SEROPREVALENCE OF TOXOPLASMA GONDII IN HEALTHY BLOOD DONORS AT EASTERN ZONE BLOOD TRANSFUSION SERVICES (EZBTS) IN DAR ES SALAAM, TANZANIA 2011

Lema, Furaha

MSc (Biochemistry) Dissertation

Muhimbili University of Health and Allied Sciences

November, 2012

SEROPREVALENCE OF TOXOPLASMA GONDII IN HEALTHY BLOOD DONORS AT EASTERN ZONE BLOOD TRANSFUSION SERVICES (EZBTS) IN DAR ES SALAAM, TANZANIA 2011

By

Lema, Furaha

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Biochemistry of the Muhimbili University of Health and Allied Sciences

Muhimbili University of Health and Allied Sciences

November, 2012

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a thesis/dissertation entitled, **Seroprevalence of *Toxoplasma gondii* in healthy blood donors at Eastern zone blood transfusion services (EZBTS) in Dar es Salaam, Tanzania 2011** in (Partial) fulfilment of the requirements for the degree of Masters of Science in Biochemistry at Muhimbili University of Health and Allied Sciences.

_____

Dr. Teddy Mselle

Supervisor

_____

Date

_____

Dr. Thomas Nyambo

Supervisor

_____

Date

DECLARATION AND COPYRIGHT

I, **Furaha Adony Lema (**Candidate MSc. Biochemistry), declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature…… Date……

This dissertation is a copyright material protected under the Berne Convention, the Copyright Act 1999 and other international and national enactments, in that behalf, on intellectual property. It may not be reproduced by any means, in full or in part, except for short extracts in fair dealing, for research or private study, critical scholarly review or discourse with an acknowledgement, without the written permission of the Directorate of Postgraduate Studies, on behalf of both the author and the Muhimbili University of Health and Allied Sciences.

ACKNOWLEDGEMENTS

I would first like to sincerely express my endlessly thanks to God, who have been always giving me strength, staying still to fulfil this hard task.

I would also like to express my special thanks to my supervisors without whom this work would not be what it is: Dr. T. Mselle and Dr. T. B Nyambo from the very initial stage of proposal development, data analysis, data interpretation and dissertation writing, specifically for your tireless support and invaluable guidance, their constructive comments and advice made this work appears as it is.

I am grateful to Programme Manager of EZBTS, the Eastern zone Manager and the Human resource Manager of the National Blood Transfusion Service in Tanzania for allowing me to conduct the study at EZBTS. Special thanks go to the head of Microbiology/Immunology Department at MUHAS who allowed me to proceed with laboratory work in Microbiology Laboratory.

Honours go to my sponsor the Ministry of Health and Social Welfare for providing me with a study leave and for the financial grant, without which this endeavour would have not been fulfilled.

I take this opportunity as well to acclaim all my teachers within and outside the Biochemistry Department for selflessly imparted to me with knowledge and skills have nothing that can equal offer than ask them to accept my humble thanks.

I would also like to acknowledge my family for their endless support to this venture morally, spiritually, materially, their love and provide me with enough time and courage during the whole process. It is not easy to mention everyone who in one way or another contributed to this work but suffice it to say thank you all.

DEDICATION

This work is dedicated to my family for all the moral support which was accorded to me throughout the period of my studies.

TABLE OF CONTENTS

CERTIFICATION II

DECLARATION AND COPYRIGHT III

ACKNOWLEDGEMENTS IV

DEDICATION V

TABLE OF CONTENTS VI

LIST OF TABLES VIII

LIST OF FIGURES IX

LIST OF ABBREVIATIONS X

ABSTRACT XI

CHAPTER ONE 1

1.1 BACKGROUND 1

1.2 PROBLEM STATEMENT 4

1.3 RESEARCH QUESTIONS 5

1.4 OBJECTIVES 5

1.5 LITERATURE REVIEW 6

1.6 RATIONALE OF THE STUDY 14

CHAPTER TWO 15

2.0 RESEARCH METHODS 15

2.1 Description of the study area 15

2.2 Study design 15

2.3 Sample size estimation 15

2.4 Recruitment plan 16

2.5 Sample collection procedures 17

2.6. Socio-demographic and behavioural data 18

2.7 Ethical consideration 18

2.8 Data management and statistical analysis 18

CHAPTER THREE 19

3.0 RESULTS..... 19

CHAPTER FOUR..... 28

4.0 DISCUSSION..... 28

CHAPTER FIVE 32

5.0 CONCLUSION 32

REFERENCES 33

APPENDICES 38

APPENDIX I: NATIONAL BLOOD TRANSFUSION SERVICE STANDARD FORM..... 38

APPENDIX II: PRIVATE HEALTH LABORATORIES (SAFETY RULES) BOARD 40

APPENDIX III.TOXOPLASMOSIS SEROLOGIC TESTING..... 41

APPENDIX IV: QUALITY CONTROL CERTIFICATE-IGM ASSAY..... 42

APPENDIX V: QUALITY CONTROL CERTIFICATE-IGG ASSAY..... 43

APPENDIX VI: PRINCIPLE OF THE ASSAY 44

LIST OF TABLES

Table 1: Distribution of anti-*toxoplasma* IgG and IgM antibodies.....19

Table 2: Demographic characteristics of the study subjects…….....20

Table 3: Relationship between Gender and *T. gondii* IgG serostatus……..…….....21

Table 4: Relationship between Age and ant- *T. gondii* antibody serostatus….....23

Table 5: Relationship between Occupation (Work status) and *T. gondii* serostatus….....25

Table 6: Distribution of *T. gondii* IgG antibody serostatus by domicile…….....26

LIST OF FIGURES

Figure 1: Life cycle of *T. gondii* .....…….....8

Figure 2: Four major advantages of ELISA…….....……12

Figure 3: Direct and Indirect Detection ELISA Formats……....13

Figure 4: Diagnostic serology findings based on IgG and IgM.....14

Figure 5: Relationship between gender with ant-*T. gondii* IgG antibody serostatus ……22

Figure 6: Relationship between age with ant-*T. gondii* IgG antibody serostatus.....24

Figure 7: Relationship between occupation and *T. gondii* IgG antibody serostatus……..25

Figure 8: Distribution of *T. gondii* antibody by domicile.....27

LIST OF ABBREVIATIONS

AIDS	-	Acquired Immunodeficiency Syndrome
CPL	-	Central Pathology Laboratory
EIA	-	Enzyme Immunosorbent Assay
ELISA	-	Enzyme-Linked Immunosorbent Assay
EZBTS	-	Eastern Zone Blood Transfusion Services
GCLP	-	Good Clinical Laboratory Practice
HB	-	Haemoglobin
HBsAg	-	Hepatitis B Surface antigen
HBV	-	Hepatitis B Virus
HCV	-	Hepatitis C Virus
HIV	-	Human Immunodeficiency Virus
ID	-	Identification Number
IgG	-	Immunoglobulin G
IgM	-	Immunoglobulin M
IRB	-	Institutional Review Board
MNH	-	Muhimbili National Hospital
MPL	-	Multi Purpose Laboratory
MUHAS	-	Muhimbili University of Health and Allied Sciences
NACP	-	National Aids Control Program
NBTS	-	National Blood Transfusion Services
OR	-	Odds Ratio
PCR	-	Polymerase Chain Reaction
r.p.m	-	Revolution Per Minute
SOP	-	Standard Operating Procedure
SPSS	-	Scientific Product for Social Statistics
T. gondii	-	*Toxoplasma gondii*
TTIs	-	Transfusion Transmitted Infections
TPTIs	-	Transmission Parasite Transmitted Infections
VNRBD	-	Voluntary-Non Remunerated Blood Donation.

ABSTRACT

Background: Blood transfusion services are required to provide blood and blood derived components which are safe in a cost effective way for transfusion into patients who require the blood products. Blood donation tends to save lives for many people but posses many challenges particularly when it comes to screening. Some blood donation centres routinely screen only limited number of infectious agents. EZBTS for example screen for HIV, Hepatitis B, Hepatitis C and Syphilis only. However, *T. gondii* infections in blood donors represent a risk of transmission of the pathogens to the recipients. There is scarce information about the prevalence of *T. gondii* in blood donors in Tanzania. A number of studies have shown that the risk for *T. gondii* infection increases with age but the difference was not significant between genders.

Study objective: The aim of this study was to determine the prevalence of *T. gondii* infection among healthy donors in Dar es Salaam and possible implications on mandatory *T. gondii* screening.

Methodology: A cross-sectional study was conducted at the EZBTS located at Ilala District, in Dar es Salaam City. One hundred and sixty donated blood samples were examined for *T .gondii* infection between November 2011 and April 2012. Donated blood was tested for anti-*T. gondii* IgG and IgM antibodies by using ELISA.

Results: Seventy six (76) of 160 donated blood tested positively for IgG anti-*T. gondii* antibodies. None of the blood donors tested positive for IgM anti-*T .gondii* antibodies. In this study 108 (67.5%) male tested positive for IgG anti-*T. gondii* and 52 (32.5%) female were IgG positive. The study found that the percentage IgG positive (seroprevalence) was much higher in male than female although the difference was not significant. (*p=0.112*)
Moreover, we observed that the frequency of seropositivity decreased with advancing age from 45-54 years and above. The highest peak for percentage IgG positive (41.2%) were in the age range of 25 to 34 years. The seroprevalence in the age of 25-34 was influenced by age, since the association was statistically significant *(p= 0.001)*. The Skilled category (non-labourers) had higher percentage IgG seroprevalence (61.2%) while the lowest was unskilled category (labourers) (15.2%). We observed that the association was statistically significant *(p= 0.024)*. Based on area of domicile, Temeke district has shown to have a higher percentage IgG seroprevalence while the lowest was Ilala district. The

seroprevalence was not influenced by area of domicile, since the association was not statistically significant (*p= 0.101*)

Conclusion: This study revealed high prevalence of *T. gondii* among blood donors, indicating potential hazard for blood transfusion and highlighting the importance of screening for *T. gondii* among blood donors. The disease toxoplasmosis should be considered to be included in pre transfusion testing schedule together with other tests i.e HIV, Hepatitis B, hepatitis C and Syphilis.

CHAPTER ONE

1.1 Background

Blood transfusion services are required to provide blood and components which are safe in cost-effective way for transfusion into patients who require the blood products. However, blood transfusion can be a potential source of infection by a variety of transmissible agents such as bacteria, viral and protozoan infections [1]. The most common parasitic organisms implicated in transfusion-transmitted infections are: *Plasmodium* spp, *Trypanosoma cruzi*, *Babesia microti*, *Toxoplasma gondii* and *Leishmania* spp [2]. For parasite to be transmitted by blood transfusion must: circulate in the blood stream of donors, comprise of certain physical characteristics and resist processing steps leading to the preparation of labile blood products (packed red blood cells, therapeutic frozen plasma, or platelet concentrates) and also should survive conservation (parasites must retain infectivity) [3].

Among all the TPTIs, *T. gondii* has recently become the focus of intense research both as a serious pathogen causing public health problem worldwide and as a model member of the phylum Apicomplexa. *T. gondii* has been a good animal model for human disease. Mainly because host crosses are feasible (Cats), can be grown in virtually any warm blooded cell line and can be maintained in indefinetive mice/cell culture. *T. gondii* is also amenable to molecular genetic manipulation and complete genome sequence known have made [4].

Toxoplasmosis is a parasitic disease caused by a single celled protozoan *T. gondii.* It is an obligate intracellular parasite that has been found in many species of birds, reptiles and mammals [5]. The organism can be transmitted through transfusion of blood, organ transplantation, vertical transmission, ingestion of rare or raw meats and contact with contaminated cat faeces [5, 6]. In adults, infection is usually benign or asymptomatic. However, symptomatic cases including fatalities do occur in immunosuppressed patients and pregnant women [7]. Vertical transmission varies according to the time of pregnancy when the mother becomes infected. Maternal infections occurring during the first trimester is less likely to pass to the fetus, however if transmission occurs severe outcomes such as spontaneous abortion, still birth and hydrocephalus are more likely to occur [8]. Infection acquired later in pregnancy where most transmission occur, tends to cause less severe, but

nonetheless serious congenital manifestations including cerebral calcifications and learning disabilities [9].

Based on blood borne disease prevalence, different studies have suggested the need for routine screening different blood borne pathogens such as HIV, HBC, HCV and syphilis [10]. For example, a study conducted in 2006 on seroprevalence of HIV, HBsAg, HCV and syphilis at MNH among blood donors, the results showed 3.8%, 8.8%, 1.5% and 4.7% respectively and these findings suggested for routine screening and strict selection of donors [11]. In another study conducted in 1999 at MNH, Dar es Salaam showed the prevalence of transfusion associated HIV-1, HCV, HBV, HAV and syphilis among blood donors, infections to be 8.7%, 8%, 20%, 11%, 0% and 12.7% respectively [12]. Another study done in Mali looking at the prevalence viral infections among blood donors showed the prevalence of HIV and HBV to be: 4.5% and 14.5% respectively suggesting that routine screening program is required [13].

The prevalence of blood borne pathogens shown in above studies, were not much higher compared to the prevalence of toxoplasmosis among healthy blood donors. Depending on geographical, climatic and socio- economic factors, the prevalence of Toxoplasmosis in blood donors could be as high as 60% [2, 14]. Despite the high prevalence in blood donors, blood transfusion centers in Tanzania, including the EZBTS screen only for HIV, HCV, HBV and syphilis.

Globally, over 6 billion people have been infected with *T. gondii* with an annual incidence ranging from 1% to 5% [2]. In some parts of the world the rate of seroprevalence can be as high as 90% as reported for North America, Western Europe and other Tropical Countries. In the USA the prevalence between 3% and 67% seropositive for antibodies against *T. gondii* has been reported in adults [15]. Other countries being reported at 23.9% Nigeria [16], 46% in Tanzania [17] and 47% in France (rural area) [18].

The prevention toxoplasmosis relies on the knowledge of the disease epidemiology which includes risk factors for the disease. Regular medications for acute toxoplasmosis involve use of pyrimethamine and sulfadiazine in combination with pyrimethamine. Clindamycin

is an antibiotic used most often for people with HIV/AIDS and spiramycin is indicated for prevention of vertical transmission.

There is limited data on the contributory factor by healthy blood donors to the rise of new cases (incidence) of *T. gondii* in Tanzania. In Tanzania routine screening for blood donors has not been done, as there is neither current surveillance study nor *T. gondii* screening program in blood donation. Primarily, this can be due to financial burden to health care and, or lack of awareness of prevalence of Toxoplasmosis in a general population.

