SEROPREVALENCE OF HUMAN T-CELL LYMPHOTROPIC VIRUS 1 AND 2 IN BLOOD DONORS AT TWO BLOOD DONOR CENTRES IN NAIROBI, KENYA.

DR. NAOMI MORAA ARIAGA MB ChB (MB.Ch.B)

H58/81322/2015

Registrar, MMed Human Pathology

Department of Human Pathology, School of Medicine,

College of Health Sciences, University of Nairobi

P.O.BOX 19676 – 00202, Nairobi, Kenya

A Research Dissertation Submitted In Part Fulfillment for the Award of Degree in Masters of Medicine in Human Pathology at the University of Nairobi.

2019

STUDENT'S DECLARATION

I declare that this proposal is my original work under the guidance of my supervisors and has not been submitted to the University of Nairobi or any other institution of higher learning.

Dr. Ariaga Naomi Moraa. (MB.Ch.B)
University of Nairobi.
Signature: ……. Date: ……

SUPERVISORS' DECLARATION

We confirm that this proposal has been developed under our guidance and approval as University supervisors.

Dr. Peter M. Maturi

Unit Head, Hematology and Blood Transfusion Unit,

Department of Human Pathology,

School of Medicine, University of Nairobi.

Signature……. Date……

Dr. Valerie K. Magutu

Tutorial Fellow, Hematology and Blood Transfusion Unit,

Department of Human Pathology,

School of Medicine, University of Nairobi.

Signature……. Date……

ACKNOWLEDGEMENTS

I would like to thank my family and friends for their constant support and encouragement throughout this study.

My supervisors Drs. Peter Maturi and Valerie Magutu for their guidance.

The Nairobi Regional Blood Transfusion Centre and Kenyatta National Hospital Blood Transfusion Unit staff for their assistance in collection of the blood sample.

Laboratory staff at UNITID for their assistance during the laboratory work.

My fellow postgraduate colleagues in the department of Human Pathology for their encouragement and support.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ATL	Adult T-cell leukemia/lymphoma
BTU	Blood Transfusion Unit
CMV	Cytomegalovirus
EIAs	Enzyme Immunoassays
ELISA	Enzyme linked immunosorbent assay
HAM/TSP	HTLV-associated myelopathy/tropical spastic paraparesis
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency virus
HRP	Horseradish peroxidase
HTLV	Human T-cell lymphotrophic virus
Ig	Immunoglobulin
KNH-UoN ERC	Kenyatta National Hospital –University of Nairobi Ethical Research Committee
MB.Ch.B	Bachelor of Medicine, Bachelor of Surgery
MMed	Masters of Medicine
MOH	Ministry of Health
NBTS	National Blood Transfusion Service
RBTC	Regional Blood Transfusion Center
STLV	Simian T-lymphotropic virus
TTIs	Transfusion-Transmissible Infections
UNITID	University of Nairobi Institute of Tropical and Infectious Disease
UON	University of Nairobi
VDRL	Venereal Disease Research

ABSTRACT

Background: Human T-cell Lymphotrophic Virus is known to cause Adult T-cell leukemia/lymphoma and HTLV-associated myelopathy/tropical spastic para-paresis. Transfusion of contaminated blood is the major mode of HTLV-1/2 transmission. Many countries have documented the prevalence of HTLV-1/2 in blood donors. This study aimed at determining the HTLV-1/2 prevalence among healthy blood donors in Blood Transfusion Center at Kenyatta National Hospital and the Regional blood Transfusion Center.

Objectives: To determine the Human T-cell Lymphotrophic Virus -1/2 seroprevalence among eligible blood donors and to correlate HTLV-1/2 seroprevalence with other routinely tested Transfusion Transmissible Infections in Kenyatta National Hospital Blood Transfusion Unit and the Nairobi Regional Blood Transfusion Centre.

Study design: A descriptive cross-sectional study.

Materials and methods: One hundred and thirty-eight (138) blood donors who met the national guidelines for blood donation were consecutively recruited into the study. A questionnaire was administered and socio-demographic data recorded. Blood samples were drawn for routine tests and HTLV-1/2 serology which was carried out using HTLV-1/2 immunoglobulin G antibody enzyme linked immunosorbent assay (ELISA) technique. The results of routinely screened transfusion transmitted infections (Human Immunodeficiency Virus, hepatitis B virus, hepatitis C virus and syphilis) were obtained from the donor registers at the Blood Transfusion Unit at Kenyatta National Hospital and the Regional Blood Transfusion Center.

Results: One hundred and thirty eight study participants were recruited, 71% (98) were male and 40 (29%) females. The age of the participants ranged between 18 to 59 years, 51% of participants' age ranged between 21 and 30 years while those above 51 years and less than 20 years were the least. None of the study participants tested positive for HTLV-1/2 yielding a seroprevalence of 0% in this population. The prevalence rates of the routinely screened transfusion transmitted infections: HIV, hepatitis B virus, hepatitis C virus and syphilis were 5.7%, 3.6%, 0.7%, 0% respectively. A correlation could not be made between routinely screened infections and HTLV-1/2 infection as the seroprevalence of HTLV was very low.

Conclusion: Human T-cell Lymphotrophic Virus-1/2 seroprevalence among eligible blood donors at the KNH BTU and Nairobi RBTC was low (0%) and may indicate that routine screening of the virus in this population of blood donors is unnecessary. Multiregional studies should be encouraged in order to expand the Kenya Transfusion Transmissible Infections Policy.

1.0 INTRODUCTION

Blood transfusion is a vital therapeutic procedure used as a lifesaving intervention in all clinical disciplines. Blood transfusion, like all medical interventions, is generally safe but carries the risk of transfusion-transmissible infections including Hepatitis B virus, Hepatitis C virus, Human Immunodeficiency Virus, syphilis, malaria, Human T cell Lymphotrophic Virus and Cytomegalovirus. A global study done by Antoine Gessain et al in 2005 showed regional disparity in prevalence of HTLV-1/2 in Sub-Saharan Africa with a seroprevalence range of 0.2-5.5% (1), the highest in Nigeria at 5.5% and the same study showed the seroprevalence in Rwanda to be low at 0.2%. In contrast, a study done in Kenya in cervical smears showed a high prevalence of HTLV/HIV coinfection at 19.5 % (2), however no studies have been done among blood donors. Many low and middle income countries including Kenya, have a higher prevalence of some TTIs and this poses a greater risk of infection (3). People HTLV and co-infected with HIV and hepatitis C have a high risk of developing peripheral neuropathy(4) and liver disease(5) respectively. HBV and HTLV-I viruses are transmitted in same mode and this increases the risk of acquiring either of the infection. HTLV-1/2 can modify the course of syphilis infection and cause severe infection (6). Blood is normally screened for HIV, HCV, HBV, Syphilis but screening of HTLV is not universal. A study done in India revealed that with each unit of blood transfused, there is 1% risk of transmitting a TTIs (7). This implies that there exists a need to expand the screening of donor blood in these countries, and Kenya in particular, beyond the routinely screened infections to include other TTIs like HTLV-1/2. HTLV-1/2 transmission through blood is only preventable by screening of donated blood.

HTLV is a Retrovirus of which four types have been documented: HTLV 1-4. Epidemics are caused by type one and two which are found globally.

Modes of HTLV-1/2 transmission include: mother to child transmission that accounts for 20% of the infections (8) and transfusion of contaminated blood accounts for 15-60% of the infections (1) and sexual transmission which occurs in people with genital sores, ulcers or through unprotected sex with an infected partner (9). Intravenous exposure of contaminated blood is the most efficient way of HTLV-1/2 infection (8). People transfused with packed red cells are at a high risk of being infected with the virus because transmission of the virus is dependent on cell to cell contact and not cell free virion (8).

HTLV-1/2 affects primarily lymphoid cells. HTLV-2 primarily infects CD8+ cells while HTLV-1 infects CD4+ cells leading to their increased proliferation (10). With regards to disease burden, HTLV-1 is more significant because it is the etiologic agent of multiple disorders. However, of the 20 million people affected with HTLV, only 3-5% develop Adult T-cell leukemia (11). HTLV-2 causes mild central nervous system disorders and lung infections. HTLV-3 and HTLV-4 are not associated with any illnesses.

Enzyme Immunoassays, particularly Enzyme-linked immunosorbent assay are the most commonly employed method used in diagnosis of HTLV-1/2 virus. The Center for Disease Control and Prevention recommended in 1988 the screening of HTLV-1/2 antibodies in various high income countries including USA, Canada and Caribbean (12).

The aim of this study was to determine the seroprevalence of HTLV 1/2 in healthy donors and further correlate HTLV-1/2 seroprevalence with other routinely tested TTIs.

2.0 LITERATURE REVIEW

Transfusion is an essential service that has been in clinical use for a very long time. In Africa approximately three million units of blood are collected annually (13). In the West red cells units are predominantly used while in Africa whole blood is the main component (13). Blood transfusion, like all medical interventions is generally safe but also carries the risk of infections, metabolic and immunologic complications. The risk of being infected with TTIs is higher in Africa and this is attributed to the high prevalence of these diseases. For instance, 5-10% of HIV cases in Africa results from transfusion of contaminated blood and this threatens the safety of blood transfusion services (14). Screening of donated blood depends on the disease prevalence in a region. In high income countries donated blood is screened for HIV, HBV, HTLV, HCV and West Nile virus. The Kenya National Blood Transfusion Service currently screens donor blood for syphilis, HBV, HCV and HIV 1 and 2.

2.1 Human T-cell Lymphotrophic Virus

Human T-cell Lymphotrophic Virus-1

Human T-cell Lymphotrophic Virus-1 was discovered in 1979 and four types have been discovered. HTLV-1 is a Retrovirus in the genus Deltaretrovirus and subfamily of Orthoretrovirinae. Seven subtypes of HTLV-1 have been reported (15). Out of the 20 million people infected with HTLV-1 globally, only 2%-8% will develop an HTLV-1–associated disease e.g Adult T-cell Leukemia/Lymphoma, Tropical spastic paraparesis/HTLV-1–associated myelopathy, uveitis, infective dermatitis during their lifetime(16).

Human T-cell Lymphotrophic Virus -2

Human T-cell lymphotrophic Virus-2 belongs to Retroviridae family and the genus Deltatrovirus. The burden of HTLV-2 infection in the world is about 6 to12 fold lower than that of HTLV-1(17).

