws or syphilis before? ? Yes ☐ No ☐
2. Did you receive any sort of treatment? ? Yes ☐ No ☐
3. What drugs were given to you?
4. Have you ever had sores or skin ulcer before? Yes ☐ No ☐
5. Have you ever had lesions that appear as bumps on the skin of the face, hands, feet, and genital area before? Yes ☐ No ☐

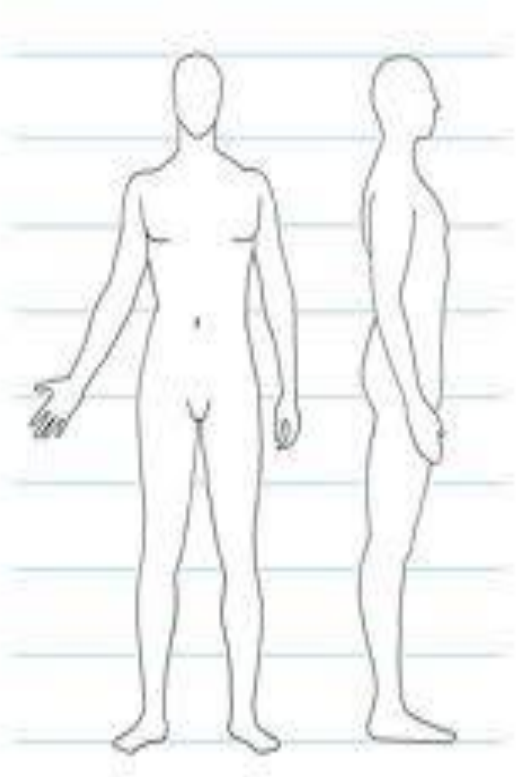
If you have ever had sores, crusts or ulcers on the skin or on mucous membranes, please:

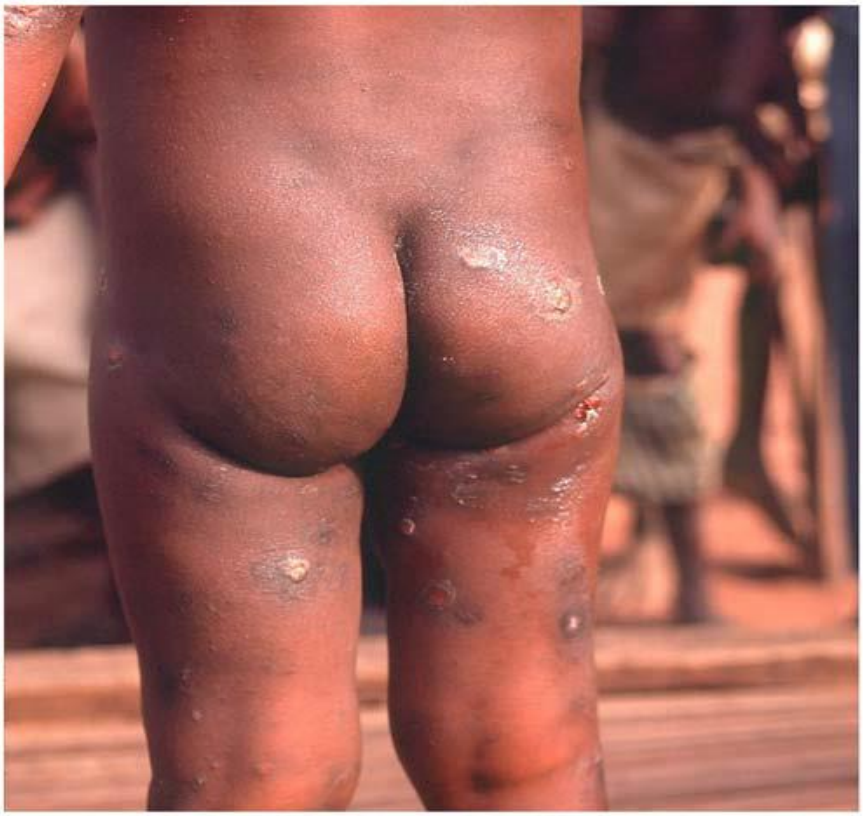
Tick at the age in the box, when it began:

Before the age of 15 years	
After the age of 15 years	

Have you ever had slow healing sores, crusts or ulcers on the skin like the ones on the photo below? or on mucous membranes (mouth, genitals)?. If yes, please draw on the figure below.







Thank you very much for your cooperation!

5. Consent forms

5.1 Informed consent 1

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

Title of Research;

Investigations of syphilis testing and of test cross reactions in Ghanaian blood donors

Name of researcher:

This study is being conducted by Mr Francis Sarkodie of the Komfo Anokye Teaching Hospital as part of requirement for an award of PhD in Public Health in Liverpool School of Tropical Medicine - UK

Background;

This study determines various syphilis test regiments and their performance across Ghana. It further describes the true prevalence of syphilis antibodies and their cross reactions in Komfo Anokye Teaching Hospital (KATH) blood donor population and intends to develop an appropriate algorithm for syphilis testing in donated blood. It is expected that at the end of the study, the costs and performance of different testing regimes, the rate of confirmed and false positives, the estimated rate of active infections and the contribution of yaws would be further known. The findings of this project will serve as a basis for further research in blood transfusion, and ultimately be used to improve and sustain the blood transfusion safety and services in Ghana and elsewhere.