1.2 PROBLEM STATEMENT

Some parasitic diseases are known to be transmitted by blood transfusion [2]. Among these is Toxoplasmosis which is caused by *T. gondii* [19]. This infection can remain dormant for a long time, however, when activated it can become clinically overt. Globally, seropositivity of the disease varies widely. It is estimated that between 30% and 65% of all individuals worldwide are infected with toxoplasmosis [2]. However, there are large regional and country variations. The prevalence of the disease in Tanzania is reported to be 46% indicating that disease is of a significant public health concern [17]. Worldwide, the *T. gondii* infection rate has been on the increase with the current estimates of about 50% prevalence. Current annual estimate of *T. gondii* incidence is estimated to be around 1 to 5%. *T. gondii* infection can be transmitted by transfusion of blood from asymptomatic, apparently healthy individuals exposed to infection [20]. The prevalence of infected donors varies ranging from 8% to 60% according to the geographical location, socio economic factors, climatic and population concerned.

In the majority of cases, those who are affected are asymptomatic. Toxoplasmosis can cause very serious health problems particularly among individuals with weakened immune system and pregnant mothers. Maternal infection occurring during the first trimester is less likely to pass to the fetus, however if transmission occurs, severe outcomes such as spontaneous abortion, miscarriage, still birth and hydrocephalus are more likely [21]. Most Infections are acquired later in pregnancy where they tend to cause less severe, but nonetheless serious congenital manifestations including cerebral calcifications and learning disabilities [22].

Studies have shown that acute infection and reactivation of latent infection cause serious complications and even death among immunosuppresed persons [16]. Therefore because of increased numbers of individuals suffering from immunosuppresing diseases including cancer, autoimmune disease, AIDS or transplant recipients, the incidence of Toxoplasmosis is on the increase.

Apparently the seroprevalence of *T. gondii* among blood donors in Tanzania is not known. The current study was therefore carried out to establish the seroprevalence of *T. gondii* in samples obtained from blood donors in Dar es Salaam. This could impose the necessity for screening for *T. gondii* before transfusing blood.

1.3 RESEARCH QUESTIONS

- Is *T. gondii* infection a public health problem in blood donated at EZBTS?
- Does blood transfusion play a significant role in the transmission of the disease?
- What is the relationship between age, sex and occupation of blood donors with *T. gondii* seroprevalence?

1.4 OBJECTIVES

1.4.1 General Objectives

- To establish the extent of *T. gondii* seroprevalence among healthy blood donors at Eastern Zone Blood Transfusion Services.

1.4.2 Specific objectives

- To determine the prevalence of *T. gondii* among healthy blood donors in Dar es Salaam.
- To determine the age, sex and occupation distribution of *T. gondii* among asymptomatic healthy blood donors.
- To determine the prevalence of recent *T. gondii* infection

1.5 LITERATURE REVIEW

1.5.1 Aetiology of Toxoplasmosis

T. gondii is a prevalent obligate intracellular parasite which chronically infects more than a third of the world's population. *T. gondii* belongs to the phylum Apicomplexa, which consists of intracellular parasites having a characteristically polarized cell structure and a complex cytoskeletal and organellar arrangement at their apical end [23]. The *T. gondii* genome consists of three DNA components, located in the nucleus, apicoplast and mitochondrion. This nuclear genome is haploid (estimated to be 87 Mb) for most of the parasite's life cycle, except for a brief diploid phase in the cat intestine before meiosis [4]. This obligate intracellular parasite can infect and replicate within virtually any nucleated mammalian or avian cell. Previously, isolates of *T. gondii* were considered to be highly similar, molecular analysis revealed that there are six major clades from a small number of distinct ancestral lineages. These isolates they display very marked clonality, notably in North America, South America and Europe [24]. The transmission involves three main critical intracellular stages. Acute infection which involve rapidly replicating tachyzoite stage, latent infection which is slower growing and cyst-forming bradyzoite stage and ingesting infectious oocysts from the environment [5].

1.5.2 Pathogenesis of Toxoplasmosis including the routes of transmission in humans

The complex life cycle of *T. gondii* facilitate both clonal and sexual modes of transmission. Many vertebrates serve as intermediated hosts for replication of haploid tissues stages. Sexual development is restricted to the intestine of the epithelium of cats which shed diploid oocyst that undergo meiosis in the environment [25, 26].(fig.1) If first contracted during pregnancy, *T. gondii* may be transmitted vertically by tachyzoites that are passed to the fetus via the placenta. Horizontal transmission of *T. gondii* may involve three life-cycle stages, i.e. tachyzoites, bradyzoites (tissue cyst) and oocysts (sporozoytes).

Following ingestion, the outer wall of cysts or oocysts is disrupted by enzymatic degradation and bradyzoites and sporozoites are liberated into intestinal lumen. Thereafter rapidly invade and multiply within surrounding host cells, tachyzoites are disseminated via blood or lymphatic system to most of the organs of human body. In this fashion, the

hallmarks of the infection develop: cell death and focal necrosis surrounded by an acute inflammatory response.

In briefly, the initial step of the parasite invasion process is recognition and attachment to the target cell. Upon encountering the host cell, the parasite will survey the membrane until an appropriate point of attachment is recognized by apical pole. Host cell laminin, parasite laminin, parasite surface lectins and major parasite surface protein may participate in preliminary attachment of parasite to host cell [27]. Two types of the organelle at the anterior end of the parasite appear to be involved, i.e. rhoptries and micronemes. Rhoptries are large club shaped anterior organelles with a slender duct through which organellar contents (ROP proteins) are discharged at the time of invasion. Micronemes are smaller, vesicular organelles that may discharge their contents immediately preceding or during invasion [28]. Rhoptry protein secretes at the time of invention and is associated with membrane of parasitophorous vacuoles. Following the attachment of parasite to its target cell, it is assumed that an annular junction is formed between parasite and host cell membrane, through which the parasite forcibly penetrates the host cell while apparently pulling the cell membrane around itself [29]. At the end, the parasite is enclosed within a parasitophorous vacuole whose membrane derives from that host cell like that phagocyte vacuoles. Extracellular tachyzoites are highly susceptible to oxygen intermediates changes in pH and osmotic fluctuation and are killed by specific antibody in the presence of complement.

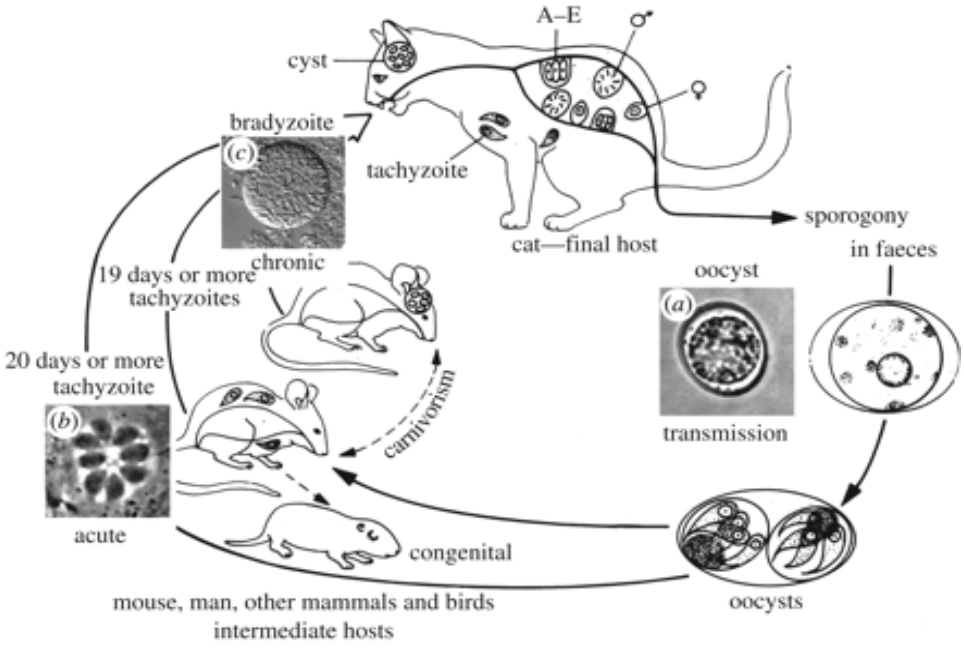


Figure 1: Life cycle of *T. gondii* . A–E refers to stages of development within enterocytes, a-diploid oocyst, b- tachyzoites and c- bradyzoites. Adapted from Frenkel J. K (1973).

1.5.3 Seroprevalence of toxoplasmosis in general and with special reference to blood transfusion

Toxoplasmosis has been found to be influenced by several factors such as geographical location, climate, socio-economic status, nutritional customs and population group at a specific area. The geographical location which facilitates more exposure to disease can be reflected due to agricultural activities and livestock keeping sustaining their life as a source of employment [14]. In climatic characteristics, warm and humid areas have been found to associate with high prevalence compared to dry climate and high altitude. Also for a society belonged to a low socio-economic level, eating roasted meat, popularly known as "*nyama choma"* is not a frequent practice among them compared to high socio-economic level [30]. Different tradition and customs may rely on a certain food than others e.g. Most societies from Northern part of Tanzania they prefer eating raw or undercooked meat as they favourite food, so the exposure to *T. gondii* infection is high.

The population status might influence the spread of Toxoplasmosis where in some parts of the world the rate of seroprevalence can be as high as 90% as reported for North America, Western Europe and other Tropical countries. In USA the prevalence between 3% and 67% seropositive for antibodies against *T. gondii* has been reported in adults [15]. Others countries reports at (47%) [18] in France and 2% up to 63% in Indonesia [31]. In Africa seroprevalence is also varied, e.g some of the reports claimed at (23.9%) Nigeria [16] and (46%) Tanzania [17].

Infection rate has been significantly increasing where about one third of the world population is infected by *T. gondii* with an annual incidence of new cases ranging from 1% to 5%. Due to the increase of incidence of *T. gondii* infection, researchers around the world are focusing on the different factors that contribute to the rise of new cases. The rise of new cases indicates the potential increase of transmission where specifically the prevalence of infected donors varies ranging from 8% to 60% [2]. *T. gondii* infection in blood donors could present a risk for transmission in blood recipients hence provide more chances to the rise of new cases. Despite these risks, neither pregnant women nor blood donors are routinely screened for toxoplasmosis in most countries including Tanzania.

Developed Countries have already started to establish screening for *T. gondii* by healthy blood donors. In Africa few countries have already determined seroprevalence of *T. gondii* infection by donated blood in their blood banks institutions. Countries like Iraq has seroprevalence of (22%) [32], Mexico (7.4%) [33], Thailand (9%) [34], Chile (21.2%) [35], India (20.3%) [36], Malaysia (28.1%) [37] and in Africa, countries like Egypt (59.6 %) [38], kenya (54%) [39] and Mali (21%) [40] seroprevalence by voluntary healthy blood donors.

1.5.4 Risk factors to Toxoplasmosis (Social demographic and behavioural characteristics)

Recent epidemiologic studies have identified the following risk factors for *T. gondii* infection: presence of cats in or around the house, cleaning the cat litter box, eating raw or undercooked meat especially pork, having 3 or more kittens, gardening. Others are through

having contact with soil, eating raw or unwashed vegetables or fruits, having poor hand hygiene and working with meats (butchery) [41]. Although the risk factors for acute infection have been reported in studies from Europe, South America, and the United States, up to 50% of acutely infected people do not have identifiable risk factors or symptoms. Generally, there was significant correlation between seropositivity of *toxoplasma* antibodies and behavioural factors in many studies. Many studies showed a significant increase in toxoplasmosis prevalence with increasing age, exposure and there were no significant difference between genders [42]. In a study conducted by Fromont et al., [18] found that the seroprevalence increased with age where the slope was the steepest up to the age of 40 years but that increase was not significant afterwards. Similar findings have also found in study conducted by Jeffery et al where the risk for *T. gondii* infection increased with age and was higher among persons with a lower educational level, those who lived in crowded conditions, and those who worked in soil-related occupations [15].

In a study conducted in Mexico(Durango City) showed that the age group of 45–60 years showed a significantly higher frequency of *T. gondii* infection than the group of 25–34 years. Blood donors without education had a significantly higher frequency of infection (15.8%) than those with 13–19 years of education). Other characteristics of blood donors including male gender, consumption of undercooked meat or blood transfusion did not show an association with infection in Durango City [33]. Furthermore, a study by Studencova et al., [43] in Slovakia showed that there were no significant differences between genders or between inhabitants of rural and urban areas but a significant increase in toxoplasmosis with increasing age was observed. The study conducted in Tanga region by Swai in 2009 showed *T. gondii* seroprevalence was slightly higher amongst males (46.2%) than females (43.3%) although the difference was not significant. Individuals, ≤20 and ≥60 years old had the highest prevalence of 60% and 61.5%, respectively [30]. The lowest prevalence of (35.7%) was observed amongst the 50-60 years age group. The seroprevalence of *toxoplasma* antibodies was significantly higher amongst individuals who keep livestock (52.2%) and abattoir workers (46.3%).

1.5.5 Methods used to diagnose the parasite in blood

The diagnosis of Toxoplasmosis may be established by serological tests, PCR, histological demonstration of the parasite and/or its antigens (i.e. immunoperoxidase stain), or isolation of the organism (culture). Although different direct identification methods of parasite are available, they are unsuitable for routine use in blood banks. The serologic tests are essential for transfusion of blood components to those of high risk group recipients and this involve analysis of serum samples for ant *T. gondii* antibodies [44].

Serology tests based on ELISA have been found to have wide range of applications, as seen in fig. 2. Other tests like PCR do not have the ability to predict the time of infection although it is helpful in diagnosis of congenital toxoplasmosis. Dye test is more reliable but they consume time and are cumbersome as they require live parasites to be analyzed under microscope [45]. Agglutination assay tend to detect IgM persisting for longer than one year after significant infection has been acquired. Therefore may detect IgM in a significant proportion of women whose fetus are not at risk from *Toxoplasma* infection [46]. Also to demonstrate tachyzoites through histological diagnosis in tissue section is always difficult.