Human T-cell Lymphotrophic Virus-3 and Human T-cell Lymphotrophic Virus-4

HTLV-3 and 4 were discovered in Cameroon in 2005 (18). These viruses infect hunters of monkey through bites and scratches. These viruses have not been associated with any human disease (19).

2.2 Epidemiology

HTLV-1/2 is present globally, with areas of both high endemicity and areas without the virus. Type one and two actively spread epidemics affecting 15-20 million individuals globally (8). HTLV-1/2 seroprevalence varies in most geographic regions. In endemic regions, the HTLV-1 seroprevalence in adults is 1–2% but it can be as high as 20–40% (8). High endemic regions include the Southwestern part of Japan with a prevalence of 15-30%, Caribbean areas with a prevalence of 3-6%, Colombia, French Guyana and Gabon (1) . HTLV-I/2 seroprevalence is age and gender dependent, prevalence is higher in advance age and in females. HTLV-1/2 seroprevalence increases with advance age and this may be due to an age-cohort effect (1). In females the high seroprevalence is because of accumulation of sexual exposures and because of the more efficient mode of sexual transmission from male to females (1). The education level of an individual is important in determining health status. A study done in Brazil showed that majority of the study subjects 67.3% who were co-infected with HIV/HTLV were either illiterate or had attained secondary education (20). The same study showed that the study participants who were infected earned less than 250 Dollars (25000 Kenyan shillings) per month.

Africa is most likely an endemic area for HTLV-1/2 infection but few countries have documented the seroprevalence of HTLV-1/2 (1). Studies done in West Africa have shown a prevalence range of 0.2-3% (1). The seroprevalence of HTLV-1in Cameroon is between 0.5 to 2% (1). Studies done in Gabon have documented a seroprevalence of 5-10% (1). Very few studies have been done in East Africa despite ATL and/or TSP/HAM cases being stated. East Africa is documented to be less endemic for HTLV-1/2 as compared to other African countries (1). For instance in Rwanda HTLV-1 seroprevalence is 0.2% and in Mozambique it is between 0.9-2.3% (1). HTLV-1 seroprevalence in Uganda is 0.5% (21). However in Kenya, a study done in cervical smears of women attending Kenyatta National Hospital showed a high prevalence of HTLV-1 at 20.4% (2).

2.3 Transmission

The main modes for HTLV-1/2 transmission include, transfusion, Mother to child and sexual.

Vertical transmission

This is mainly seen in mother to infant transmission. About 25 % of infants who are breast fed also become infected (1). High HTLV-1/2 pro-viral load in milk and prolonged breast feeding are risk factors for transmission (1). The seroconversion time frame ranges between 1-3 years (22).

Sexual transmission

Sexual transmission is responsible for the HTLV-1/2 seroprevalence in women because of the efficient mode of transmission from male to female (1).

Transfusion

A study done in Japan revealed that transfusion of blood products contaminated with HTLV-1 is the major mode of transmission and this accounts for 15-60% of the infections (1). Transmission occurs through transfusion of cellular blood products (whole blood, red blood cells and platelets) but not with the plasma derivatives (23).

Transplant

HTLV-1/2 causes diseases that are associated with high risk of morbidity and mortality as it infects CD 4+ and CD 8+ T cells leading to impaired cellular immunity. This is made worse especially in immunocompromised patients like transplant patient who can also acquire HTLV-1/2 through transplant organs e.g liver transplant and kidney transplant (24). The risk of transmission of HTLV varies with the prevalence of the HTLV1/2 infection in the population.

2.4 Human T-cell Lymphotrophic Virus associated diseases

Adult T cell leukemia/lymphoma

Adult T-cell leukemia or lymphoma was discovered in 1977 in Southwestern Japan (25). Globally 15 to 20 million patients are affected by HTLV-1/2 and 90% of the infected people are asymptomatic (26). Out of 20 million people who are infected approximately 2% to 6% will develop this aggressive adult T-cell leukemia (26). Blood transfusion accounts for 15-60 % of infections especially in areas of high endemicity. Screening of blood is the only effective strategy in prevention of HTLV virus infection as no vaccine has been developed (12). The latency period for developing ATL is approximately 30–50 years after infection.

Pathogenesis

HTLV-1 infects many cell types, including lymphoid cells, fibroblasts and macrophages. HTLV-1 is transmitted by contact of an infected cell with uninfected cell and not by extracellular virus (27). The single-stranded RNA virus within infected cells is then converted to pro-viral DNA and eventually integrated into host DNA by the enzyme viral integrase.

Adult T cell Leukemia can present as acute or chronic. The acute disease presents in approximately 60% of the patients as marked leukocytosis with atypical lymphocytes and eosinophilia. Chronic disease presents with progressively enlarging lymph nodes and increased lymphocytes in blood. Once infected, HTLV persists in CD4+ cells leading to a prolonged interaction between the virus and the immune system. Infection with HTLV-1/2 cause's immune dysfunction which leads to various diseases and in people already immunocompromised like HIV/AIDS patients, patients on therapy for various malignancies this can worsen the already elevated rate of opportunistic coinfections (29).

Human T-cell Lymphotrophic Virus myelopathy/tropical spastic para-paresis

Human T-cell Lymphotrophic Virus-I-associated myelopathy/tropical spastic para-paresis is a progressive neurological illness that presents with spastic para-paresis, bowel/bladder dysfunction and lower limb sensory changes. 0.25–3.8% of the people infected with HTLV-1/2 will develop HAM/TSP (23).

HTLV-1 uveitis

HTLV-1 uveitis presents unilaterally as blurred vision and floaters. Optic atrophy, cataract, vitreous opacities, glaucoma and retinal vascular occlusion are the complication that occur following HU.

2.5 HTLV /HIV, Hepatitis B virus, Hepatitis C virus, Syphilis Coinfection

Human T-cell Lymphotrophic Virus, syphilis, HIV, HBV, HCV have common transmission routes. Human T-cell Lymphotrophic Virus and HIV are both retroviruses that have the same mode of transmission and can coexist. Human Immunodeficiency Virus infected people are at risk for human T-lymphotrophic virus coinfection and central nervous system disease (28). The prevalence of HIV/HTLV coinfection is 10.9% (28). A study done in cervical smears in women in Kenya showed the prevalence of HTLV/HIV coinfection of 19.5% (2). HTLV-1/HIV coinfection increases the risk of developing myelopathy and it also cause rapid progression to Acquired Immune Deficiency Syndrome (29). Blood is routinely screened for HIV but screening of HTLV is not universal because the prevalence of HTLV-1/2 varies in regions. In middle and low income countries with low prevalence of HTLV-1/2, blood is not screened for the virus instead resources are allocated to more prevalent TTIs e.g HIV.

2.6 Rationale

Very few studies have been done in East Africa on the seroprevalence of HTLV-1/2 virus. In Kenya only one study has been done in cervical smears of women attending Kenyatta National Hospital. No study had been done among blood donors and therefore there is paucity of data. This study will add to the available evidence from the world, provide local data and also form the basis for future studies.

The prevalence of HTLV-1/2 varies globally. Africa is an endemic area for HTLV-1/2 with a prevalence of 6.6% to 8.5% (30). Infection with HTLV-1/2 causes immune dysfunction which leads to various diseases like ATL, HAM/TSP that cause significant morbidity and mortality. The immunocompromised patients have an increased risk of TTIs and a high demand for blood transfusion, enhance their need for safe blood products.

2.7 Broad Objective

To determine the seroprevalence of HTLV-1/2 among blood donors at the KNH Blood Transfusion Unit and the Nairobi RBTC, Kenya.

2.8 Specific Objectives

1. To determine the seroprevalence of HTLV-1/2 among blood donors at the KNH Blood Transfusion Unit and the Nairobi RBTC, Kenya.
2. To determine the correlation between HTLV-1/2 and routinely screened infections (hepatitis B and C virus, HIV and syphilis) among blood donors at the KNH Blood Transfusion Unit and the Nairobi RBTC, Kenya.

3.0 METHODOLOGY

3.1 Study Design

The study was a descriptive cross-sectional study carried out to determine the prevalence of HTLV-1 and 2 at the KNH Blood Transfusion Unit and the Nairobi RBTC, Kenya.

3.2 Study Setting

The study was conducted at Kenyatta National Hospital Blood Transfusion Unit and the Nairobi Regional Blood Transfusion Centre and UNITID laboratory. Approximately 30 donors visit the KNH BTU daily and 100 donors per day on average are recruited during blood drives carried out by Nairobi RBTC.

The Blood Transfusion Unit is located within Kenyatta National Hospital which is the largest referral hospital in Kenya located along Hospital Road off Ngong Road. Kenyatta National Hospital Blood transfusion Unit mainly deals with blood banking and donation service dealing with replacement donors. KNH BTU provides blood donation services seven days a week from 8 a.m. to 5 p.m in the evening. The Regional Blood Transfusion Centre is situated next to KNH. Most of its blood supply is obtained during blood drives to various institutions like schools and open air events. The blood drives occur on average two times a week and the donors are entirely voluntary donors who satisfy the criteria for blood donation.

3.3 Study Population

One hundred and thirty eight blood donors at KNH BTU and the Nairobi RBTC who met the preset criteria for blood donation were recruited. The Kenyan blood donor population includes students from secondary and tertiary institutions and the general public recruited during blood donation campaigns conducted in various parts of Nairobi County by the RBTC.

3.4 Inclusion/Exclusion Criteria

Inclusion Criteria

Blood donors who met the pre-set national guidelines for blood donation (individuals aged between 18-65 years, weighing a minimum of 50kg with Hb of 12.5 g/dl and above, Appendix 1 for national guidelines) in KNH BTU and the Nairobi RBTC.

Eligible blood donors who gave consent to participate in this study were recruited.

Exclusion Criteria

Hemolyzed or lipemic samples and blood donors who declined to participate in the study.

3.5 Sample size

The sample size was 138 blood donors. This was derived from a study done by Gessain et al that found HTLV-1/2 seroprevalence rate of 10% in Gabon (1). The study was done in blood donors in Gabon where the seroprevalence of HTLV-1/2 is highest in Africa. The sample size was calculated using the Fisher's formula (31).