Purpose(s) of research:

The purpose of this research is to determine the prevalence of syphilis, its cross reactions in Ghanaian blood donor population and the potential infectivity of the confirmed positives.

Consent:

Our screening indicates that you may have had syphilis. We ask for your consent to a research project where we will draw an extra blood sample of 5 ml. With this we will determine if you truly have had syphilis. We will also determine if there is a risk of active infection. If our tests indicate that you have active infection, we will refer you to a medical doctor in-charge of transfusion transmissible infections for further investigation and treatment.

Risk(s):

There will be no risk to the person to be interviewed as well as the participants because the research assistants have been trained for the interviews and blood collection.

Benefit(s):

The goal of this study is to develop an appropriate algorithm for syphilis testing in donated blood and the findings of this project will serve as a basis for further research in blood transfusion, and ultimately be used to improve and sustain the blood transfusion safety and services in Ghana and elsewhere. The participants would be referred for clinical advice.

Confidentiality:

A coding system shall be introduced as part of quality assurance and control. The participants shall be systematically coded to prevent mixing of samples and results, and these shall be used as processing numbers. No name or identifier will be used in any publication or reports from this study. However, as part of our responsibility to conduct this research properly, we may allow officials from the National Blood Service and ethics committees to have access to your records.

Voluntariness:

Taking part in this study should be out of your own free will. You are not under any obligation to participate. Research is entirely voluntary.

Alternatives to participation:

If you choose not to participate, this will not affect the blood donor clinical services that shall be offered to you in this hospital in any way.

Withdrawal from the research:

You may choose to withdraw from the research at any time without having to explain yourself. You may also choose not to answer any question you find uncomfortable or private.

Consequence of Withdrawal:

There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study. Please note however, that some of the information that may have been obtained from you without identifiers (name etc), before you chose to withdraw, may have been modified or used in analysis reports and publications. These cannot be removed anymore. We do promise to make good faith effort to comply with your wishes as much as practicable.

Costs/Compensation:

There shall be no cost or compensation for the participants. However, any Information that shall be received shall be conveyed to the participants.

Contacts:

If you have any question concerning this study, please do not hesitate to contact Mr Francis Sarkodie (Name of Researcher or PI) on 0244371770.

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Chairman

Committee on Human Research and Publication Ethics

Kumasi

Tel: 22301-4 ext. 1098 or 020 5453785

CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information, including that about risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

Name_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

5.2 Informed consent 2

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

Title of Research;

Investigations of syphilis testing and of test cross reactions in Ghanaian blood donors

Name of researcher:

This study is being conducted by Mr Francis Sarkodie of the Komfo Anokye Teaching Hospital as part of requirement for an award of PhD in Public Health in Liverpool School of Tropical Medicine - UK

Background;

This study determines the relative proportions of those with clinical histories attributable to yaws rather than syphilis in confirmed syphilis antibody positive donors.

It is expected that at the end of the study, the rate of confirmed and false positives, estimated rate of active infections and the contribution of yaws would be known. The findings of this project will serve as a basis for further research in blood transfusion, and ultimately be used to improve and sustain the blood transfusion safety and services in Ghana and elsewhere.

Purpose(s) of research:

The purpose of this research is to determine the prevalence of syphilis, test cross reactions in Ghanaian blood donor population and the potential infectivity of the confirmed positives.

Consent:

Our screening indicates that you have a suspected active syphilis. We ask for your consent to be interviewed. With this we will determine if you truly have had syphilis rather than yaws. We will refer you to a medical doctor in-charge of transfusion transmissible infections for further investigation and treatment after this interview.

Risk(s):

There will be no risk to the person to be interviewed as well as the participants because the research assistants have been trained for the interviews and blood collection.

Benefit(s):

The participants would be referred for clinical advice for treatment.

Confidentiality:

A coding system shall be introduced as part of quality assurance and control. The participants shall be systematically coded to prevent mixing of results, and these shall be used as processing

numbers. No name or identifier will be used in any publication or reports from this study. However, as part of our responsibility to conduct this research properly, we may allow officials from the National Blood Service and ethics committees to have access to your records.

Voluntariness:

Taking part in this study should be out of your own free will. You are not under any obligation to participate. Research is entirely voluntary.

Alternatives to participation:

If you choose not to participate, this will not affect the blood donor clinical services that shall be offered to you in this hospital in any way.

Withdrawal from the research:

You may choose to withdraw from the research at any time without having to explain yourself. You may also choose not to answer any question you find uncomfortable or private.

Consequence of Withdrawal:

There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study. Please note however, that some of the information that may have been obtained from you without identifiers (name etc), before you chose to withdraw, may have been modified or used in analysis reports and publications. These cannot be removed anymore. We do promise to make good faith effort to comply with your wishes as much as practicable.

Costs/Compensation:

There shall be no cost or compensation for the participants. However, any Information that shall be received shall be conveyed to the participants.

Contacts:

If you have any question concerning this study, please do not hesitate to contact Mr Francis Sarkodie (Name of Researcher or PI) on 0244371770.

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Chairman

Committee on Human Research and Publication Ethics

Kumasi

Tel: 22301-4 ext. 1098 or 020 5453785

CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information, including that about risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

Name_____

DATE: _____ SIGNATURE/THUMB PRINT: _____