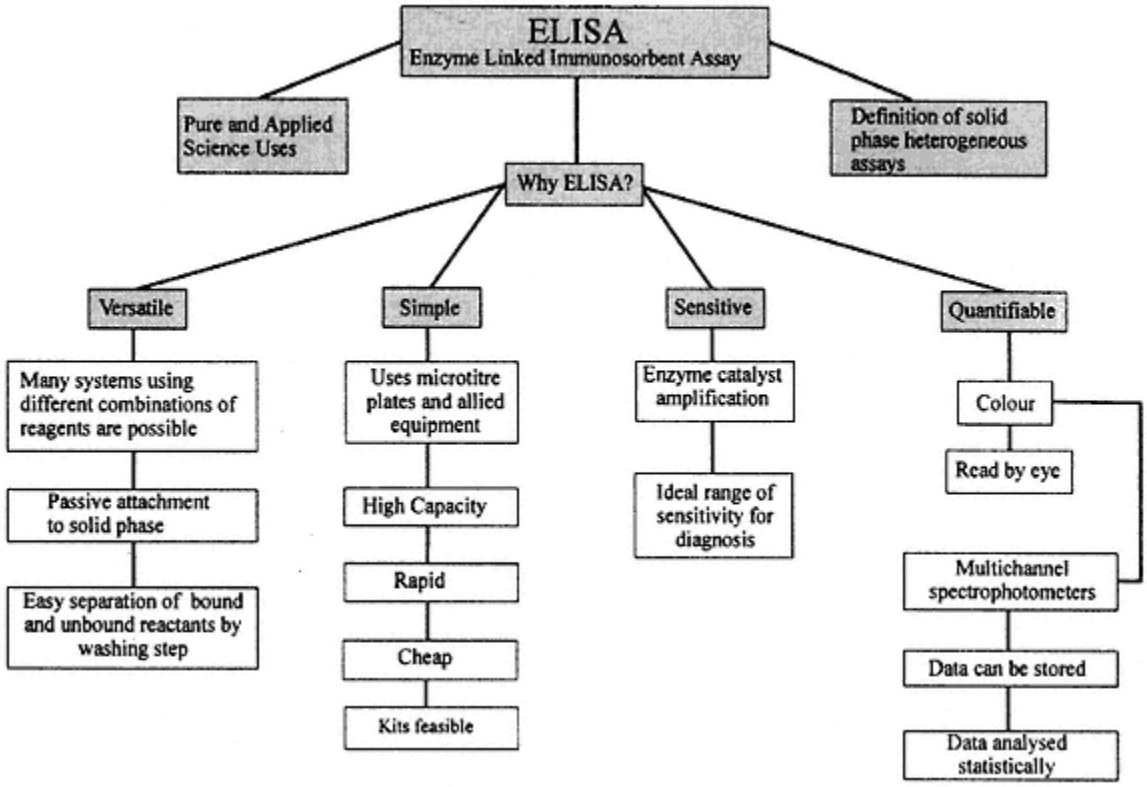


Figure 2. Four major advantages of ELISA that are promoted and all of which add to the reasons that this form of ELISA has been, and will continue to be successful. Adapted from Crowther J.R (2002).

ELISA formats can be direct detection**,** indirect detection or sandwich assay. The direct detection method uses a labeled primary antibody that reacts directly with the antigen. The direct assay can be performed with antigen that is directly immobilized on the assay plate or with the capture assay format. The indirect detection method uses a labeled secondary antibody for detection and is the most popular format for ELISA. The sensitivity is increased because each primary antibody contains several epitopes that can be bound by the labeled secondary antibody, allowing for signal amplification [47]. In a sandwich ELISA, it is critical that the secondary antibody be specific for the detection of the primary antibody only or the assay will not be specific for the antigen (fig. 3). ELISA may be run in a qualitative or quantitative format. Qualitative formats provide a simple positive or negative result. A quantitative ELISA is used to seek empirical support where the optical density of the sample is compared to a standard curve.

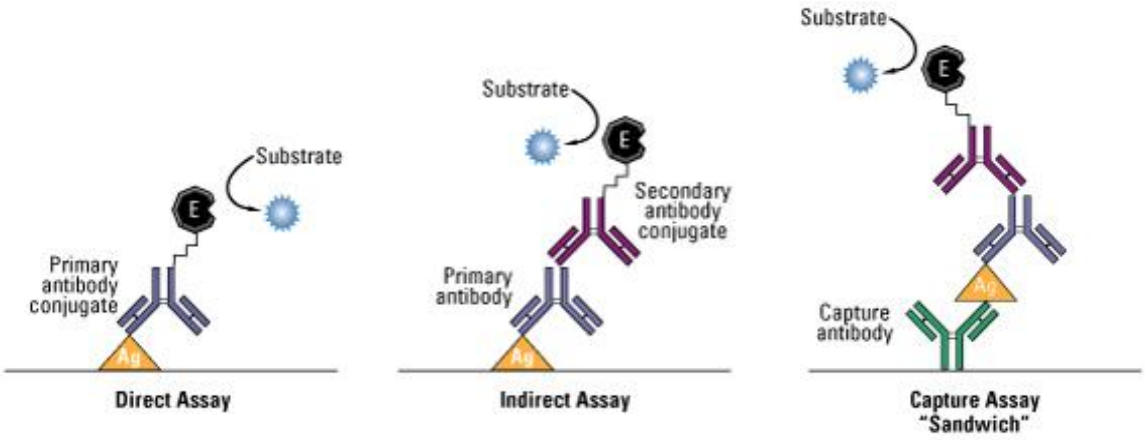


Figure 3. Direct and Indirect detection ELISA formats. Adapted from Scientific Thermo Fisher (2011).

ELISA tests for IgG and IgM antibodies are the tests that most commonly used today to quantitate the specific analytes especially during blood transfusion. Persons should be initially tested for the presence of *toxoplasma*-specific IgG antibodies to determine their immune status. A positive IgG titer indicates infection with the organism at some time. If more precise knowledge of the time of infection is necessary, then an IgG positive person should have an IgM test performed by a procedure with minimal nonspecific reactions, such as IgM-capture ELISA [48](fig. 4). The major problem with IgM testing is lack of specificity, where two situations might occur frequently, persons with a positive IgM but negative IgG and Individuals with positive IgG and IgM results. [48]. IgM antibodies are measured by the "double-sandwich" or "immuno-capture" IgM-ELISA method. This method avoids false positive results due to the presence of rheumatoid factor and antinuclear antibodies and in patients with recently acquired infection [49]. IgM *T. gondii* antibodies are detected initially and, in most cases, these titers become negative within a few months.

Normally a positive IgG test with a negative IgM test in a donor will be interpreted as chronic infection and a positive for both IgG and IgM in a donor will be interpreted as recently infection [33, 50].

IgG result	IgM result	Report/interpretation for humans*
Negative	Negative	No serological evidence of infection with *Toxoplasma*.
Negative	Equivocal	Possible early acute infection or false-positive IgM reaction. Obtain a new specimen for IgG and IgM testing. If results for the second specimen remain the same, the patient is probably not infected with *Toxoplasma*.
Negative	Positive	Possible acute infection or false-positive IgM result. Obtain a new specimen for IgG and IgM testing. If results for the second specimen remain the same, the IgM reaction is probably a false-positive.
Equivocal	Negative	Indeterminate: obtain a new specimen for testing or retest this specimen for IgG in a different assay.
Equivocal	Equivocal	Indeterminate: obtain a new specimen for both IgG and IgM testing.
Equivocal	Positive	Possible acute infection with *Toxoplasma*. Obtain a new specimen for IgG and IgM testing. If results for the second specimen remain the same or if the IgG becomes positive, both specimens should be sent to a reference laboratory with experience in diagnosis of toxoplasmosis for further testing.
Positive	Negative	Infected with *Toxoplasma* for more than 1 year.
Positive	Equivocal	Infected with *Toxoplasma* for probably more than 1 year or false-positive IgM reaction. Obtain a new specimen for IgM testing. If results with the second specimen remain the same, both specimens should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Positive	Positive	Possible recent infection within the last 12 months, or false-positive IgM reaction. Send the specimen to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.

*except infants

Figure 4: The Diagnostic serology findings which might provide positive, negative or equivocal based on IgG and IgM. Adapted from Diagnostic serology-Wikipedia (2009).

1.6 RATIONALE OF THE STUDY

The current study will establish the necessary need for screening of *T. gondii* which could stop the transmission from the one individual to another. This will ensure better treatment which is very important for recently infected pregnant women and immunosupressed patients. Since a baby's immune system does not develop fully for the first year of life and the resilient tissue cysts that form throughout the body are very difficult to eradicate with anti-protozoans, so the infection can be very serious in the young [51]. Basically, because it is difficult to eradicate the parasites after long-term chronic infection, early detection is very crucial for effective therapy. Late diagnosis of toxoplasmosis usually results into death and costly management [52].

CHAPTER TWO

2.0 RESEARCH METHODS

2.1 Description of the study area

This study was carried out at the headquarter office which is the Eastern zone blood transfusion centre (EZBTC) located at Mchikichini area in Ilala district in Dar es Salaam region. EZBTC is one of the eight zones of blood transfusion centres in Tanzania serving four regions which are Dar es Salaam, Coast, Morogoro and Dodoma and 68 hospitals. The institution has so far achieved to increase blood collection from 32,000 in year 2005 to 120,000 units in year 2009 with the target to collect 250,000 units by the year 2015. The number of voluntary-non remunerated blood donation increased from less than 5% to 25%**.**

2.2 Study design

This was a cross-sectional study conducted between November 2011 and April 2012. Simple random selection technique was used to select 160 blood bags from the refrigerator containing maximum of 500 blood bags. The blood bags were tagged using a specific ID number 1-500 on a slip of paper. Lottery method was conducted which allows to select one sample after every mixing. The selected numbers of particular blood units was matched with the donor's records following the registration number. Questionnaire was provided by the EZBTS which contain both demographic and clinical information of the donor. A small volume of blood was withdrawn from each of the selected blood unit. The selected samples which met the study inclusion criteria were also arranged with a given unique ID number by EZBTS. ID number will identify and link to the respective donor's information contained in a form.

2.3 Sample size estimation

The study involved sampling for a convenient sample from voluntary healthy donors attending EZBTS. Study sample size formula; [53].

$$N = \frac{t^2 \times P(1-P)}{d^2}$$

Whereby N= required sample

t= confidence level at 95% (1.96)

P= Prevalence of the disease (46%)

d= margin of error at 8% (0.08)

$$N = \frac{1.96 \times 1.96 \times 0.46(1-0.46)}{0.08 \times 0.08} = \sim 149+10\% = \sim 160$$

Then, total of 160 voluntary blood donors were required in this study.

2.4 Recruitment plan

2.4.1 Inclusion and exclusion criteria for blood donors

The study included blood donors with the following criteria**;** voluntary healthy blood donor, donors aged 15 years and above and those who successful pass the donor health questionnaire form for all questions. The study did not include blood donors with any of the following criteria; voluntary blood donor below 15 years old, voluntary blood donors with a history of immunocompromised condition such as Cancer patient, Organ transplant patient's donors, autoimmune patients and AIDS patients. Also Voluntary blood donor with a haemoglobin level < 12.5dl/L and with a weight < 50 kg were not included in the study. The EZBTS questionnaire form is attached as appendix 1.

2.4.2 Inclusion and exclusion criteria for control subjects

The study controls were chosen randomly from the Central pathology laboratory (CPL) at the Muhimbili National hospital (MNH). A questionnaire was used to collect necessary background information of the recruited control subjects who met the study inclusion criteria. The study included controls with the following criteria, those who did not take any drugs to treat toxoplasmosis, voluntary blood donors who do not take any drugs to treat immunosuppressed conditions. Control subjects exclusion criteria include patients known to be HIV positive, voluntary blood donors who are taken drugs to treat immunosuppressed conditions.

2.5 Sample collection procedures

2.5.1 Blood sample collection

Plasma samples were obtained from voluntary blood donors collected by EZBTS at Ilala district. Vials which contain about 1-3ml blood sample from each donor were collected into the red top tube, which were labelled with participant's ID. Serum was separated from blood cells as soon as possible by centrifugation at 3000 *r.p.m* for five minutes, and serum separated and stored at -20ºC until the day of analysis.

2.5.2 Specimens and laboratory investigations

The frozen sample were slowly thawed and brought to room temperature. The sera were examined for anti-*toxoplasma* IgG and IgM antibody using a commercial ELISA kit (Dour Lab Company) and ELISA reader machine (Multscan Ex). Positive and negative controls were included in each batch of test run and the assay carried out according to the manufacturer's instructions.

Briefly, washing buffer was first prepared; serum samples, positive and negative control and standards added into respective wells in the 96-well plate and incubated for 30 min at room temperature followed by washing the microplate 5 times using washing buffer. After the washing step, 100 ul of enzyme conjugate (peroxidase-labeled anti-human IgG) was added and incubated for 30 min at room temperature, followed by washing. Thereafter, 50 ul of chromogen/substrate A was added followed by 50ul chromogen/Substrate B solution into each well, After 10 min of incubation at room temperature, 50 ul of stop solution was added and then the plate read in an ELISA reader at 450 nm and 620 nm.

The sample that had *Toxoplasma* IgG index value less than 9.9 was considered as negative whereas one with value above 1.1 was considered positive. Samples that read between 9.9 to 1.1 were regarded as equivocal.

Serum that tested anti- *T. gondii* IgG antibody positive was further analyzed for anti-*T. gondii* IgM antibody by ELISA. For both IgG and IgM tests, the Toxo G index and Toxo M index were calculated as shown in assay procedure in appendix VI. Briefly, these indices were determined by dividing the mean values of each sample with the cut-off

calibrator mean value. A sample was considered positive for IgG or IgM when a Toxo G index or a Toxo M index was equal or greater than 1.1

2.6. Socio-demographic and behavioural data

A blood donor questionnaire form was used to explore the socio-demographic characteristics of blood donors. Data including age, residence place, marital status, sex, occupation and educational level index were obtained from all participants as shown in appendix 1.

2.7 Ethical consideration

Ethical clearance to conduct this study was obtained from the Higher Degree Research and Publication Committee of Muhimbili University of Health and Allied Health Sciences (MUHAS), Dar es Salaam Tanzania. Further, permission to conduct the study was obtained from the EZBTS blood collection authority and all donor information, procedures and test results were confidentially kept.

2.8 Data management and statistical analysis

The collected data were entered into the computer Microsoft Excel program, followed by data coding, checking and cleaning where current SPSS v16 statistical software was used to analyze the entered study data. We used a references seroprevalence of 46% as expected frequency of the factor under the study, 120,000 as the size of population from which the sample was selected, a worst acceptable result of 8% and a confidence level of 95%. The result of the calculation was 160 subjects. Descriptive statistics were used for categorical (frequency or percentage) variables. Chi-square test ($\chi 2$) was used to explore the association between prevalence of infection in which the significant test was at $p \leq 0.05$.