Where:

n= minimum sample size

Z= Z value (1.96 at 95% confidence level)

P=prevalence rate of 10 %

D= degree of precision set at 5 %

Sample size = 138

Approximately 30 blood donors donate blood daily at the BTU, KNH.

3.6 Sampling method

Every 5th donor at KNH BTU and 5th donor at Nairobi RBTC that met the preset national guidelines for blood donation (Appendix 1) and gave consent to participate in this study was recruited into the study until the sample size was achieved.

3.7 Recruitment

Blood donors eligible for blood donation at the two donor sites were recruited into the study. The rationale of the study was explained to the study participants by the principal investigator or the research assistant. They were informed about the advantages, disadvantages and side effects of taking part in the study and that their participation was entirely on voluntary basis after which they signed the consent form if they agreed to participate in the study (Appendix III). A questionnaire assessing the socio-demographic factors was then administered and the data was recorded on each questionnaire by the principal investigator or the research assistant (Appendix II). The test results for the routinely screened TTIs were obtained later and recorded in the questionnaires.

3.8 Laboratory methods

Specimen collection, transport and storage

Approximately 4 ml of blood was drawn from every participant through the same venipuncture used for blood donation into serially labeled plain vacutainers, placed in cool boxes and transported to UNITID laboratory. Serum was then separated from the blood samples into aliquot vials which were labeled with the same serial numbers and stored at -80ºC awaiting testing for HTLV-1/2 IgG antibodies once the sample size was achieved.

HTLV-1/2 testing procedure

The ELISA kits components and samples were retrieved from the refrigerators and allowed to thaw at room temperature. Wantai Bio-Pharm HTLV-1/2 IgG ELISA kits were used and the manufacturer's manual was followed during the entire testing process (Appendix IV).

The positions of the positive, negative, blank and test sample wells were recorded on the precoated ELISA plate. 50µl of HRP-Conjugate was added into all the wells excluding the blank. 50µl of Positive control, Negative control, and Specimen was added into the respective wells. The plates were then sealed and incubated at 37°C for one hour. The plates were then washed five times to remove excess unbound antibodies. 50µl of Chromogen Solution A and Chromogen Solution B was added into each well including the Blank. This was incubate at 37°C for 30 minutes. Positive control well produced a blue color. 50µl of Stop solution was added into each well and mix gently. The positive control well showed yellow color. The absorbance was measured at 450nm using a microplate reader.

Each sample was tested once on the 96 well microplate and each plate include 2 positive and 3 negative controls. Optical densities obtained from the negative controls were used to calculate the critical value (Cut Off) from which the test sample results were interpreted. A Cut Off value of 0.222 was obtained.

Testing for routinely screened Transfusion Transmitted Infections that include antibodies against HCV, HIV1/2, HBsAg and syphilis (Treponema pallidum) was performed at the NBTS and Kenyatta National Hospital Immunology laboratories. The corresponding results were obtained from the donor registers at KNH BTU and the Nairobi RBTC.

3.9 Variables

Independent outcome measures

Age, marital status, sex, Income, donor category, HIV, HBsAg, VDRL and HCV status.

Dependent variables

HTLV-1 and HTLV-2 antibody titers.

3.10 Quality assurance

Pre-analytical stage

After study participants' recruitment, blood samples were collected by qualified and competent personnel. The samples were clearly labeled using serial numbers and transported in cool boxes to UNITID laboratory. Centrifugation was performed and serum was separated into aliquot vials labeled with corresponding serial numbers and stored at -80ºC awaiting testing.

Analytical stage

The samples were analyzed in UNITID laboratory which is a research laboratory. The reagents were stored at 2-8º C according to manufacturer's instructions. Kits were checked for expiry dates. The kits and samples were allowed to attain room temperature before analysis. Standard operating procedures (SOPs) were followed in analyzing the samples using the ELISA technique with inbuilt positive and negative internal controls after calibration of the equipment. For each 96 well plate 2 positive and 3 negative controls were included.

Post analytical stage

Expected values for positive and negative controls were used to validate the laboratory test results obtained. Proper calculation of cut offs was done to ensure correct interpretation of the results. Care was taken to avoid transcription errors during data entry and analysis.

3.11 Ethical consideration

The study approval was obtained from KNH/UON Ethical and Research Committee before conducting the study (Appendix V). Clearance from National Blood Transfusion Service was obtained so as to recruit blood donors during blood drives organized by Nairobi RBTC (Appendix VI). Study participation and blood sample collection was carried out after obtaining a written informed consent from each participant. There was no extra prick made to obtain blood and only

4ml of blood was drawn for testing hence no adverse effects to the study subjects. Study participation by blood donors was on voluntary basis and no form of incentives was given to the study participants. Confidentiality was ensured when conducting interviews and no personal identifying data was recorded rather serial labels were used. Total confidentiality was maintained throughout the study results handling process with only the principal investigator, supervisors and statistician accessing the data.

Data collected on soft copies was protected using passwords from access by unauthorized persons while information on hard copies was secured in lockable cabinets.

3.12 Data management and analysis

Socio-demographic data and laboratory results were entered into predesigned study questionnaires. This data was then entered and analyzed with the use of Excel 2013. Demographic data that was categorical was summarized and presented as frequencies and proportions.

4.0 RESULTS

4.1 Introduction

138 study participated were recruited in the study. 69 study participants were recruited from KNH BTU and the rest from Nairobi RBTC. This section presents the socio-demographic information of the donors and also looked at the relationship between the donors' demographic status.

4.2 Study Participants Characteristics.

The age of the participants ranged between 18-59 years. Those aged 18 to 20 years accounted for only 7% and all of them were voluntary donors. 51% of the voluntary blood donors were between 21 and 30 years while 52% were replacement donors. The study participants whose age ranged between 31-40 years constituted 29% among voluntary donors and 35% in replacement donors. 7% of the voluntary donors and 13% of the replacement donor's age ranged between 41-50 years. Those above 51 years were voluntary donors being the least at 6%. Figure 1 shows the study participants characteristics.

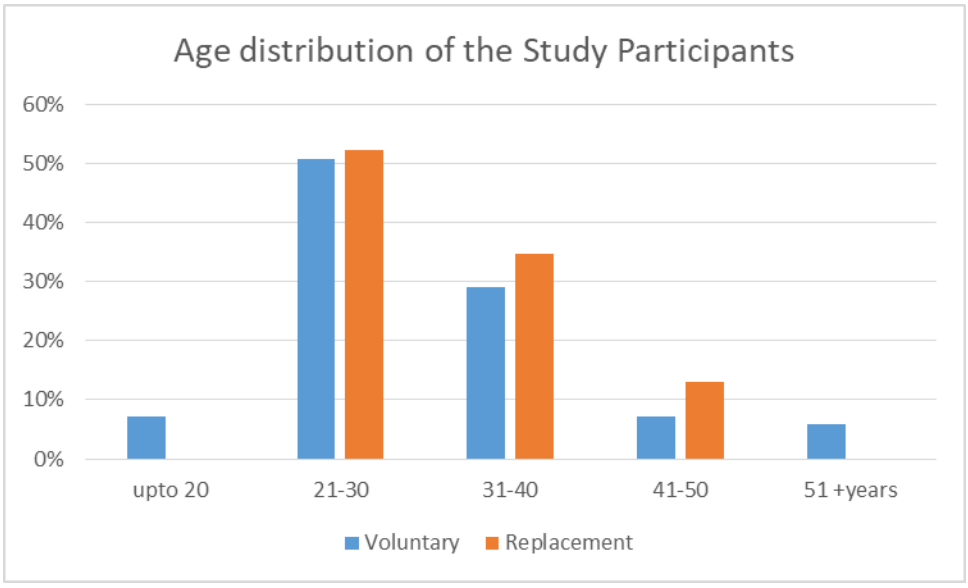


Figure 1 Age distribution by Donor type *(n=138)*

The male participants were predominant as they accounted for 71 % (98) compared to 29 % (40) females as illustrated by the figure 2 below.

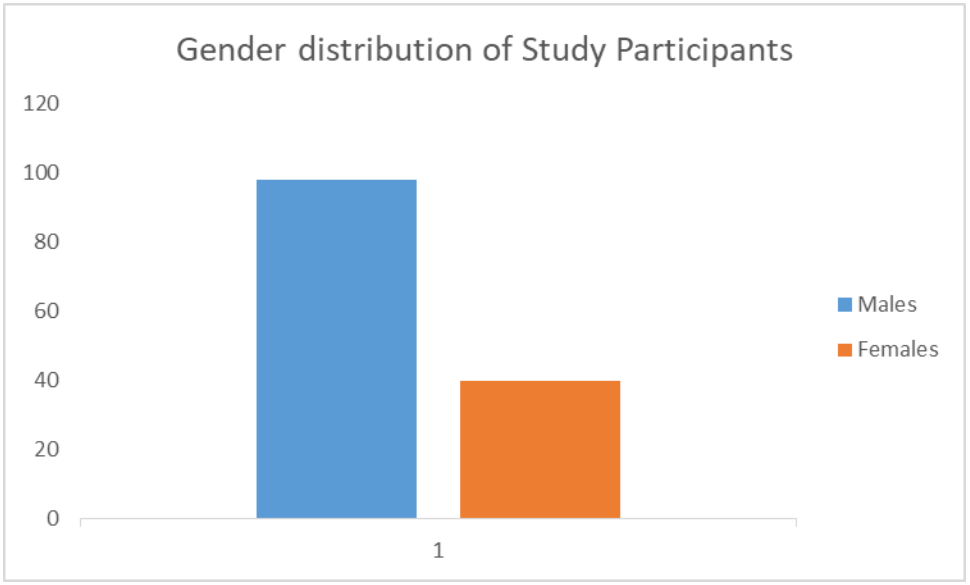


Figure 2 Gender Distribution of the Study Participants. ***(n=138)***

7% of the study participants were voluntary donors and had attained primary education. 12% of the study participants were replacement donors and had attained primary education. 30% of the voluntary donors and 29% of the replacement donors had attained secondary education. Those who had attained tertiary education constituted the majority, accounting for 63% voluntary donors and 59% replacement donors as illustrated by figure 3.