CHAPTER THREE

3.0 RESULTS

3.1 Seroprevalence of anti-*T. gondii* antibodies

All blood samples in the study were tested for anti-*T. gondii* IgG antibodies, where the seroprevalence was 47.5% (76/160). Further analysis of all samples that were IgG antibodies positive were tested for anti- *T. gondii* IgM antibodies and found to be all negative as shown in table 1 below.

Table 1: Distribution of anti- *T. gondii* IgG and IgM antibodies

Antibody	Positive(n)	Negative(n)	Total (%)
IgG	76	84	47.5
IgM	-	76	0

Note: Samples were analyzed by anti-*T. gondii IgG* ELISA kit where the sera positive for anti- *T. gondii* antibodies were further analyzed by anti -*T. gondii* IgM ELISA kit.

3.2 Social-demographic characteristics associated with seropositivity

The study recruited a total of 160 healthy blood donors, where the male participants appeared to be almost as twice as female of which 108 (67.5%) were males and 52 (32.5%) were females. (Table: 2 and fig.5). The study also included different age strata ranging from 18-62. Most of the sampled subjects were found in the age group 25-34 years old with high frequency of participation (28.1%) while the lowest frequency of participation (7.5%) were in the age group of 55 years and above (Table:2). In frequency distribution based on occupation, skilled category (Non-labourers) was having the highest frequency of participation (41.9%) and the lowest were unskilled (labourers) (20.6%) as shown in table: 2. In area of domicile, Ilala district has the highest frequency of participation (44.4%) where Temeke has the lowest frequency of participation (22.5%) (Table:2)

*Chi-square test ($\chi 2$) was used to explore the association between prevalence of infection in which the significant test was at $p \leq 0.05$.

Table 2: Demographic characteristics of the study subjects

Characteristics.		No. of blood Samples exam.	(%)	No. positive for ant-*T. gondii*	Prevalence (%)
Gender	Male	108	67.5	56	51.8
	Female	52	32.5	20	38.5
Age groups (years)					
	15-24	44	27.5	10	22.7
	25-34	45	28.1	32	71.1
	35-44	36	22.5	22	61.1
	45-54	23	14.4	7	30.4
	55 and above	12	7.5	7	58.3
Occupation					
	Unskilled	33	20.6	5	15.2
	Skilled	67	41.9	41	61.2
	Students	60	37.5	30	50.0
Domicile					
	Ilala	71	44.4	28	39.4
	Kinondoni	53	33.1	26	49.1
	Temeke	36	22.5	22	61.1

The overall seroprevalence of anti-*T. gondii* antibodies in blood samples in the study was 47.5% whereas the male and female IgG seropositivity were 56/108 (51.8%) and 20/52 (38.5%) respectively as shown in table 3, and fig 5. However, this gender difference in seroprevalence was not statistically significant ($p= 0.112$) as shown below.

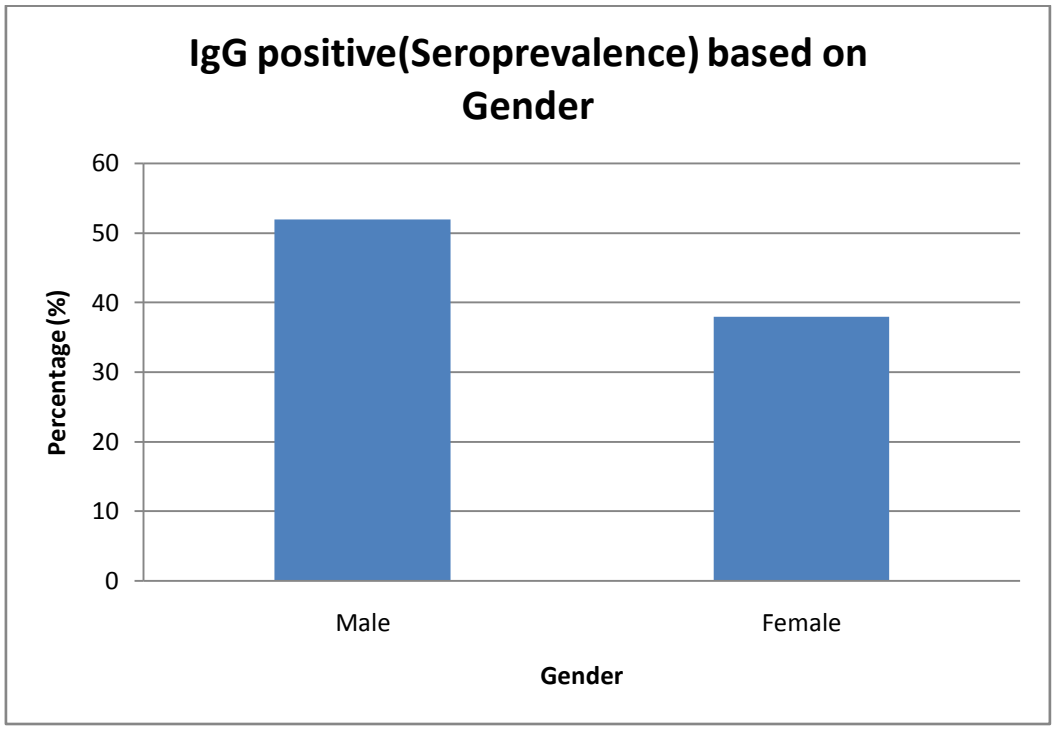
Table 3: Relationship between Gender and *T. gondii* IgG seropositive

Cross tabulation

		Status		
		+ve	-ve	Total
Gender	F	20	32	52
		38.5%	61.5%	100.0%
	M	56	52	108
		51.9%	48.1%	100.0%
Total		76	84	160
		47.5%	52.5%	100.0%

Chi-square 2.524, df 1, *p= 0.112*

Figure 5: Relationship between Gender with anti- *T. gondii* IgG antibody serostatus



The age (in years) ranged from 18 to 62, and the highest seropositivity of (71.1%) and the lowest (22.7%) were between 25–34 years and 15-24 years respectively. Moreover, there was a significant difference in the prevalence of toxoplasmosis between the age groups above at (*p= 0.001*) as shown in table 4, and fig. 6.

Table 4: Relationship between Age and anti- *T. gondii* IgG antibody serostatus

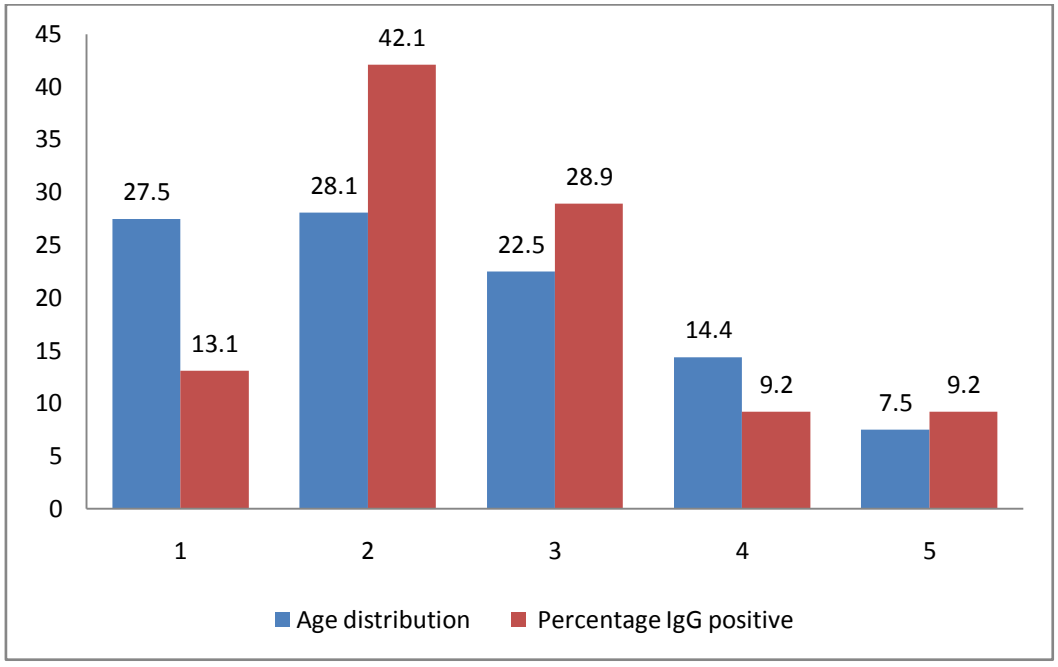
Age groups * Status cross tabulation

		Status		
		+ve	-ve	Total
age groups	15-24	10	30	40
		25.0%	75.0%	100.0%
	25-34	30	17	47
		63.8%	36.2%	100.0%
	35-44	22	16	38
		57.9%	42.1%	100.0%
	45-54	7	16	23
		30.4%	69.6%	100.0%
	55+	7	5	12
		58.3%	41.7%	100.0%
Total		76	84	160
		47.5%	52.5%	100.0%

Chi- square 18.043, df 4, *p = 0.001*

The seroprevalence was influenced by age, we observed that the association with age was statistically significant (*p= 0.001*).

Figure 6: Relationship between Age with anti- *T. gondii* IgG antibody serostatus



o2ng2e0 (–li3n0e]),

Analysis of the effect of occupation on *T. gondii* serostatus among blood donors revealed that skilled workers had the highest 41/71 (61.2%) seroprevalence, whereas the lowest was among unskilled workers/labourers 5/33 (15.2%) and chi square analysis revealed that the association was statistically significant (*p= 0.024*) as shown in the table 5. With regards to the area of domicile, the vast majority of IgG positive donors were from Temeke (61.1%) and lowest was Ilala (39.4%). The seroprevalence was not influenced by area of domicile, since the association was not statistically significant (*p= 0.101*) as shown in the table 6, and figure 8.

Table 5: Relationship between Occupation (Work status) and anti- *T.gondii* serostatus

Occupation * Status cross tabulation

		status1		
		+ve	-ve	Total
Occupation	Unskilled	5	15	20
		25.0%	75.0%	100.0%
	Skilled	41	30	71
		57.7%	42.3%	100.0%
	Students	30	39	69
		43.5%	56.5%	100.0%
Total		76	84	160
		47.5%	52.5%	100.0%

Chi-square 7.497, df 2, *p=0.024*, the seroprevalence was influenced by occupation, the association was statistically significant (*p= 0.024*).

Figure 7: Relationship between Occupation and *T. gondii* IgG antibody serostatus



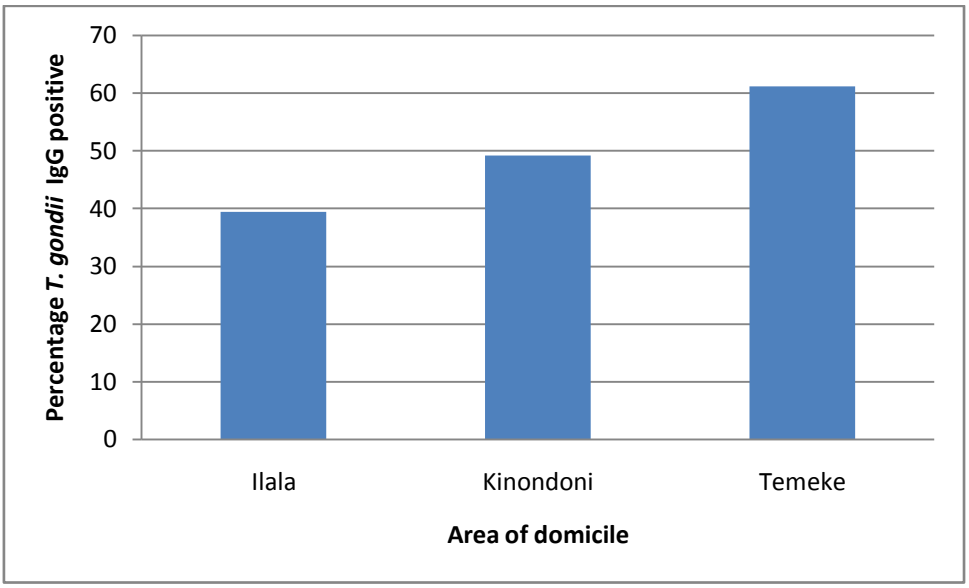
Table 6: Distribution of *T. gondii* IgG antibody serostatus by domicile

Districts * Status cross tabulation

			Status		Total
			+ve	-ve	
District	ILALA	Count	28	43	71
		% within district	39.4%	60.6%	100.0%
	KINONDONI	Count	26	27	53
		% within district	49.1%	50.9%	100.0%
	TEMEKE	Count	22	14	36
		% within district	61.1%	38.9%	100.0%
Total		Count	76	84	160
		% within district	47.5%	52.5%	100.0%

Chi-square 4.577, df 2, p= 0.101, there was no statistical difference in seroprevalence of *T. gondii* among the three Dar es Salaam districts *(p= 0.101)*.

Figure 8: Distribution of *T. gondii* IgG antibody by domicile



Note: Temeke district has shown to have a higher percentage IgG seroprevalence while the lowest was Ilala district. The seroprevalence was not influenced by area of domicile, since the association was not statistically significant (*p= 0.101*).

CHAPTER FOUR

4.0 DISCUSSION

This study revealed the seroprevalence of anti- *T. gondii* antibodies in blood samples from the blood bank to be 47.5% which is much higher than all the other four mandatory tests carried out in different blood banks, *i.e.,* antibodies to HIV, HBV, HBC and syphilis. Our findings among healthy blood donors are consistent with a previous study by Furtado et al., 2011 which found the seroprevalence of *T. gondii* in the general Tanzania population which was found to be 46%. The ELISA test is regarded as the best reference method for the serodiagnosis of Toxoplasmosis as it has got high specificity and sensitivity.

The current study of Toxoplasmosis may pave a way towards establishing screening for *T. gondii* before giving blood to the recipients. Incidentally this could reduce the country effort dealing with the disease complications for example, if a woman gets infected with *T. gondii* for the first time in her life during pregnancy, she may pass infection to the fetus which might also lead to miscarriage, still birth, abortion or even death. Also severe complications such as damage to brain (Toxoplasmosis encephalitis) and eyes (Ocular toxoplasmosis) have been found to immunosupressed patients [54]. Late diagnosis of Toxoplasmosis normally results into death and costly management.

Also, the current study is the leading research to try to find the contributory factors for the increase of transmission rate of *T. gondii* through healthy blood donors in Tanzania. In worldwide, the establishment of *toxoplasma* seroprevalence by blood donors is based on regions and it was known to vary widely among different regions of the globe. The seroprevalence of *T. gondii* in this study was much higher than that reported in different countries in North America, South America, Europe, Asia and some parts of Africa. Countries like Chile has a seroprevalence of 21.2% [35] , Malaysia (28.1%) [37], Slovakia (24.2%) [43]. Iraq has the seroprevalence of (22%). Another study placed seroprevalence in Mexico (7.4 %) [33] by IgG and (1.9%) by IgM, Czech Republic (2.4%) by IgM and (32.1%) by IgG, respectively, with the (9%) [34] in Thailand. Also in study conducted by Sundar et al., in India claimed a seroprevalence of IgG (20.3%) and IgM (3.6%) [36] and Turkish has claimed seroprevalence of (19.5%) by IgG [55]. In African countries like Mali has seroprevalence of (21%) [40] and Nigeria has a seroprevalence of (20.8%) [16]. A

significant association between *T. gondii* seropositivity and eating meats by-products have been found in many studies [14, 41]. High socio-economic society has habit of consuming raw or undercooked meat e.g "Nyama choma (barbequing meat) and pig meat. Dar es Salaam City has high socio-economic society compared with other regions and cities in which similar study were conducted. Regions and cities like Bangalore-India, Urban Karnataka-South India and Osorno-Chile have low socio-economic level hence have a reduced exposure status to Toxoplasmosis. Climatic characteristics such as hot, arid climate and high altitude is associated with a low prevalence of infection as seen in cities like Urban Karnataka-India, Bratislava-Slovakia, Loei province-Thailand and Durango City-Mexico. Dar es Salaam City has warm and humid climate which support more exposure to *T. gondii* infection. The possibility that different regions, countries and continents could harbour different strains (genotypes) of *toxoplasma* parasite with different virulent capability might also affect the status of seroprevalence.

In contrast, the seroprevalence that we found correlates with the results of studies done in different countries and regions, this reflect similarity in geographical, climatic, and socioeconomic conditions between our study and others. Studies conducted by Swai et al., in Tanga City showed prevalence of (46%) [30], Zarkovic et al., in New Zealand (42.9%) [56] and a study by Sarwat et.al., in Saud Arabia (46.7%) [57] showed similar findings with the current study.

In general, the highly abundant clonal genotypes of *T. gondii* continue to dominate in North America and Europe and show a very high virulent capability e.g Brazil has a seroprevalence of (75%) [58]. Some of the African countries showed higher seroprevalence such as Egypt (59.6%) [59], Kenya (54%) [39] and a study by Al-amiri in Abha City-Saudi Arabia (52.1%) [38]. The possible difference could be climatic condition by these countries as warm and humid areas facilitate higher exposure to *T. gondii* infection. The possibility that, the use of different methods for diagnosis might affect the findings should be considered. e,g in a study done in Kenya using hemagglutination test which is not recommended for detection of ant-*T. gondii* antibodies. The possibility that most of blood donors from regions above belonged to a high socio-economic level and eating meat like cow, sheep and pigs is a frequent practice among them. Also different

traditions and customs may rely on a certain food which might facilitate more exposure to disease than others.

In this study, we found only IgG antibodies seropositivity where IgM was negative among all blood donors. This findings was similar to studies conducted by Maiga [40] and Studencova [43], and by and large many studies have focused only on IgG seropositivity because the IgM appeared to be rarely seen. Because IgM antibody is a marker of recent *T. gondii* infection the absence of anti- *T. gondii* positive among blood donors indicate that the positive donors had chronic infection and none of the donors had recent *T. gondii* infection. In this study, we performed detection of IgM antibodies only in IgG positive samples because the presence of IgM antibodies alone is rarely seen. Also the window period between the appearance of IgM and the appearance of IgG is extremely short as IgM has narrow window period. Moreover, studies have shown that the probability of finding an IgM positive-IgG negative infected individuals seems to be quite low to financially justify a systematic IgM screening in a cross sectional study [33]. Through focusing on healthy blood donors it is likely to eliminate different recent infections.

IgM negativity or lower prevalence of IgM have been found in studies like Studencova et al., in Slovakia, Sundar et al., [36], Alavarado et al., [33], Elhence et al., [7], others are Sarwat et.al., [57] and Yazar et al., [55]. This could also be explained by the fact that the group examined consisted only of healthy persons, and IgG-positive persons were infected with latent toxoplasmosis without a persistence of IgM and IgA antibodies after acute infection in the past.

With respect to the socio-demographic characteristics of the blood donors, the number of males who participated in this study was much higher compared to females. In this study males were 108 (67.5%) and females 52 (32.5%) and their percentage IgG positive (seroprevalence) was much higher in male than female although the difference was not significant (*p=0.112*) (fig. 5). Similar findings have been reported to several studies like Studencova et al., [43], Alvarado et al., [33], Fromont et al., [18] and Nissapatorn et al., [37]. In this study majority of participants were in a middle from 18-34 years where most of the female were in reproductive stage or pregnant. This reflects that they are likely to receive the blood than to donate.

We observed that the frequency of seropositivity decreased with age, mostly percentage IgG positive (41.2%) were in 25–34 years (fig. 6). The seroprevalence was influenced by age, since the association was statistically significant (*p= 0.001*). This observation agrees with those reported in other studies like Coelho et al., [58] and Alvarado et al., [33]. Generally, the age between 25–34 years and 35-44 years it showed higher risk (exposure status) of seroprevalence when compared to the other age groups. This could be due to the fact this age group is more socio -economically more active consequently carrying a higher exposure risk. Also low level of hygiene during food preparation could result to low level of awareness of toxoplasmosis. The number of participants has shown to decrease as the age increases, where the age of 55 and above have the lowest number of participation. Normally as the age increases the immunity tends to decrease, hence the chance to donated blood will be low. These findings are not similar with Studencova et al., [43] or Nissapatorn et al., [37] which showed a significance increase in seroprevalence with advancing age. The possible reasons for this discrepancy could be due differences in *T. gondii* exposure in different geographical regions because of differences in socio-economic and cultural activities, geographical location and population growth.

Paradoxically, this study found that skilled workers had significantly higher IgG seroprevalence compared to the unskilled workers (labourers), indicating that professionals who are in high socio economic bracket are at higher risk of having the disease. It is unexpected for a higher socioeconomic status group to be much affected than labourers, however perhaps this could be attributed to the habit by the majority of middle class individuals of consuming undercooked meat and barbequing e.g. “Nyama choma and pork” especially in Dar es Salaam City [14, 41]. Further the fewer number of unskilled workers in the study population could have skewed the results further, and the need to sensitize this group of workers on the importance of blood donation.

Our study showed that the prevalence of anti- *T.gondii* antibodies was highest in Temeke district, whereas Ilala blood donors had the lowest IgG seroprevalence. However, this difference was not statistically significant (*p= 0.101*), and moreover, it is not surprising because these districts are close to one another, suggesting that a more extensive study recruiting samples from diverse regional locations should be conducted to evaluate more on possible risks factors to the disease in general population.

CHAPTER FIVE

5.0 CONCLUSION

Screening for *T. gondii* is not routinely performed in Tanzania due to limited health resources and lack of awareness of the prevalence of the disease and its mode of transmission. Lack of awareness is likely to expose them to increased risk of contracting toxoplasmosis, as they might not take proper precaution. The current study shows a high seroprevalence of *T. gondii* antibodies among blood donors at EZBTS in Dar es Salaam and illustrates the current transfusion transmissible risk of *T. gondii*. Thus, it may be appropriate and beneficial to include *T. gondii* screening as part of pre transfusion blood testing schedule. Moreover, there is a significant age but not gender difference in the prevalence of toxoplasmosis among blood donors.

5.1 Limitation of the study.

The study recruited a small sample size limited to Dar es Salaam due to lack of enough funds to conduct the study and also schedule of activities were limited.

5.2 Recommendations

- This study was limited to the EZBTS, an extensive study of a large population involving a large population from rural and urban areas in all eight zones.
- Considering the relative high seroprevalence of Toxoplasmosis as revealed by this study it would be important to increase public awareness about different routes of transmission of *T. gondii*.
- Periodic studies to investigate other transfusion-transmissible infectious diseases are required

REFERENCES

1. Singh, G. and R. Sehgal, *Transfusion-transmitted parasitic infections.* Asian J Transfus Sci, 2010. 4(2): p. 73-7.
2. Wendel Neto, S., *Current concepts on the transmission of bacteria and parasites by blood components.* Sao Paulo Med J, 1995. 113(6): p. 1036-52.
3. Garraud, O., *Mechanisms of transfusion-linked parasite infection.* Transfus Clin Biol, 2006. 13(5): p. 290-7.
4. Sibley, L.D. and J.C. Boothroyd, *Construction of a molecular karyotype for Toxoplasma gondii.* Mol Biochem Parasitol, 1992. 51(2): p. 291-300.
5. Tenter, A.M., A.R. Heckeroth, and L.M. Weiss, *Toxoplasma gondii: from animals to humans.* Int J Parasitol, 2000. 30(12-13): p. 1217-58.
6. Remington, J.S., *Toxoplasmosis in the adult.* Bull N Y Acad Med, 1974. 50(2): p. 211-27.
7. Elhence, P., et al., *Seroprevalence of Toxoplasma gondii antibodies in North Indian blood donors: implications for transfusion transmissible toxoplasmosis.* Transfus Apher Sci, 2010. 43(1): p. 37-40.
8. Flatt, A. and N. Shetty, *Seroprevalence and risk factors for toxoplasmosis among antenatal women in London: a re-examination of risk in an ethnically diverse population.* Eur J Public Health, 2012.
9. Jones, J.L., et al., *Toxoplasmosis prevention and testing in pregnancy, survey of obstetrician-gynaecologists.* Zoonoses Public Health, 2010. 57(1): p. 27-33.
10. Ampofo, W., et al., *Prevalence of blood-borne infectious diseases in blood donors in Ghana.* J Clin Microbiol, 2002. 40(9): p. 3523-5.
11. Matee, M.I., P.M. Magesa, and E.F. Lyamuya, *Seroprevalence of human immunodeficiency virus, hepatitis B and C viruses and syphilis infections among blood donors at the Muhimbili National Hospital in Dar es Salaam, Tanzania.* BMC Public Health, 2006. 6: p. 21.
12. Matee, M.I., et al., *Prevalence of transfusion-associated viral infections and syphilis among blood donors in Muhimbili Medical Centre, Dar es Salaam, Tanzania.* East Afr Med J, 1999. 76(3): p. 167-71.
13. Tounkara, A., et al., *Seroprevalence of HIV/HBV coinfection in Malian blood donors.* J Int Assoc Physicians AIDS Care (Chic), 2009. 8(1): p. 47-51.

14. Schoonman, L.B., T. Wilsmore, and E.S. Swai, *Sero-epidemiological investigation of bovine toxoplasmosis in traditional and smallholder cattle production systems of Tanga Region, Tanzania.* Trop Anim Health Prod, 2010. 42(4): p. 579-87.
15. Jones, J.L., et al., *Toxoplasma gondii infection in the United States: seroprevalence and risk factors.* Am J Epidemiol, 2001. 154(4): p. 357-65.
16. Uneke, C.J., et al., *Seroprevalence of acquired toxoplasmosis in HIV-infected and apparently healthy individuals in Jos, Nigeria.* Parassitologia, 2005. 47(2): p. 233-6.
17. Furtado, J.M., et al., *Toxoplasmosis: a global threat.* J Glob Infect Dis, 2011. 3(3): p. 281-4.
18. Fromont, E.G., B. Riche, and M. Rabilloud, *Toxoplasma seroprevalence in a rural population in France: detection of a household effect.* BMC Infect Dis, 2009. 9: p. 76.
19. Raisanen, S., *Toxoplasmosis transmitted by blood transfusions.* Transfusion, 1978. 18(3): p. 329-32.
20. Werk, R. and W. Bommer, *[Toxoplasmosis transmission by blood transfusion? (author's transl)].* Dtsch Med Wochenschr, 1978. 103(41): p. 1598, 1601-2.
21. Holliman, R.E., *Congenital toxoplasmosis: prevention, screening and treatment.* J Hosp Infect, 1995. 30 Suppl: p. 179-90.
22. Ayi, I., et al., *Sero-epidemiology of toxoplasmosis amongst pregnant women in the greater accra region of ghana.* Ghana Med J, 2009. 43(3): p. 107-14.
23. Weiss, L.M. and J.P. Dubey, *Toxoplasmosis: A history of clinical observations.* Int J Parasitol, 2009. 39(8): p. 895-901.
24. Su, C., et al., *Globally diverse Toxoplasma gondii isolates comprise six major clades originating from a small number of distinct ancestral lineages.* Proc Natl Acad Sci U S A, 2012. 109(15): p. 5844-9.
25. L. David Sibley, A.K., James W. Ajioka and a.B.M. Rosenthal, *Genetic diversity of Toxoplasma gondii in animals and humans.* 2009. 364: p. 2749-2761.
26. Frenkel, J.K., *Toxoplasma in and around us.* Bioscience, 1973. 23: p. 343-352.
27. Pavesio, C.E. and S. Lightman, *Toxoplasma gondii and ocular toxoplasmosis: pathogenesis.* Br J Ophthalmol, 1996. 80(12): p. 1099-107.

28. Striepen, B., et al., *Targeting of soluble proteins to the rhoptries and micronemes in Toxoplasma gondii.* Mol Biochem Parasitol, 2001. 113(1): p. 45-53.
29. Lingelbach, K. and K.A. Joiner, *The parasitophorous vacuole membrane surrounding Plasmodium and Toxoplasma: an unusual compartment in infected cells.* J Cell Sci, 1998. 111 (Pt 11): p. 1467-75.
30. Swai, E.S. and L. Schoonman, *Seroprevalence of Toxoplasma gondii infection amongst residents of Tanga district in north-east Tanzania.* Tanzan J Health Res, 2009. 11(4): p. 205-9.
31. Gandahusada, S., *Study on the prevalence of toxoplasmosis in Indonesia: a review.* Southeast Asian J Trop Med Public Health, 1991. 22 Suppl: p. 93-8.
32. Amany M, A.K.a.A.M.H., *Serological and biochemical study of HBV, HCV, HIV and Toxoplasmosis infection among blood donors in Iraq.* 2008. 23: p. 154.
33. Alvarado-Esquivel, C., et al., *Seroepidemiology of infection with Toxoplasma gondii in healthy blood donors of Durango, Mexico.* BMC Infect Dis, 2007. 7: p. 75.
34. Pinlaor, S., et al., *Seroprevalence of specific total immunoglobulin (Ig), IgG and IgM antibodies to Toxoplasma gondii in blood donors from Loei Province, Northeast Thailand.* Southeast Asian J Trop Med Public Health, 2000. 31(1): p. 123-7.
35. Zamorano, C.G., et al., *[Seroepidemiological survey of human toxoplasmosis in Osorno, Region X, Chile, 1998].* Bol Chil Parasitol, 1999. 54(1-2): p. 33-6.
36. Sundar, P., et al., *Toxoplasma seroprevalence in healthy voluntary blood donors from urban Karnataka.* Indian J Med Res, 2007. 126(1): p. 50-5.
37. Nissapatorn, V., et al., *Seroepidemiology of toxoplasmosis among HIV-infected patients and healthy blood donors.* Med J Malaysia, 2002. 57(3): p. 304-10.
38. Al-Amari, O.M., *Prevalence of antibodies to Toxoplasma gondii among blood donors in Abha, Asir Region, south-western Saudi Arabia.* J Egypt Public Health Assoc, 1994. 69(1-2): p. 77-88.
39. Griffin, L. and K.A. Williams, *Serological and parasitological survey of blood donors in Kenya for toxoplasmosis.* Trans R Soc Trop Med Hyg, 1983. 77(6): p. 763-6.