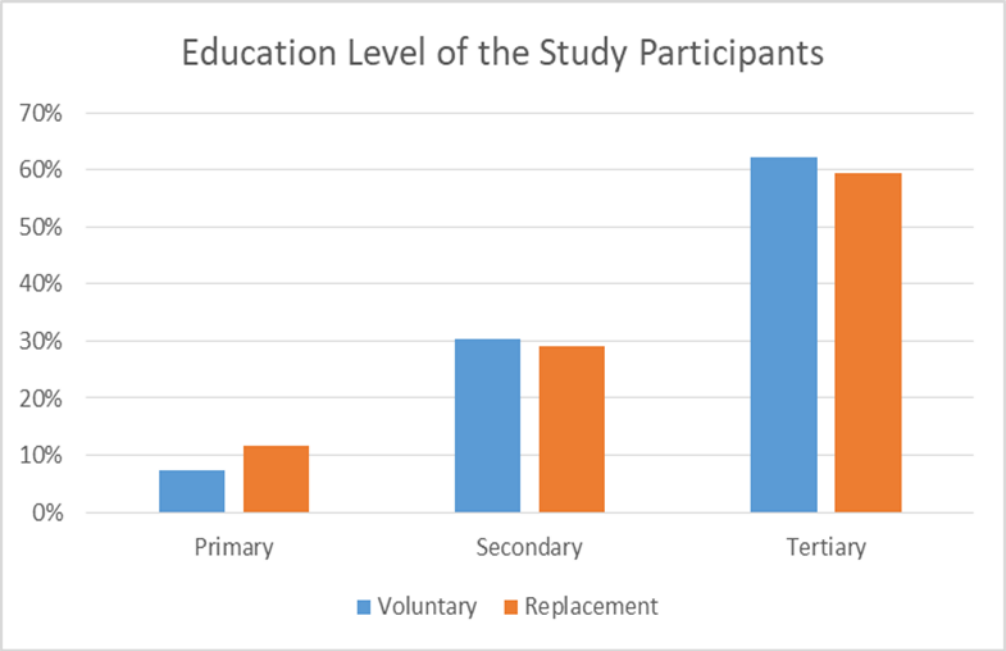


Figure 3 Education level of the Study Participants *(n=138)*

46% of the study participants earned between 10000 to 50000 KSh and only 32% of the study participants earned less than 10000 KSh.

Table 1 Socio-Economic Status of the Study Participants *(n=138)*

Social Economic Status (KSh)	**Frequency N (%)**
<10000	**30% (42)**
10000-50000	**46%(63)**
50000-100000	**8%(11)**
>100000	**5%(7)**
Student	**11%(15)**

The marital status of the study participant's revealed that 51 % (71) were single, while 49 % (67) were married as indicated by figure 4. None of the study participants indicated that they were widows or divorced.

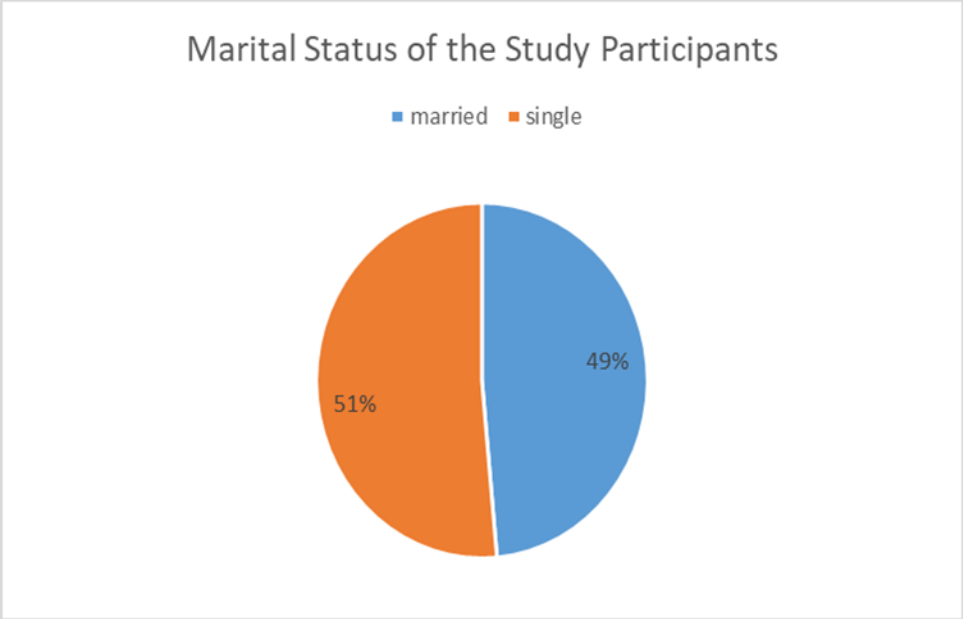


Figure 4 Marital Status of the Study Participants (*n=138)*

4.3 Laboratory Findings

Figure 5 below shows 3.6% (5) of the study participants were seropositive for hepatitis B, 0.7 % (1) was positive for hepatitis C and 5.7 % (8) were positive for HIV antibodies. None of the study participants was seropositive for HTLV-1/2. Therefore, the prevalence of HTLV-1/2 among the blood donors in this study was zero (0%). A correlation could not therefore be made between routinely screened infections (hepatitis B and C virus, HIV and syphilis) and HTLV-1/2 infection.

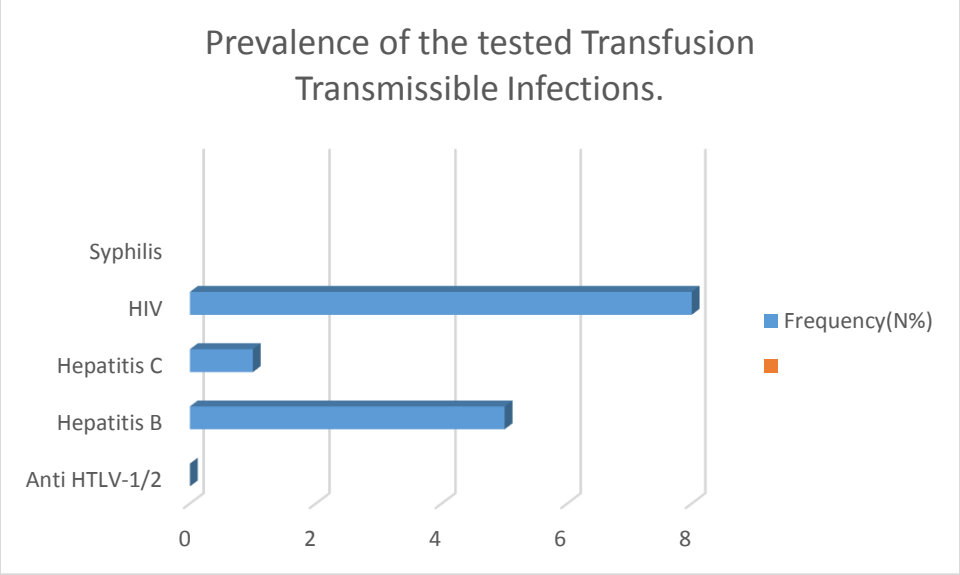


Figure 5 Prevalence of the Transfusion Transmissible Infections *(n=138)*

5.0 DISCUSSION

Transfusion of blood contaminated with HTLV-1/2 accounts for 15-60% of infection in areas of high endemicity and screening of blood is the most effective strategy in prevention of transmission (1). In Kenya, only one study has been done on HTLV-1/2 coinfection with HIV and no study has been done in blood donors. This study found HTLV-1/2 seroprevalence of 0% and this is lower than 19.5% prevalence obtained from a study done in cervical smears of women (2). Unlike prior studies, this study was the first to be done in blood donors who are certified fit to donate blood. Screening of blood is the most effective strategy in prevention of transmission (12). In Kenya, only one study has been done on the prevalence of HTLV-1/2 coinfection with HIV and little is known about the seroprevalence among blood donors. The seroprevalence of HTLV-1/2 infection among blood donors in this study was 0%.

The 0% prevalence obtained is similar to the HTLV-1/2 seroprevalence in Rwanda of 0.2 % (1) and Uganda with a prevalence of 0.5 % (21). This findings agree with prior studies that indicated the seroprevalence of HTLV-1/2 in East Africa to be low or totally absent in some regions (1). The findings in this study are also similar to the seroprevalence obtained in Enugu, Nigeria 0% (32). Europe is not endemic for HTLV virus and the seroprevalence is very low. For instance, in France the seroprevalence is 0.004% and the seroprevalence is 0.0% in Spain (33). The 0% seroprevalence obtained is comparable to European rates.

Prior studies have shown certain sociodemographic factors such as advance age, gender, marital status, low income, geographical region influence HTLV-1/2 seroprevalence (33) . According to Kenyan national blood transfusion service guideline, donors should be between18-65years.In this study 51% of the participants were between 21-30 years as compared to those with 40 years and above. This is also reflects the donor population of the whole country in general .The age of the study participants in this study are similar to those in Enugu Nigeria where 53% of the study participants were 21-30 years (32). HTLV-1/2 prevalence increases with advance age with a prevalence of 1.6% in those between 18-30 years and 2.9% in those more than 50 years old years (32). HTLV-1/2 prevalence increases with increase in age with a prevalence of 1.6% in those between 18-30 years and 2.9% in those more than 50 years old (34). A possible explanation for the association is the greater length of exposure to events such as sexual exposure that may result

in acquiring HTLV-1/2. Once infected with the virus the infection is carried forward as there is no cure for HTLV-1/2.

Majority of the study participants were males at 71% and this is a reflection of the Kenya donor population. This is because women tend to have lower hemoglobin level and are disqualified as potential blood donors (35). In a study done in Enugu 96% of the study participants were male while only 4% were females and these findings are similar to this study. This findings not only represent the blood donor population in Africa but also in Europe. A study done in Europe showed that 58% of blood donors Germany were male and 53% were male in Switzerland (36).

The 0% seroprevalence observed could be attributed to gender disparity. Study done in Nigeria indicated that the prevalence of HTLV-1/2 is higher in females as opposed to males (32). This is because of the efficient mode of transmission from males to females during sexual intercourse. In both Africa and Europe Males donate blood more than females and for this HTLV-1/2 positive cases may be missed.

In Kenya the free primary education has improved the level of literacy in the country and this has been further strengthen by easy access to student loans thus enabling Kenyans to further their education. In this study 61% of the study subjects had acquired tertiary education, 30% had attained secondary education with those only with primary education being the least at 9%. These findings are not similar in the neighboring country, Uganda where 99.7% of the participants had attained secondary education, 0.3% primary education and none had attained tertiary education. This can be explained by the fact that the education system of a country is determined mainly by the governing policies of a country. A study done in Brazil showed that majority of HIV/HTLV-1/2-coinfected cases were either illiterate or had only secondary education (20). The donors from this study have tertiary education (90%), this can explain the low seroprevalence and also adults with tertiary level of education are less likely to engage in risky sexual activities.

Higher income together with social status are associated with better health. The same study done in Brazil indicated HTLV/HIV coinfection cases belonged to social classes that earn less than US$250 (25000 shillings). From this study majority of the blood donors had an income between 10000-50000KSh and this could explain the 0% seroprevalence obtained.

51% of the study subjects were single with only 49% being married. This is similar to finding from Uganda and Nigeria where majority of the participants were single. In Uganda 98.9% of the study participants were single while 1.2% (21) were married which is similar to Enugu Nigeria where 76.7% of the participants were single and 22.3% married (32). The seroprevalence of HTLV virus is much higher in married individuals as opposed to single people and this is explained by the higher frequency of exposure in marriage (37).

Human Immunodeficiency Virus prevalence was 5.8% which is similar to the Kenya HIV estimate report 2018 prevalence of 6.1% in Nairobi County (38). The prevalence of hepatitis B virus was 3.6% compared to 5.2% from the Kenya national estimates for the periods 2011–2012 (39). This decline can be attributed to strategies to curb transmission include behavioral changes, education among at-risk populations and vaccination. HCV seroprevalence is 0.79–0.99% according to national estimates for the period 2007–2010 (39) and this is comparable to the seroprevalence obtained of 0.7%. The national estimates indicate the prevalence of syphilis to be 0.15–0.28% (39) which is similar to findings obtained in this study of 0%. A correlation cannot be done between routinely screened infections (hepatitis B and C, HIV and syphilis) and HTLV-1/2 infection because of the 0% seroprevalence of HTLV-1/2.

Lastly, a low seroprevalence of HTLV-1/2 infection was observed in this study but multicenter studies are required to establish the seroprevalence of HTLV-1/2 different regions in Kenya.

5.1 Limitations

The prevalence of HTLV-1/2 in this study cannot be generalized as it varies between regions and positive cases may be missed, however this study is the first of its kind and it forms the basis for comparison for future studies.

ELISA method, which has been validated for widespread donor screening in China, was used. Nuclei Acid testing has been used to screen and confirm positive HTLV virus antibodies but it's expensive and has not been validated for wide spread use.

The blood donor population age ranges from 18 to 65 years but majority of donors in this study were between 21-30 years and this is limitation as prevalence of HTLV virus is high in older age groups.

The sample size used in this study was 138 and 368 sample size was used in a study done in Uganda but despite the large sample size only 2 cases were positive.

5.2 Conclusion

The seroprevalence of HTLV-1/2 infection among the blood donors who participated in this study was 0%. The low prevalence obtained cannot justify the routine screening of the virus in blood donors in Nairobi County but the prevalence of HTLV varies in different region.

5.3 Recommendations

HTLV-1/2 prevalence varies in different regions and multicenter studies should be encouraged in order to establish the seroprevalence of the virus. The low seroprevalence obtained does not justify routine screening of HTLV-1/2 in Nairobi County.

REFERENCES

1. Gessain A, Cassar O. Epidemiological aspects and world distribution of HTLV-1 infection. 2012;3(388):1–23.

2. He X, Maranga IO, Oliver AW, Gichangi P, Hampson L, Hampson IN. Analysis of the prevalence of HTLV-1 proviral DNA in cervical smears and carcinomas from HIV positive and negative Kenyan women. Viruses. 2016;8(9).

3. Zerihun A, Ataro Z, Urgessa F, Wasihun T. Prevalence and Trends of Major Transfusion Transmissible Infections among Blood Donors in Dire Dawa Blood bank , Eastern Ethiopia : Retrospective Study.2018;28(6):701.

4. Increased risk of developing peripheral neurophaty in patients coinfected with HIV 1 and HTLV 2.1992;174:891-3.

5. Hisada M, Chatterjee N, Zhang M, Battjes RJ, Goedert JJ. Increased hepatitis C virus load among injection drug users infected with human immunodeficiency virus and human T lymphotropic virus type II. J Infect Dis [Internet]. 2003;188(6):891–7.

6. Carnaúba D, Bittencourt A, Brites C. Atypical presentation of syphilis in an HTLV-I infected patient. Braz J Infect Dis [Internet]. 2003;7(4):273–7.

7. Nizam SV, Sciences M. Prevalence of Transfusion Transmissible Infections in a Hospital Attached Blood Bank. 2015;108:42-4.

8. Carneiro-proietti F, Catalan-soares BC, Proietti FA, Ba A, Murphy EL. Global epidemiology of HTLV-I infection and associated diseases. 2005;6058–68.

9. Sexual transmission of human T-cell lymphotropic virus type 1. Rev Soc Bras Med Trop [Internet]. 2014;47(3):265–74.

10. Feuer G, Green PL. Comparative biology of human T-cell lymphotropic virus type 1 (HTLV-1) and HTLV-2. Oncogene. 2005;24(39):5996–6004.

11. Giam CZ, Semmes OJ. HTLV-1 infection and adult T-cell leukemia/ lymphoma-A tale of two proteins: Tax and HBZ. Viruses. 2016;8(6).

12. Gonçalves DU, Proietti FA, Ribas JGR, Araújo MG, Pinheiro SR, Guedes AC, et al.

Epidemiology, treatment, and prevention of human T-cell leukemia virus type 1-associated diseases. Clin Microbiol Rev. 2010;23(3):577–89.

13. Bloch EM, Vermeulen M, Murphy E. Blood Transfusion Safety in Africa: A Literature Review of Infectious Disease and Organizational Challenges. Transfusion Medicine Review. 2012;26(2):164–80.

14. Fessehaye N, Naik D, Fessehaye T. Transfusion transmitted infections - A retrospective analysis from the National Blood Transfusion Service in Eritrea. Pan Afr Med J. 2011;9:1–6.

15. Zhang LL, Wei JY, Wang L, Huang S Le, Chen JL. Human T-cell lymphotropic virus type 1 and its oncogenesis. Acta Pharmacol Sin [Internet]. 2017;38(8):1093–103.

16. O'Connor OA. Getting the Facts: Adult T cell leukemia/lymphoma (HTLV-1). Lymphoma Res Found. 2008;1;2-3.

17. Murphy EL, Cassar O, Gessain A. Estimating the number of HTLV-2 infected persons in the world. Retrovirology. 2015;12(S1):2015.

18. Mahieux R, Gessain A. HTLV-3/STLV-3 and HTLV-4 viruses: Discovery, epidemiology, serology and molecular aspects. Viruses. 2011;3(7):1074–90.

19. Larocque E, Halin M, Landry S, Marriott SJ, Switzer WM, Barbeau B. Human T-Cell Lymphotropic Virus Type 3 (HTLV-3)- and HTLV-4-Derived Antisense Transcripts Encode Proteins with Similar Tax-Inhibiting Functions but Distinct Subcellular Localization. J Virol. 2011;85(23):12673–85.

20. Morimoto HK, Caterino-De-Araujo A, Morimoto AA, Reiche EMV, Ueda LT, Matsuo T, et al. Seroprevalence and Risk Factors for Human T Cell Lymphotropic Virus Type 1 and 2 Infection in Human Immunodeficiency Virus-Infected Patients Attending AIDS Referral Center Health Units in Londrina and Other Communities in Paraná, Brazil. AIDS Res Hum Retroviruses. 2005; 21(4):256-262.

21. Uchenna Tweteise P, Natukunda B, Bazira J. UgandaHuman T-Cell Seropositivity among Blood DoLymphotropic Virus Types 1 and 2nors at Mbarara Regional Blood Bank, South Western. Leuk Res Treatment [Internet]. 2016;2016:1–6.

22. John-olabode S, Akinbami A, Durojaiye I, dosunmu adedoyin, Adediran A, Oshinaike O, et al. Seroprevalence of human T-lymphotropic virus antibodies among patients with lymphoid malignancies at a tertiary center in Lagos, Nigeria. J Blood Med.2014:5;169–17.

23. Switzer WM, Shankar A, Trimble SR, Thompson AA, Giardina PJ, Cohen AR, et al. Human T Cell Lymphotropic Virus Type 1 Infection Among U.S. Thalassemia Patients. AIDS Res Hum Retroviruses. 2013;29(7):1006–9.

24. Zarranz Imirizaldu JJ, Gomez Esteban JC, Rouco Axpe I, Perez Concha T, Velasco Juanes F, Allue Susaeta I, et al. Post-transplantation HTLV-1 myelopathy in three recipients from a single donor. J Neurol Neurosurg Psychiatry. 2003;74(8):1080–4.

25. Takatsuki K. Discovery of adult T-cell leukemia. Retrovirology. 2005;12(2):1–3.

26. Ireland G, Croxford S, Tosswill J, Raghu R, Davison K, Hewitt P, et al. Human T-lymphotropic viruses (HTLV) in England and Wales, 2004 to 2013: Testing and diagnoses. Eurosurveillance. 2017;22(21).

27. Pique C, Jones KS. Pathways of cell-cell transmission of HTLV-1. Front Microbiol. 2012;3(OCT):1–14.

28. Abdel-Fattah M, Meligy B, El-Sayed R, El-Naga YA, Gupta V, Sharma P, et al. Research letters. Indian Pediatr. 2015;52(9):521–6.

29. Laher AE, Ebrahim O. HTLV-1, ATLL, severe hypercalcaemia and HIV-1 co-infection: An overview. Pan Afr Med J. 2018;30:1–9.

30. Report T. Risk assessment of HTLV-I / II transmission by tissue / cell transplantation.

31. Fischer, Laing Js and T. Handbook for Family Planning Operations Research. Popul Counc. 1991;2nd edition:45–6.

32. Onoh R, Okoye A, Ibegbulam GO, Ugwu NI, Anigbo CS, Nonyelu CE. Seroprevalence of human T-cell lymphoma/leukemia virus type-1 (HTLV-1) antibodies among blood donors at Enugu, Nigeria. J Blood Med. 2015;31.