40. Maiga, I., P. Kiemtore, and A. Tounkara, *[Prevalence of antitoxoplasma antibodies in patients with acquired immunodeficiency syndrome and blood donors in Bamako]*. Bull Soc Pathol Exot, 2001. 94(3): p. 268-70.
41. Jones, J.L., et al., *Risk factors for Toxoplasma gondii infection in the United States.* Clin Infect Dis, 2009. 49(6): p. 878-84.
42. Moradali Fouladvand, A.B., Behrouz Naeimi, Keivan Zandi and Saeed Tajbakhsh, *seroprevalence of Toxoplasmosis in high school girls in Bushehr City, South west of Iran. 2009.* Microbiology, 2010. 4(11): p. 1117-1121.
43. Studenicova, C., G. Bencaiova, and R. Holkova, *Seroprevalence of Toxoplasma gondii antibodies in a healthy population from Slovakia.* Eur J Intern Med, 2006. 17(7): p. 470-3.
44. Scientific, T.F., *Life Science Research Products,Pierce Proteins.* 2011.
45. Dando, C., et al., *Simple and efficient method for measuring anti-toxoplasma immunoglobulin antibodies in human sera using complement-mediated lysis of transgenic tachyzoites expressing beta-galactosidase.* J Clin Microbiol, 2001. 39(6): p. 2122-5.
46. Lappin, M.R. and C.C. Powell, *Comparison of latex agglutination, indirect hemagglutination, and ELISA techniques for the detection of Toxoplasma gondii-specific antibodies in the serum of cats.* J Vet Intern Med, 1991. 5(5): p. 299-301.
47. Crowther, J.R., *Methods in Molecular Biology.* 2002. 149.
48. Findings, D.S.W., *Toxoplasmosis.* 2009.
49. Kaul, R., P. Chen, and S.R. Binder, *Detection of immunoglobulin M antibodies specific for Toxoplasma gondii with increased selectivity for recently acquired infections.* J Clin Microbiol, 2004. 42(12): p. 5705-9.
50. Sahwi, S.Y., et al., *Toxoplasmosis as a cause of repeated abortion.* J Obstet Gynaecol (Tokyo 1995), 1995. 21(2): p. 145-8.
51. Kamerkar, S. and P.H. Davis, *Toxoplasma on the brain: understanding host-pathogen interactions in chronic CNS infection.* J Parasitol Res, 2012. 2012: p. 589295.
52. Zumla, A., et al., *Toxoplasma serology in Zambian and Ugandan patients infected with the human immunodeficiency virus.* Trans R Soc Trop Med Hyg, 1991. 85(2): p. 227-9.

53. L.Naing, T.W., B.N.Rusli, *Practical issues in calculating the sample size for prevalence studies,Medical statistics.* 2006. 6.

54. Montoya, J.G. and J.S. Remington, *Management of Toxoplasma gondii infection during pregnancy.* Clin Infect Dis, 2008. 47(4): p. 554-66.

55. Yazar, S., B. Eser, and M. Yay, *Prevalence of anti-toxoplasma Gondii antibodies in Turkish blood donors.* Ethiop Med J, 2006. 44(3): p. 257-61.

56. Zarkovic, A., et al., *Seropositivity rates for Bartonella henselae, Toxocara canis and Toxoplasma gondii in New Zealand blood donors.* Clin Experiment Ophthalmol, 2007. 35(2): p. 131-4.

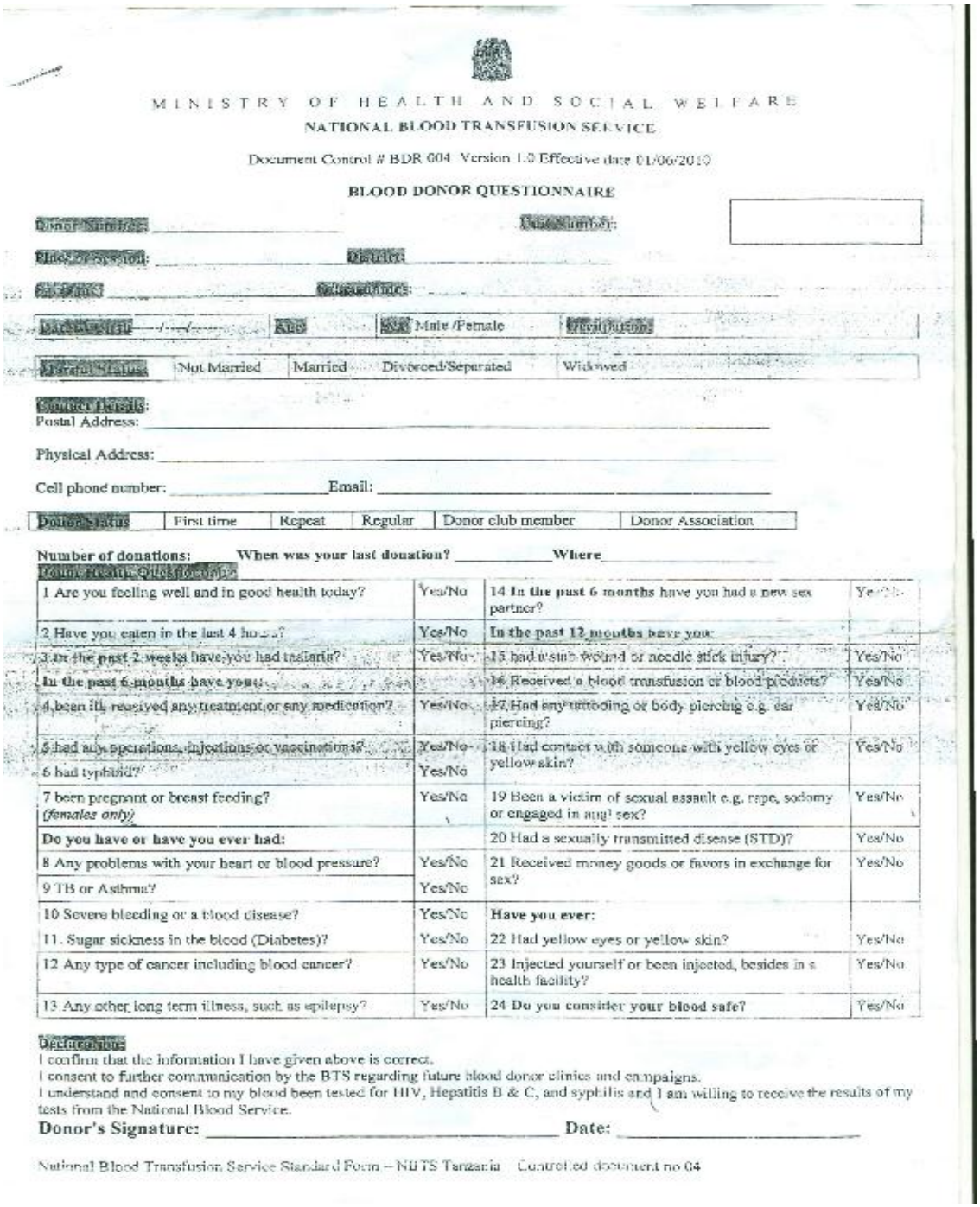
57. Sarwat, M.A., et al., *Toxoplasma gondii in Saudi blood donors a serological study using three tests.* J Egypt Soc Parasitol, 1993. 23(3): p. 751-7.

58. Coelho, R.A., M. Kobayashi, and L.B. Carvalho, Jr., *Prevalence of IgG antibodies specific to Toxoplasma gondii among blood donors in Recife, Northeast Brazil.* Rev Inst Med Trop Sao Paulo, 2003. 45(4): p. 229-31.

59. Elsheikha, H.M., et al., *Seroprevalence of and risk factors for Toxoplasma gondii antibodies among asymptomatic blood donors in Egypt.* Parasitol Res, 2009. 104(6): p. 1471-6.

APPENDICES

Appendix I: National blood Transfusion Service Standard Form



MINISTRY OF HEALTH AND SOCIAL WELFARE

NATIONAL BLOOD TRANSFUSION SERVICE

Document Control # BDR 004 Version 1.0 Effective date 01/06/2010

BLOOD DONOR QUESTIONNAIRE

Donor Surname: _____ Unit Number:

Place of Operation: _____ District: _____

Ward/Mtaa: _____ Village/Kitongoji: _____

Date of Birth: ___/___/_____ Age: _____ Sex: Male /Female Occupation: _____

Marital Status: Not Married | Married | Divorced/Separated | Widowed

Contact Details:

Postal Address: _____

Physical Address: _____

Cell phone number: _____ Email: _____

Donor Status: First time | Repeat | Regular | Donor club member | Donor Association

Number of donations: **When was your last donation?** _____ **Where** _____

For Health Officer/Nurse Only

1 Are you feeling well and in good health today?	Yes/No	14 **In the past 6 months** have you had a new sex partner?	Yes/No
2 Have you eaten in the last 4 hours?	Yes/No	**In the past 12 months have you:**	
3 In the past 2 weeks have you had malaria?	Yes/No	15 had a skin wound or needle stick injury?	Yes/No
In the past 6 months have you:		16 Received a blood transfusion or blood products?	Yes/No
4 been ill, received any treatment or any medication?	Yes/No	17 Had any tattooing or body piercing e.g. ear piercing?	Yes/No
5 had any operations, injections or vaccinations?	Yes/No	18 Had contact with someone with yellow eyes or yellow skin?	Yes/No
6 had typhoid?	Yes/No		
7 been pregnant or breast feeding? *(females only)*	Yes/No	19 Been a victim of sexual assault e.g. rape, sodomy or engaged in anal sex?	Yes/No
Do you have or have you ever had:		20 Had a sexually transmitted disease (STD)?	Yes/No
8 Any problems with your heart or blood pressure?	Yes/No	21 Received money goods or favors in exchange for sex?	Yes/No
9 TB or Asthma?	Yes/No		
10 Severe bleeding or a blood disease?	Yes/No	**Have you ever:**	
11. Sugar sickness in the blood (Diabetes)?	Yes/No	22 Had yellow eyes or yellow skin?	Yes/No
12 Any type of cancer including blood cancer?	Yes/No	23 Injected yourself or been injected, besides in a health facility?	Yes/No
13 Any other long term illness, such as epilepsy?	Yes/No	24 Do you consider your blood safe?	Yes/No

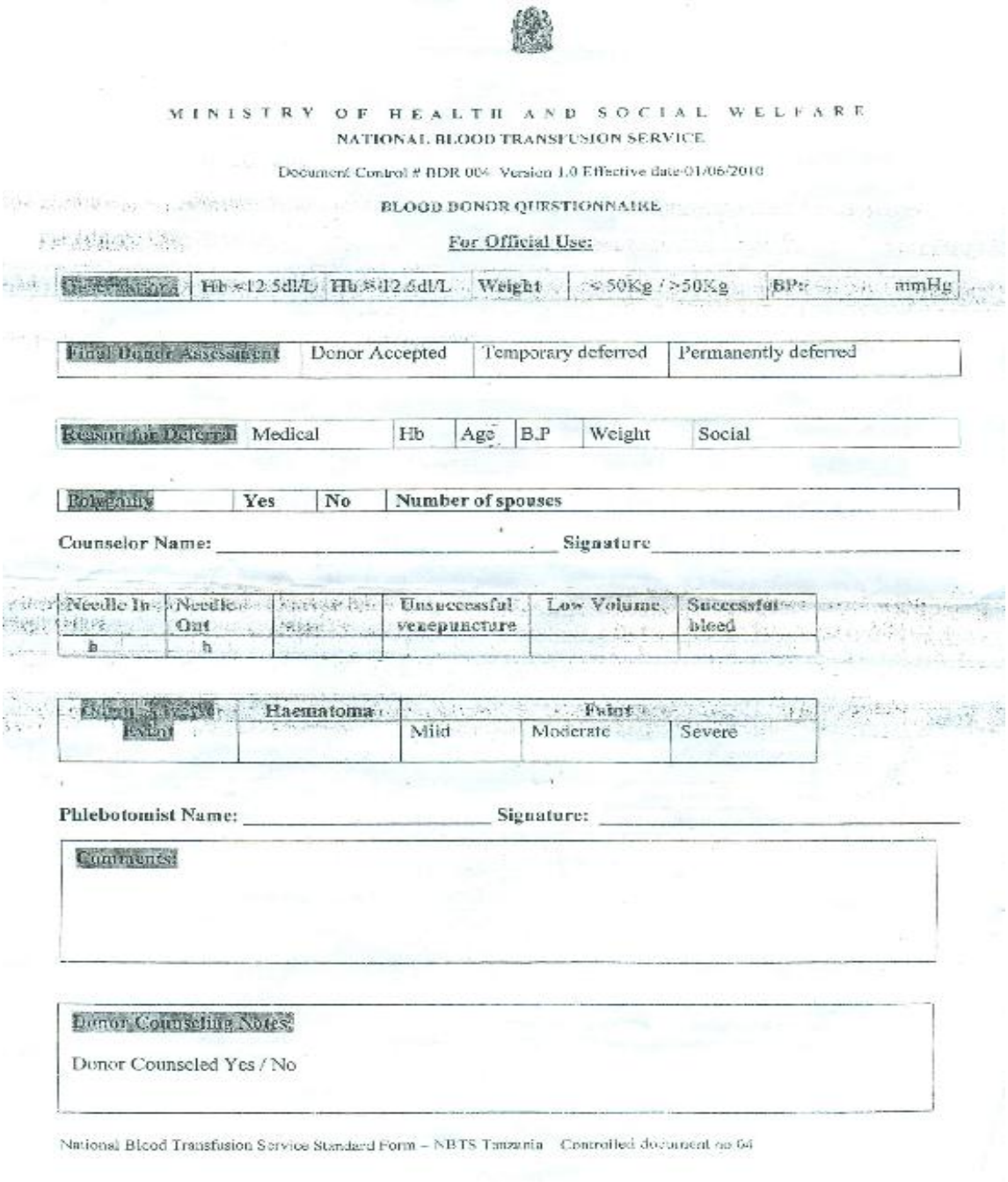
Declaration

I confirm that the information I have given above is correct.
I consent to further communication by the BTS regarding future blood donor clinics and campaigns.
I understand and consent to my blood been tested for HIV, Hepatitis B & C, and syphilis and I am willing to receive the results of my tests from the National Blood Service.