33. Chaudhari CN, Shah T, Misra RN. Prevalence of human T cell leukaemia virus amongst blood donors. Med J Armed Forces India [Internet]. 2009;65(1):38–40.

34. Santos RFS, Conceição GC, Martins MS, Kraychete A, Penalva MAC, Carvalho EM, et al. Prevalence and risk factors for Human T-Lymphotropic Virus Type 1 (HTLV-1) among maintenance hemodialysis patients. BMC Nephrol. 2017;18(1):1–7.

35. Valerian DM, Mauka WI, Kajeguka DC, Mgabo M, Juma A, Baliyima L, et al. Prevalence and causes of blood donor deferrals among clients presenting for blood donation in northern Tanzania. PLoS One. 2018;13(10):1–12.

36. Volken T, Weidmann C, Bart T, Fischer Y, Klüter H, Rüesch P. Individual characteristics associated with blood donation: A cross-national comparison of the German and swiss population between 1994 and 2010. Transfus Med Hemotherapy. 2013;40(2):133–8.

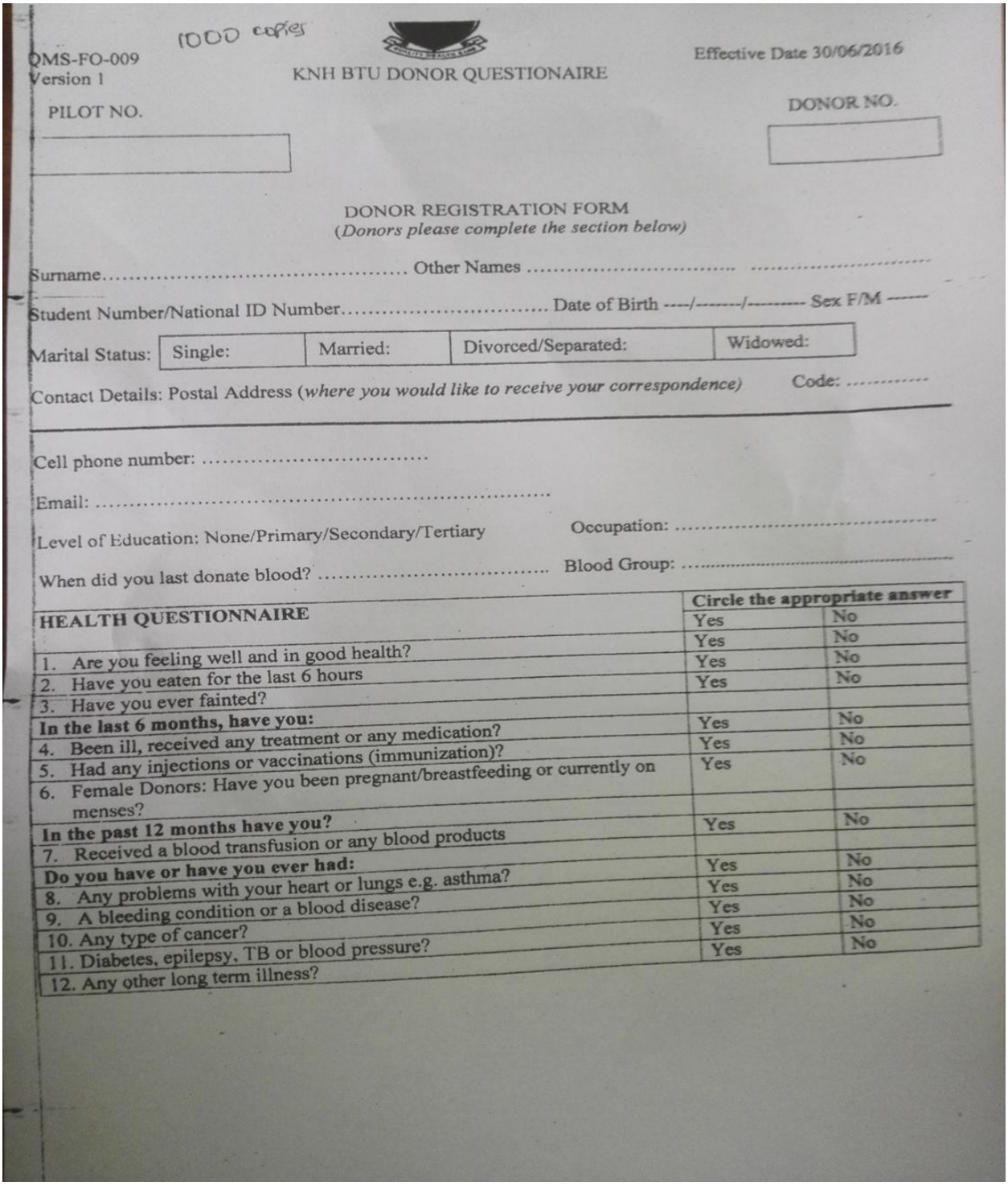
37. Karimi G, Zadsar M, Pourfathollah AA. Seroprevalence and geographical distribution of human T-lymphotropic virus type 1 among volunteer blood donors in endemic areas of Iran. Virol J [Internet]. 2017;14(1):1–9.

38. National AIDS and STI Control Programme. Kenya HIV Estimates 2015. 2016;1–28.

39. Onyango CG, Ogonda L, Guyah B, Okoth P, Shiluli C, Humwa F, et al. Seroprevalence and determinants of transfusion transmissible infections among voluntary blood donors in Homabay, Kisumu and Siaya counties in western Kenya. BMC Res Notes [Internet]. 2018;11(1):1–6.

APPENDICES

Appendix I: Criteria for donor screening



1000 copies

QMS-FO-009
Version 1

KNH BTU DONOR QUESTIONAIRE

Effective Date 30/06/2016

PILOT NO.

DONOR NO.

DONOR REGISTRATION FORM
(Donors please complete the section below)

Surname..... Other Names ....

Student Number/National ID Number..... Date of Birth ----/-------/-------- Sex F/M ------

Marital Status:	Single:	Married:	Divorced/Separated:	Widowed:

Contact Details: Postal Address (*where you would like to receive your correspondence)* Code: .....

Cell phone number: .....

Email: .....

Level of Education: None/Primary/Secondary/Tertiary Occupation: .....

When did you last donate blood? ..... Blood Group: .....

HEALTH QUESTIONNAIRE	**Circle the appropriate answer**	
1. Are you feeling well and in good health?	Yes	No
2. Have you eaten for the last 6 hours	Yes	No
3. Have you ever fainted?	Yes	No
In the last 6 months, have you:		
4. Been ill, received any treatment or any medication?	Yes	No
5. Had any injections or vaccinations (immunization)?	Yes	No
6. Female Donors: Have you been pregnant/breastfeeding or currently on menses?	Yes	No
In the past 12 months have you?		
7. Received a blood transfusion or any blood products	Yes	No
Do you have or have you ever had:		
8. Any problems with your heart or lungs e.g. asthma?	Yes	No
9. A bleeding condition or a blood disease?	Yes	No
10. Any type of cancer?	Yes	No
11. Diabetes, epilepsy, TB or blood pressure?	Yes	No
12. Any other long term illness?	Yes	No

QMS-FO-009
Version 1

KNH BTU DONOR QUESTIONAIRE

Effective Date 30/06/20

RISK ASSESSMENT QUESTIONNARE

The lives of patients who receive your blood are totally dependent on your honesty in answering the questi
below. Your answers will be treated in a confidential manner.

In the past 12 months have you:		
1. Received or given money, goods or favours in exchange for sexual activities?	Yes	No
2. Had sexual activity with a person whose sexual background you did not know?	Yes	No
3. Been raped or sodomized?	Yes	No
4. Had a stab wound or had an accidental needle stick injury e.g. injection needle?	Yes	No
5. Had any tattooing or body piercing e.g. ear piercing?	Yes	No
6. Had a sexually transmitted disease (STD)?	Yes	No
7. Live with or had sexual contact with someone with yellow skin?	Yes	No
8. Had sexual activity with anyone besides your regular sex partner?	Yes	No
Have you ever:		
9. Had yellow eyes or yellow skin?	Yes	No
10. Injected yourself or been injected, besides in a health facility?	Yes	No
11. Used non-medical drugs such as marijuana, cocaine etc?	Yes	No
12. Have you or your partner been tested for HIV?	Yes	No
13. Do you consider your blood safe to transfuse to a patient?	Yes	No

DECLARATION:

I declare that the information I have given above is correct. I understand that my blood will be tested for H
Hepatitis B & C and Syphilis and the results of my tests may be obtained from the Blood Transfusion Uni

Signature---------- **Date**---------/----------/----------

For Official Use:

Weight (kg)	Hb>12.5g/dl	BP	Pulse

Donor is accepted	
Yes:	**No:**

Low Volume	Venepuncture	Haematoma	Faint		
			Mild	Moderate	Sev

Time Needle In		Time Needle Out	

Report

Name of Interviewer: ..... Date: .....

Appendix II: Study Questionnaire

Seroprevalence of HTLV-1/2 among blood donors in KNH BTU and Nairobi RBTC

Study No……

Donor Ref No…….

1. Personal details

Physical address…...

Postal address.....

E-mail address.....

Mobile number….....

2. Donor type

□ Voluntary

□ Replacement

3. Socio-demographic data

Age.....

Sex

□ Male

□ Female

4. Marital status

□ Single

□ married

□ divorced

□ widowed

□ Other…….

5. Education level (tick as appropriate)

□ Primary level

□ Secondary level

□ Tertiary level

6. Socio- economic status; Net income per month

□ <10000

□ 10000- 50000

□ 50000- 100000

□ >100000

7. Laboratory results

HIV status

□ Positive

□ Negative

Syphilis test

□ Positive

□ Negative

Hepatitis B

□ Positive

□ Negative

3.4 Hepatitis C

□ Positive

□ Negative

HTLV-1/2 antibodies

□ Positive

□ Negative

Appendix III: Study Participation Informed Consent Form
Title: Seroprevalence of HTLV-1/2 in blood donors at two donor centers in Nairobi, Kenya.

Introduction: My name is Dr. Naomi Moraa, I am postgraduate student in the Department of Human Pathology, University of Nairobi.

Information about the study

The purpose of this study is to find out the proportion of blood donors who are positive for human T-cell lymphotrophic virus type 1 and 2 (HTLV-1/2). HTLV-1/2 is a virus found in most parts of the world even in the African populations. There are several modes of transmission including through blood transfusion. Though it does not cause serious infection in most people it can result in serious sickness or even cause death in immunocompromised patients hence the need to protect them through the use of safe blood products.

This study will involve collection of approximately 4mls of blood from eligible blood donors that will be sent to the laboratory for analysis.

Voluntary participation

Your participation in this study is entirely voluntary and it is you can choose to participate or not to participate.

Procedure

Eligible blood donors will be requested to take part in the study. Those who agree to participate will be requested to sign a consent form. You will then be asked some questions that will be filled in the questionnaire. Thereafter, approximately 4mls of blood will be drawn that will be sent to the laboratory for testing for antibodies against HTLV-1/2.

Benefits

Participating in this study will go a long way in ensuring safety of blood transfusion in our country.

Harmful effects

There are no harmful effects expected in this study.

Risks

You will experience some slight pain as an extra blood volume is drawn for testing in the laboratory to check for HTLV-1/2 antibodies. You are not likely to encounter any major risks during the procedure.

Reimbursements

You will not be given any gifts monetary or otherwise for participating in this study.

Confidentiality

You will be identified using study numbers and no names will be required. The information that you provide will not be disclosed to anyone and test results can be obtained at the post donor care center. However, the findings of the study will be presented in medical conferences and published in medical journals so that other interested people may learn from it.

Request to participate in this study

Kindly indicate if you are interested in taking part in this study by signing the consent form provided. Please note you have a right to decline participating in this study.

Participant declaration

I have read the details of the study or the details of the study have been explained to me and I agree to participate in the study. I have had the opportunity to ask questions regarding my participation in this study and the questions have been answered to my satisfaction. My rights have also been explained and I consent to voluntarily participate in this study.

Participant's name …… Signature……. Date……

Witness's name……. Signature……. Date……

Principal investigator name……Signature……..Date……

If you have any queries regarding participation in the study, kindly contact us.

Principal investigator: Dr. Naomi Moraa 0720 769 058

Supervisors: Dr. Peter M Maturi 0722 400 128

Dr. Valarie K. Magutu 0721 250 089

You can also contact the Ethics and Research Committee at Kenyatta National Hospital (KNH/UON-ERC): P.O Box 20723-00202, Nairobi. Telephone number +254 020 726300-9

FOMU YA IDHINI

Kichwa cha utafiti: Utafiti unaohusu idadi ya kingamwili za HTLV-1/2 katika wafadhili wa damu katika vituo viwili vya wafadhili wa damu hapa Nairobi, Kenya. Jina langu ni Dr. Naomi Moraa. Mimi ni mwanafunzi wa chuo kikuu cha Nairobi idara ya Human Pathology. Ningependa kufanya utafiti ambao nitawaelezea. Tafadhali soma ujumbe ufuatao kwa makini. Ujumbe huu utaelezwa kwa njia ya Kiingereza na Kiswahili. Una uhuru wa kuchagua lugha ambayo unaielewa vyema.

Maelezo kwa Ufupi

Kusudi la utafiti huu ni kujua idadi ya wafadhili wa damu ambao wana kingamwili za HTLV-1/2. HTLV-1/2 ni virusi ambavyo hupatikana katika sehemu nyingi za dunia sanasana sehemu za kiafrika. Kuna njia mbali mbali za maambukizi ikiwemo kutiwa damu mishipani. Ingawa haisababishi magonjwa kali kwa watu wengi inaweza sababisha maambuki makubwa n ahata vifo kwa walio na upungufu wa kinga mwilini. Kwa hiyo kuna haja ya kuwakinga kwa kuhakikisha usalama wa damu inayotiwa mishipani. Utafiti huu utahusisha mkusanyiko wa takriban 4mls ya damu kutoka kwa wafadhili wa damu wanaostahiki ambayo itatumwa kwenye maabara kwa ajili ya uchambuzi

Ushiriki wa hiari

Ushiriki wako katika utafiti huu ni kikamilifu kwa hiari na unaweza kuchagua kushiriki au usijihusishe.

Utaratibu

Watoaji wa damu wanaohitajika watatakiwa kushiriki katika utafiti

Wale ambao watakubali kushiriki wataombwa kusaini fomu ya ridhaa. Basi utaulizwa baadhi ya maswali ambayo yatajazwa katika daima. Baadaye, takribani 4ml ya damu itatengwa ambayo itatumwa kwa maabara kwa ajili ya kupimwa kwa kinga dhidi ya HTLV-1/2.

Faida

Kushiriki katika utafiti huu kutasaidia pakubwa ili kuhakikisha usalama wa damu katika nchi yetu.

Madhara

Hakuna madhara yanayosababishwa katika utafiti huu.

Hatari

Utapata maumivu machache wakati kiasi cha ziada cha damu kinapotolewa kwa ajili ya kupima katika maabara ili tuangalie kinga za HTLV-1/2. Huwezi patana na hatari yoyote kubwa wakati wa utaratibu huu.

Kulipia

Hutapewa zawadi yoyote ya fedha au vinginevyo kwa kushiriki katika utafiti huu.

Siri

Utatambuliwa kwa kutumia nambari za kujifunza na hakuna majina yatahitajika. Maelezo ambayo utatoa hayatafunuliwa kwa mtu yeyote. Hata hivyo, matokeo ya utafiti itawasilishwa katika mikutano ya matibabu na kuchapishwa katika magazeti ya matibabu ili watu wengine wenye nia waweza kujifunza kutoka kwao.

Ombi la kushiriki katika utafiti huu

Tafadhali onyesha ikiwa una nia ya kushiriki katika utafiti huu kwa kusaini fomu ya kibali iliyotolewa. Tafadhali kumbuka una haki ya kutoshiriki katika utafiti huu.

Azimio la mshiriki

Nimesoma maelezo ya utafiti au maelezo ya utafiti yameelezwa kwangu na nimekubali kushiriki katika utafiti huu. Nimekuwa na nafasi ya kuuliza maswali kuhusu ushiriki wangu katika utafiti huu na maswali yamejibiwa kwa kuridhika kwangu. Haki zangu pia zimeelezwa na nina nia ya kushiriki kikamilifu katika utafiti huu.

Jina la mshiriki…… Sahihi……. Tarehe……

Jina la Shahidi……..Sahihi……. Tarehe……

Jina la ordodha mkuu……Sahihi…….Tarehe……

If you have any queries regarding participation in the study, kindly contact us. Ikiwa una maswali yoyote kuhusu ushiriki katika utafiti huu, wasiliana nasi.

Mtafiti mkuu: Dr. Naomi Moraa 0720 769 058. Chuo Kikuu cha Nairobi

Wasimamizi: Dr. Peter M. Maturi 0722 400 128

Dr. Valarie K. Magutu 0721 250 089

Unaweza pia kuwasiliana na Kamati ya Maadili na Utafiti katika Hospitali ya Taifa ya Kenyatta (KNH/UON-ERC): P.O Box 20723-00202, Nairobi. Telephone number +254 020 726300-9.

Appendix IV: HTLV-1/2 IGG ELISA KIT (Wantai)
Protocol

Preparation of sample: Isolate the test samples soon after collecting and analyze immediately (within 2 hours) or aliquot and store at -20º C or -80º C for long term storage. Avoid multiple freeze thaw cycles.

Assay procedure

Equilibrate the kit components and samples to room temperature prior to use

1. Mark three wells as Negative control, two wells as Positive control and one Blank, neither specimens nor HRP-Conjugate should be added into the Blank well
2. Add 50µl of HRP-Conjugate into each well except the Blank.
3. Add 50µl of Positive control, Negative control, and Specimen into their respective wells except the Blank. Shake the plate mildly to mix the contents.
4. Seal the plate with a cover and incubate at 37º C for 60 minutes
5. Remove the cover and discard the plate contents by tapping the plate on absorbent filter papers or other absorbent material.
6. Wash the plate 5 times with diluted wash buffer.
7. Add 50µl of Chromogen Solution A and then 50µl of Chromogen Solution B into each well including the Blank, mix gently. Incubate the plate at 37°C for 30 minutes avoiding light.
8. Add 50 µl of stop solution into each well. The color should change to yellow. Gently tap the plate to ensure thorough mixing.
9. Measure the absorbance at 450nm.

Wantai Human T-cell Lymphotropic Virus Diagnostics

WANTAI anti-HTLV 1+2 ELISA

HTLV Antibodies Diagnostic ELISA Kit

REF WH-9196 | V. 2017-01 [Eng.] | 96 Tests | IVD

Read the package insert carefully and completely before performing the assay. Follow the instructions and do not modify them. Only by strict adherence to these instructions, the erroneous results can be avoided and the optimal performance of WANTAI anti-HTLV 1+2 ELISA achieved.

INTENDED USE

WANTAI anti-HTLV 1+2 ELISA is an enzyme-linked immunosorbent assay (ELISA) for qualitative detection of antibodies to Human T-Cell Lymphotropic Virus types 1 and/or 2 (HTLV-1/2) in human serum or plasma. It is intended for screening of blood donors and as an aid for the diagnosis of clinical conditions related to infection with HTLV-I and/or HTLV-2.

SUMMARY

The human T-cell lymphotropic viruses (HTLV) is a member of the family of Retroviridae, consisting of enveloped double stranded RNA viruses and genetically not related to HIV1&2; however, they have similar routes of transmission and can have extremely long period of latency prior to manifestation of disease. HTLV type I was reported in 1980 as the first retrovirus shown to be pathogenic to humans. The virus preferentially infects CD4+ lymphocytes while the infections of CD8+ T lymphocytes are rare. In contrast to HTLV I, HTLV type 2 can infect all type of lymphocytes as well as the macrophages. HTLV 1&2 is transmitted transplacentally, parenterally, by sexual contacts and by infected blood. The diseases associated with HTLV infection are usually classified as malignant or nonmalignant clinical presentations. HTLV I is endemic in southern Japan, the Caribbean and the US and many other scattered population through the world. HTLV 2 is endemic in some North American Indian tribes but is detected mostly in intravenous drug users and their sexual partners.