Donor's Signature: _____ **Date:** _____

National Blood Transfusion Service Standard Form – NBTS Tanzania Controlled document no 04

Appendix II: Private Health Laborats (Safety rules) Board



MINISTRY OF HEALTH AND SOCIAL WELFARE

NATIONAL BLOOD TRANSFUSION SERVICE

Document Control # BDR 004 Version 1.0 Effective date 01/06/2010

BLOOD DONOR QUESTIONNAIRE

For Official Use:

[illegible]	Hb <12.5dl/L	Hb ≥12.5dl/L	Weight	<50Kg / >50Kg	BP= mmHg

Final Donor Assessment	Donor Accepted	Temporary deferred	Permanently deferred

Reason for Deferral	Medical	Hb	Age	B.P	Weight	Social

Polygamy	Yes	No	Number of spouses

Counselor Name: _____ Signature _____

Needle In	Needle Out		Unsuccessful venepuncture	Low Volume	Successful bleed
h	h				

[illegible] Point	Haematoma	Faint		
		Mild	Moderate	Severe

Phlebotomist Name: _____ Signature: _____

Comments:

Donor Counseling Notes:

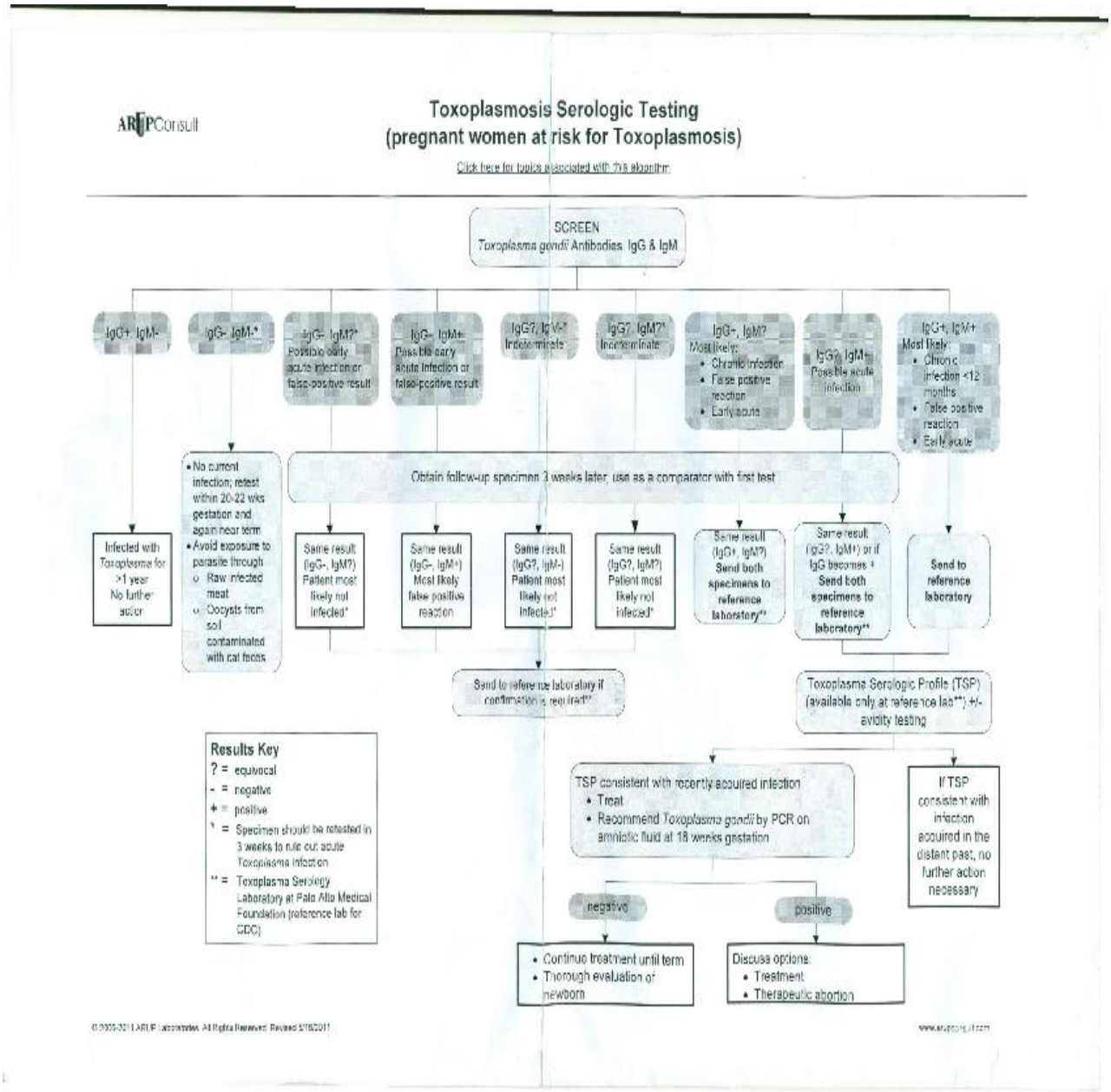
Donor Counseled Yes / No

National Blood Transfusion Service Standard Form – NBTS Tanzania Controlled document no 04

Appendix II: Private Health Laboratories (Safety rules) Board

1. Eating, drinking, smoking and applying cosmetics are prohibited in the laboratory.
2. Mouth pipetting is prohibited.
3. Staff must behave in safe and responsible manner at all times.
4. Appropriate protective clothing must be worn at all times when in the laboratory and whenever necessary, gloves should be worn.
5. The laboratory must be kept clean and tidy and should only contain items necessary for the work carried out.
6. All work surfaces must be appropriately decontaminated at the end of each working day and after any spillages.
7. All staff must wash their hands when starting to work and whenever they leave the laboratory.
8. Care must be taken to avoid the formation of aerosols or the splashing of material.
9. All contaminated waste must or reusable material must be appropriately decontaminated before disposal or reuse.
10. Access to the laboratory must be restricted to authorized personnel only.
11. All accidents must be recorded and reported immediately and appropriate action taken to prevent further occurrence.
12. All staff working in laboratories must be adequately trained both in the duties they perform and in all safety aspects of laboratory work.
13. Precaution against fire e.g keeping the fire extinguisher, bucket full of sand is mandatory in any laboratory.
14. First aid boxes to cover accidental swallowing or splashing with chemicals must always be at hand and should be replenished regularly.

Appendix III. Toxoplasmosis serologic testing



Appendix IV. Quality control certificate-IgM Assay

DIALAB ELISA TEST

DIALAB GmbH
Austria, A-2351 Wr. Neudorf
IZ-NÖ Süd
Hondastrasse, Objekt M55
Phone: ++43(0)2236 660910-0
fax: ++43(0)2236 660910-30
office@dialab.at
>>>> www.dialab.at

Quality Control Certificate

Qualitative (Cut-Off) Assay

Product: Toxoplasma IgM
Cat. No(s): V05079/V05079LV/V05079V
Exp. Date: 2012/12
Lot. No: 0053/1111007

Qualitative Test Evaluation
QC Panel (not included in the kit)

Specification		Standard	Pass
Blank		OD < 0,050	Y
Sensitivity	ToxoM-H	OD > 0,500	Y
	ToxoM-M	OD > 0,300	Y
	ToxoM-L	OD > 0,200	Y
Cut-Off Control		0,200 < OD < 0,450	Y
Specificity		≥ 19/20 OD < cut-off	Y
Positiv Control		OD > 0,500	Y
Negative Control		OD < 0,200	Y
Precision		CV < 15%	Y

Immunological reaction: 60 min at 37°C
Substrate reaction: 10 min at 37°C

[X] APPROVED
[ ] NOT APPROVED

DIALAB certifies that the product(s) identified above have been manufactured, tested and assembled according to internal procedures consistant with the requirements of EN ISO 13485:2003 and that the product(s) meet established Quality Control Specifications

2012-01-30
Date

Michael Akpetsi
Quality Control

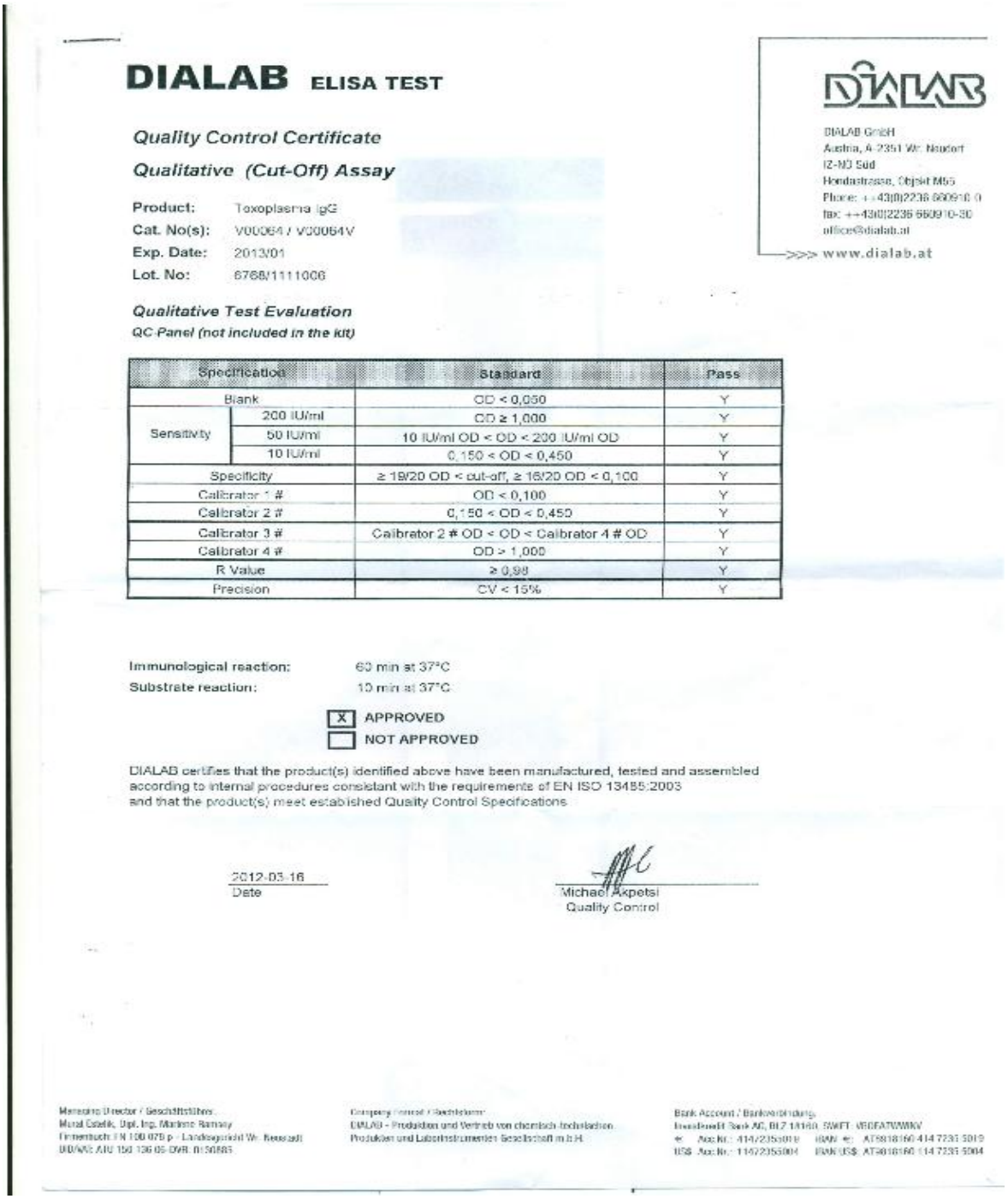
Rev. 04, 2009.02.10

Managing Director / Geschäftsführer:
Marcel Balaik, Dipl. Ing. Markus Ramsey
Firmenbuch FN 105 026 p - Landesgericht Wr. Neustadt
UID/VAT: ATU 150 136 06-DVR: 0130383

Company Format / Rechtsform:
DIALAB - Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.

Bank Account / Bankverbindung:
Investkredit Bank AG, BLZ 15130, SWIFT: WIDEATWWKV
€ Acc.Nr.: 414723350019 IBAN: € AT961813 0414 7235 0019
US$ Acc.Nr.: 114723350004 IBAN US$: AT961813 0114 7235 0004

Appendix V. Quality control certificate-IgG Assay



DIALAB ELISA TEST

DIALAB GmbH
Austria, A-2351 Wr. Neudorf
IZ-NO Süd
Hondastrasse, Objekt M55
Phone: ++43(0)2236 660910-0
fax: ++43(0)2236 660910-30
office@dialab.at
>>> www.dialab.at

Quality Control Certificate

Qualitative (Cut-Off) Assay

Product: Toxoplasma IgG
Cat. No(s): V00064 / V00064V
Exp. Date: 2013/01
Lot. No: 6768/1111006

Qualitative Test Evaluation
QC Panel (not included in the kit)

Specification		Standard	Pass
Blank		OD < 0,050	Y
Sensitivity	200 IU/ml	OD ≥ 1,000	Y
	50 IU/ml	10 IU/ml OD < OD < 200 IU/ml OD	Y
	10 IU/ml	0,150 < OD < 0,450	Y
Specificity		≥ 19/20 OD < cut-off, ≥ 16/20 OD < 0,100	Y
Calibrator 1 #		OD < 0,100	Y
Calibrator 2 #		0,150 < OD < 0,450	Y
Calibrator 3 #		Calibrator 2 # OD < OD < Calibrator 4 # OD	Y
Calibrator 4 #		OD > 1,000	Y
R Value		≥ 0,98	Y
Precision		CV < 15%	Y

Immunological reaction: 60 min at 37°C
Substrate reaction: 10 min at 37°C

[X] APPROVED
[ ] NOT APPROVED

DIALAB certifies that the product(s) identified above have been manufactured, tested and assembled according to internal procedures consistant with the requirements of EN ISO 13485:2003 and that the product(s) meet established Quality Control Specifications.