PRINCIPLE OF THE TEST

This kit uses one-step incubation, antigen "sandwich" enzyme immunoassay (ELISA) method, which uses polystyrene microwell strips pre-coated with recombinant HTLV antigens expressed in E.coli. Patient's serum/plasma specimen is incubated in the microwells together with second recombinant HTLV antigens conjugated to horseradish peroxidase (HRP-Conjugate). The pre-coated antigens express the same epitopes as the HRP-Conjugate antigens, but are expressed in different hosts. In case of presence of anti-HTLV in specimen, the pre-coated and HRP-conjugated antigens will be bound to the two variable domains of the antibody and during incubation, the specific antigen-antibody immunocomplex is captured on the solid phase. After washing to remove specimen and unbound HRP-Conjugate, Chromogen solutions containing tetramethylbenzidine (TMB) and urea peroxide are added to the wells. In presence of the antigen-antibody-antigen(HRP) "sandwich" complex, the colorless Chromogens are hydrolyzed by the bound HRP-Conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the specimen respectively. Wells containing specimens negative for anti-HTLV remain colorless.

COMPONENTS

IVD In Vitro Diagnostic Use Only

This kit contains reagents sufficient for testing of maximum of 91 specimens in a test run.

MICROWELL PLATE Code 6 (1x96wells) 8 X 12/12 X 8-well per plate	**MICROWELL PLATE:** Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. Each well contains recombinant HTLV 1/2 antigens. The microwell strips can be broken to be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2-8°C. Once opened, stable for 4 weeks at 2-8°C.
CONTROL [-] Code 8 (1x0.6ml per vial) preserv.0.1% ProClin™ 300	**NEGATIVE CONTROL:** Yellowish liquid filled in a vial with green screw cap. Protein-stabilized buffer tested non-reactive for anti-HTLV 1/2. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
CONTROL [+] Code 7 (1x0.6ml per vial) preserv.0.1% ProClin™ 300	**POSITIVE CONTROL:** Red-colored liquid filled in a vial with red screw cap. Antibodies to HTLV 1/2 diluted in protein-stabilized buffer. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
HRP | CON Code 6 (1x8ml per vial) preserv.0.1% ProClin™ 300	**HRP-CONJUGATE:** Red-colored liquid in a white vial with red screw cap. Horseradish peroxidase-conjugated HTLV 1/2 antigens. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
WASH | BUF | 20X Code 1 (1x60ml per bottle) DILUTE BEFORE USE! detergent Tween-20	**WASH BUFFER:** Colorless liquid filled in a white bottle with white screw cap. Buffer solution containing surfactant. The concentrate must be diluted 1 to 20 with distilled/deionized water before use. Once diluted, stable for 1 week at room temperature, or for 2 weeks when stored at 2-8°C.
CHROM | SOL | A Code 2 (1x7ml per vial)	**CHROMOGEN SOLUTION A:** Colorless liquid filled in a white vial with green screw cap. Urea peroxide solution. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
CHROM | SOL | B Code 3 (1x7ml per vial)	**CHROMOGEN SOLUTION B:** Colorless liquid filled in a black vial with black screw cap. TMB (Tetramethyl benzidine), N,N- dimethylformamide. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
STOP | SOL | Code 4 (1x7ml per vial)	**STOP SOLUTION:** Colorless liquid in a white vial with white screw cap. Diluted sulfuric acid solution (0.5M H_2SO_4). Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.

- **PLASTIC SEALABLE BAG:** For enclosing the strips not in use — 1 unit
- **PACKAGE INSERT** — 1 copy
- **CARDBOARD PLATE COVER** — 2 sheets

To cover the plates during incubation and prevent evaporation or contamination of the wells.

MATERIALS REQUIRED BUT NOT PROVIDED

Freshly distilled or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing system and/or pipette, disposable pipette tips, absorbent tissue or clean towel, dry incubator or water bath, 37±1°C, plate reader, single wavelength 450nm or dual wavelength 450/600-650nm, microwell aspiration/wash system.

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

1. **Specimen Collection:** No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the specimen should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, but *highly lipaemic, icteric, or hemolytic specimens should not be used* as they can give false results in the assay. *Do not heat inactivate specimens*. This can cause deterioration of the target analyte. Specimens with visible microbial contamination should never be used.
3. WANTAI anti-HTLV 1+2 ELISA is intended ONLY for testing of individual serum or plasma specimens. Do not use the assay for testing of cadaver specimens, saliva, urine or other body fluids, or pooled (mixed) blood.
4. **Transportation and Storage:** Store specimens at 2-8°C. Specimens not required for assaying within 1 week should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, specimens should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical specimens and etiological agents.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, do not freeze. To assure maximum performance of WANTAI anti-HTLV 1+2 ELISA, during storage, protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

TO BE USED ONLY BY QUALIFIED PROFESSIONALS

The ELISA assays are time and temperature sensitive. To avoid incorrect result, *strictly follow the test procedure steps and do not modify them.*

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.
4. Use only sufficient volume of specimen as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.
5. Do not touch the exterior bottom of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
7. Avoid long time interruptions of assay steps. Assure same working conditions for all wells.
8. Calibrate the pipette frequently to assure the accuracy of specimens/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.
9. Assure that the incubation temperature is 37°C inside the incubator.
10. When adding specimens, do not touch the well's bottom with the pipette tip.
11. When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.
12. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.
13. If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.
14. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
15. **WARNING:** Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for HBsAg and antibodies to HIV 1/2, HCV, TP. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
16. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
17. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
18. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.
19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.
20. The Stop solution 0.5M H_2SO_4 is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.
21. ProClin™ 300 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

INDICATIONS OF INSTABILITY DETERIORATION OF THE REAGENT: Values of the Positive or Negative controls, which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the specimens must be retested. In case of constant erroneous results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one or contact Wantai technical support for further assistance.

Warning:
H317, P280, P333+P313, P363
ProClin™ 300

Danger:
H360D, P201, P280, P308+P313
N,N- dimethylformamide

PROCEDURE

Reagents preparation: Allow the reagents to reach room temperature (18-30°C). Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and only clean vessels to dilute the buffer. All other reagents are **READY TO USE AS SUPPLIED.**

Step 1 **Preparation:** Mark three wells as negative control (e.g. B1, C1, D1), two wells as Positive control (e.g E1, F1) and one Blank (e.g. A1, neither specimens nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

Step 2 **Adding HRP-Conjugate:** Add 60µl of HRP-Conjugate into each well except the Blank.

Step 3 **Adding Specimen:** Add 60µl of Positive control, Negative control, and Specimen into their respective wells except the Blank. **Note: Use a separate disposal pipette tip for each specimen and standard to avoid cross-contamination.** Mix by tapping the plate gently.

Step 4 **Incubating:** Cover the plate with the plate cover and incubate at 37°C **for 60 minutes.**

Step 5 **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **6 times** with diluted Wash Buffer. Each time allow the microwells to soak for **30-60 seconds.** After the fina washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.

Step 6 **Coloring:** Add 50µl of Chromogen Solution A and then 50µl of Chromogen Solution B into each wel including the Blank, mix gently. Incubate the plate at 37°C **for 30 minutes avoiding light.** The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and in anti-HTLV 1/2 positive specimen wells.

Step 7 **Stopping Reaction:** Using a multichannel pipette or manually, add 50µl of Stop Solution into each wel and mix gently. Intensive yellow color develops in Positive control and anti-HTLV 1/2 positive specimer wells.

Step 8 **Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbance at **450nm.** If a dual filter instrument is used, set the reference wavelength at **600~650nm.** Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within **10 minutes** after stopping the reaction).

INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential in order to obtain correct and precise analytical data.
2. It is therefore, recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than **6 automatic washing** cycles of **350-400µl/well** are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with specimen or HRP-conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to carry out **6 washing** cycles, dispensing **350-400µl/well** and aspirating the liquid for **6 times.** If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before they are wasted in an appropriate way.
7. The concentrated Wash buffer should be diluted 1 to 20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

QUALITY CONTROL AND CALCULATION OF THE RESULTS

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well A value from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and controls.

Calculation of the Cut-off value (C.O.) = No + 0.18
(No = the mean absorbance value for three negative controls).

Quality control (assay validation): The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient specimen being analyzed.

Appendix V: Ethical approval

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/417

16th November 2018

KENYATTA NATIONAL HOSPITAL APPROVED 16 NOV 2018 KNH/UoN-ERC P. O. BOX 20723

Dr. Naomi Moraa Ariaga
Reg. No.H58/81322/2015
Dept. of Human Pathology
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Moraa

RESEARCH PROPOSAL – SEROPREVALENCE OF HUMAN T-CELL LYMPHOTROPIC VIRUS I AND 2 IN BLOOD DONORS AT TWO BLOOD DONOR CENTRES IN NAIROBI, KENYA (P541/08/2018)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and approved your above research proposal. The approval period is 16th November 2018 – 15th November 2019.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Protect to discover

Yours sincerely,

PROF. M.L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Chairperson, KNH-UoN ERC
The Assistant Director, Health Information, KNH
The Dean, School of Medicine,UoN
The Chairperson, Dept. of Human Pathology, UON
supervisors: Dr. Peter M. Maturi, Dr.Valerie K. Magutu

Appendix VI: Clearance from NBTS

MINISTRY OF HEALTH

KENYA NATIONAL BLOOD TRANSFUSION SERVICE – HQS.
LOCATION: KENYATTA NATIONAL HOSPITAL, NPHLS GROUNDS
P.O.BOX 29804-00202
NAIROBI

Ref: MOH/KNBTS/RES&DEV/VOL.1/61

2nd May, 2019

Dr Naomi Ariga
Department of Human Pathology,
School of Medicine
College of Health Sciences,
University of Nairobi
NAIROBI

RE: LETTER OF NO OBJECTION -RESEARCH AUTHORIZATION

I refer to your research letter dated 19th Feb 2019 on the above subject.

KNBTS has no objection for you to carry out the study at RBTC Nairobi on **"Seroprevalence of HTLV 1 & 2 in Blood Donors"**

You are expected to give feedback of the study findings to the office of the undersigned before publication.

Dr. Josephine Githaiga
HEAD – KNBTS

Cc: Head RBTC Nairobi

ISO 9001 : 2015 Certified