2012-03-16
Date

Michael Aspetsi
Quality Control

Managing Director / Geschäftsführer:
Murat Cetelik, Dipl. Ing. Marlene Ramsey
Firmenbuch: FN 100 079 p · Landesgericht Wr. Neustadt
UID/VAT: ATU 150 136 05 DVR: 0150885

Company Consist / Rechtsform:
DIALAB - Produktion und Vertrieb von chemisch technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.

Bank Account / Bankverbindung:
Investkredit Bank AG, BLZ 19160, SWIFT: VBOEATWWINV
€ Acc.No.: 414/23500 IBAN €: AT581916041472315000
US$ Acc.No.: 1147/23500 IBAN US$: AT301916041472315004

Appendix VI: Principle of the Assay

The *toxoplasma* IgG EIA Test Kit is a solid phase enzyme immunoassay based on indirect principle for the qualitative and quantitative detection of IgG antibodies to *toxoplasma* in human serum or plasma. The microwell plate is coated with *toxoplasma* recombinant antigens. During testing, the specimen diluent and the specimens are added to the antigen coated microwell plate and then incubated. If the specimens contain IgG antibodies to *toxoplasma*, it will bind to the antigens coated on the microwell plate to form immobilized antigen-*toxoplasma* IgG antibody complexes. If the specimens do not contain IgG antibodies to *toxoplasma*, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. The enzyme-conjugated anti-human IgG antibodies are added to the microwell plate and then incubated. The enzyme-conjugated anti-human IgG antibodies will bind to the immobilized antigen-*toxoplasma* IgG antibody complexes present. After the second incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue colour indicating the amount of *toxoplasma* IgG antibodies present in the specimens. Sulphuric acid solution is added to the microwell plate to stop the reaction producing a colour change from blue to yellow. The colour intensity, which corresponds to the amount of *toxoplasma* IgG antibodies present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 nm.

ASSAY PROCEDURE FOR IgG

MATERIALS PROVIDED

Microwell plate: 12x8-wells strips coated with recombinant *Toxoplasma gondii* antigens.

Enzyme Conjugate: 1 vial of 12 ml; Anti-human IgG antibody bound to peroxidase; Preservative: 0,1 % ProClinTM 300

Wash Buffer conc.: 1 vial of 50 ml; 25x conc., Tris-HCl buffer containing 0.1 % Tween 20; Preservative: 0.1 % ProClinTM 300

Specimen Diluent: 1 vial of 12 ml; Tris buffer, Preservative: 0.1 % ProClinTM 300

Substrate A: 1 vial of 8 ml; Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0.1 % ProClinTM 300

Substrate B: 1 vial of 8 mL; Buffer containing tetramethylbenzidine (TMB); Preservative: 0.1 % ProClinTM 300

Stop Solution: 1 vial of 8 ml; 2M Sulphuric acid

Calibrators: 4 vials of 1 ml each. Diluted human serum containing the following amounts of Toxoplasma IgG antibodies; Preservative: 0.1 % ProClinTM 300:

1) Std. 0 IU/ml 2) Std 10 IU/ml

3) Std. 50 IU/ml 4) Std. 200 IU/ml

9. Plate sealer

10. Package Insert

REAGENTS PREPARATION

WASH BUFFER:

Working Wash Buffer was prepared by diluting the Concentrated Wash Buffer 1:25 and poured the contents of the bottle in a graduated cylinder and filled it with freshly distilled water to 1250 mL. It was stable for 2 weeks at 15-30°C.

Note: If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C

until all crystals dissolve.

All reagents and specimens were allowed to reach room temperature (15-30°C) prior to testing. The procedures were strictly followed. The calibrators so were arranged so that well A1 is the Blank well. From well A1, arrange the calibrators were in a vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration.

1. Leave A1 as Blank well.

2. Add 100 µL of Calibrator 1 in wells B1 and C1. (Blue Reagent)

Add 100 µL of Calibrator 2 in wells D1 and E1. (Blue Reagent)

Add 100 µL of Calibrator 3 in wells F1 and G1. (Blue Reagent)

Add 100 µL of Calibrator 4 in wells H1 and A2. (Blue Reagent)

3. Add 100 µL of Specimen Diluent to assigned wells starting at B2. The colour of Specimen Diluent is green.

Add 5 µL of specimen to assigned wells starting at B2. Then a colour change from green to blue will occur to verify that the specimen has been added.

Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.

4. Mix gently by swirling the microwell plate on a flat bench for 30 seconds.

Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.

5. Remove the Plate Sealer.

Wash each well 5 times with 350 µL of Working Wash Buffer per well, and then remove the liquid.

Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried. Note: Improper washing may cause false positive results.

6. Add 100 µL of Conjugate to each well except for the Blank well. The colour of Conjugate is red.

7. Cover the microplate plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.

8. Repeat Step 5.

9. Add 50 µL of Substrate A to each well. (Clear Reagent)

Add 50 µL of Substrate B to each well. (Clear Reagent)

Then a blue color should develop in wells containing Positive specimens.

10. Mix gently then cover microwell plate with Plate Sealer and incubate in a water bath or incubator at 37°C ± 2°C for 10 minutes ± 1 minute.

11. Remove the Plate Sealer.

Add 50 µL of Stop Solution to each well. (Clear Reagent)

Then a yellow colour should develop in wells containing Positive specimens.

12. Read at 450/630-700 nm in 30 minutes.

Note: Microwell plate can also be read at 450 nm, but it is strongly recommended

to read it at 450/630-700 nm for better results.

Check the validation requirements below to determine if the test results are valid

Item	Validation Requirements
Blank Well	Blank Absorbance should be < 0.050 if read at 450/630-700 nm Note: It should be < 0.100 if read at 450 nm
Calibrator 1	Mean Absorbance after subtraction of Blank Absorbance should be < 0.100
Calibrator 2	Mean Absorbance after subtraction of Blank Absorbance should be > 0.150 and <0.450
Calibrator 3	Mean Absorbance after subtraction of Blank Absorbance should be > Calibrator 2 and < Calibrator 4
Calibrator 4	Mean Absorbance after subtraction of Blank Absorbance should be > 1.000

NOTE: The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

INTERPRETATION OF RESULTS-IgG

Qualitative- Calculate the Index Value to obtain qualitative specimen results.

1. If the test is valid, obtain Cut-Off Value by subtracting the Blank Absorbance from the Mean Absorbance of Calibrator 2. See an example of Cut-Off Value calculation below.

ITEM	ABSORBANCE
Blank Absorbance: Well A1	**0.001**
Cut-Off Value: Mean Absorbance of **Calibrator 2 – Blank Absorbance**	**0.255 – 0.001 = 0.254**

2. Calculate the Index Value by dividing the Specimen Absorbance by the Cut-Off Value, and then read the results by referring to the Interpretation of Results table below

ITEM	ABSORBANCE
Specimen: Well B2	**0.779**
Cut-Off Value	**0.254**
Index Value: Specimen/Cut-Off Value	**0.779/0.254 = 3.067**

Interpretation of Results – Qualitative and Quantitative

RESULTS	**Qualitative**	**Quantitave**
	Index value	Concentration
Negative	< 0,9	< 9,0 U/mL
Positive	> 1,1	≥ 11,0 U/mL
Equivocal	≥ 0,9 and ≤ 1,1	9,0 – 11,0 U/mL

NOTE: For Equivocal results, the specimen should be retested. Specimens that are repeatedly Equivocal after retest should be confirmed using an alternate method. If the results remain Equivocal; collect a new specimen in two weeks. If the new specimen is Positive, the specimen is presumed to be Positive.

PRINCIPLE OF THE ASSAY

The *Toxoplasma* IgM EIA Test Kit is a solid phase enzyme immunoassay based on immunocapture principle for the qualitative detection of IgM antibodies to *toxoplasma* in human serum or plasma. The microwell plate is coated with antihuman IgM antibodies. During testing, the specimen diluent and the specimens are added to the antibody coated microwell plate and then incubated. If the specimens contain IgM antibodies to *toxoplasma,* it will bind to the antibodies coated on the microwell plate to form immobilized anti-human IgM antibody-*toxoplasma* IgM antibody complexes. If the specimens do not contain IgM antibodies to *toxoplasma*, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. The enzyme conjugated recombinant *toxoplasma* antigens are added to the microwell plate and then incubated. The

enzyme-conjugated recombinant *toxoplasma* antigens will bind to the immobilized anti-human IgM antibody-*toxoplasma* IgM antibody complexes present. After the second

incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of *toxoplasma* IgM antibodies present in the specimens. Sulphuric acid solution is added to the microwell plate to stop the reaction producing a colour change from blue to yellow. The colour intensity, which corresponds to the amount of *toxoplasma* IgM antibodies present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450nm.

ASSAY PROCEDURE FOR IgM

MATERIALS PROVIDED

1. Microwell plate: 12x8-wells strips coated with anti-human IgM antibodies

2. Enzyme Conjugate: 1 vial of 12 ml; Recombinant *Toxoplasma gondii* antigens bound to peroxidase; Preservative: 0,1 % ProClinTM 300

3. Wash Buffer conc.: 1 vial of 50 ml; 25x conc., Tris-HCl buffer containing 0,1 % Tween 20; Preservative: 0.1 % ProClinTM 300

4. Specimen Diluent: 1 vial of 12 ml; Tris buffer, Preservative: 0,1 % ProClinTM 300

5. Substrate A: 1 vial of 8 ml; Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0,1 % ProClinTM 300

6. Substrate B: 1 vial of 8 mL; Buffer containing tetramethylbenzidine (TMB); Preservative: 0,1 % ProClinTM 300

7. Stop Solution: 1 vial of 8 ml; 2M Sulfuric acid

8. *Toxoplasma* IgM Negative Control: 1 vial of 1 mL; Diluted human serum nonreactive for *Toxoplasma* IgM antibodies; Preservative: 0,1 % ProClinTM 300

9. *Toxoplasma* IgM Cut-Off Calibrator: 1 vial of 1 mL; Diluted human serum weakly reactive for Toxoplasma IgM antibodies; Preservative: 0,1 % ProClinTM 300

10. *Toxoplasma* IgM Positive Control: 1 vial of 1 mL; Diluted human serum highly reactive for *Toxoplasma* IgM antibodies; Preservative: 0,1 % ProClinTM 300

9. Plate sealer

10. Package Insert

REAGENTS PREPARATION

WASH BUFFER:

Working Wash Buffer was prepared by diluting the Concentrated Wash Buffer 1:25 and poured the contents of the bottle in a graduated cylinder and filled it with freshly distilled water to 1250 mL. It was stable for 2 weeks at 15-30°C.

Note: If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C

until all crystals dissolve.

All reagents and specimens were allowed to reach room temperature (15-30°C) prior to testing. The procedures were strictly followed. The calibrators so were arranged so that well A1 is the Blank well. From well A1, arrange the calibrators were in a vertical

The procedure below assigns specific wells arranged in a vertical configuration.

1. Leave A1 as Blank well.

2. Add 100 µL of Negative Control in wells B1 and C1. (Blue Reagent)

Add 100 µL of Cut-Off Calibrator in wells D1 and E1. (Blue Reagent)

Add 100 µL of Positive Control in wells F1 and G1. (Red Reagent)

3. Add 100 µL of Specimen Diluent to assigned wells starting at H1. The colour of Specimen Diluent is green.

Add 5 µL of specimen to assigned wells starting at H1. Then a colour change from green to blue will occur to verify that the specimen has been added. Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.

4. Mix gently by swirling the microwell plate on a flat bench for 30 seconds. Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.

5. Remove the Plate Sealer.

Wash each well 5 times with 350 µL of Working Wash Buffer per well, and then remove the liquid. Turn the microwell plate upside down on absorbent tissue for a few seconds.

Ensure that all wells have been completely washed and dried.

Note: Improper washing may cause false positive results.

6. Add 100 µL of Conjugate to each well except for the Blank well. The color of Conjugate is red.

7. Cover the microplate plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.

8. Repeat step 5.

9. Add 50 µL of Substrate A to each well. (Clear Reagent)

Add 50 µL of Substrate B to each well. (Clear Reagent)

Then a blue color should develop in wells containing Positive specimens.

10. Mix gently then cover microwell plate with Plate Sealer and incubate in a water bath or incubator at 37°C ± 2°C for 10 minutes ± 1 minute.

11. Remove the Plate Sealer.

Add 50 µL of Stop Solution to each well. (Clear Reagent)

Then a yellow colour should develop in wells containing Positive specimens.

12. Read at 450/630-700 nm within 30 minutes.

Note: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450/630-700 nm for better results.

Check the validation requirements below to determine if the test results are valid.

Item	Validation Requirements
Blank Well	Blank Absorbance should be < 0.050 if read at 450/630-700 nm Note: It should be < 0.100 if read at 450 nm
Negative Control	Mean Absorbance after subtraction of Blank Absorbance should be < 0.200
Cut-Off Calibrator	Mean Absorbance after subtraction of Blank Absorbance should be > 0.200 and < 0.450
Positive Control	Mean Absorbance after subtraction of Blank Absorbance should be > 0.500

INTERPRETATION OF RESULTS-IgM

Qualitative

Calculate the Index Value to obtain qualitative specimen results.

1. If the test is valid, obtain Cut-Off Value by subtracting the Blank Absorbance from the Mean Absorbance of Cut-Off Calibrator. See an example of Cut-Off calculation below.

ITEM	ABSORBANCE
Blank Absorbance: Well A1	0.001
Cut-Off Value: Mean Absorbance of Cut – Off Calibrator – Blank Absorbance	0.311 – 0.001 = 0.310

2. Calculate the Index Value by dividing the Specimen Absorbance by the Cut-Off Value, and then read the results by referring to the Interpretation of Results table below.

ITEM	ABSORBANCE
Specimen: Well H1	0.779
Cut-Off Value	0.310
Index Value: Specimen/Cut-Off Value	0.779/0.310 = 2.513

18. Interpretation of Results – Qualitative

RESULTS	**QUALITATIVE**
NEGATIVE	< 0,9
POSITIVE	>1.1
EQUAVOCAL Between 0.9 and 1.1	

*NOTE: For Equivocal results, the specimen should be retested. Specimens that are repeatedly Equivocal after retest should be confirmed using an alternate method. If the results remain Equivocal, collect a new specimen in two weeks. If the new specimen is Positive, the specimen is presumed to be Positive.