PREVALENCE OF CYTOMEGALOVIRUS ANTIBODIES IN BLOOD DONORS AT THE NATIONAL BLOOD TRANSFUSION CENTRE, NAIROBI - KENYA

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DECLARATION

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I **Dr. Dorothy G. Njeru**, declare that this dissertation for Master of Medicine in Human Pathology is my original work and has not, to the best of my knowledge, been presented by any other individual at any other institution of higher learning.

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DEDICATION

This work is dedicated to my parents, Mr and Mrs Francis N. Macharia.

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ABBREVIATIONS

AABB	American Association of Blood Banks
AIDS	Acquired immunodeficiency syndrome
BP	Blood pressure
CMV	Cytomegalovirus
CDC	Centres for disease control
CSF	Cerebrospinal fluid
Cc	Centilitres
°C	Degrees centigrade
$CuSO_4$	Copper sulphate
DNA	Deoxyribonucleic acid
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
FHI	Family Health International
G (g)	Grams
G20	Gauge 20
Hb	Haemoglobin
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HHV-5	Human herpes virus type 5
HIV	Human immunodeficiency virus
HPC	Haematopoietic progenitor cells
HTLV	Human T-cell leukaemia virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
JICA	Japan International Cooperation Agency
KEMRI	Kenya Medical Research Institute
KNH	Kenyatta National Hospital
KNH/ERC	Kenyatta National Hospital Ethics and Research Committee
KSH	Kenya shillings
KTS	Kenya Transfusion Service
M.B.Ch.B	Bachelor of Medicine and Surgery

MHC	Major Histocompatibility Complex
MMed	Master of Medicine
MOH	Ministry of Health
mRNA	Messenger ribonucleic acid
NASCOP	National AIDS and STD Control Programme
NBTC	National blood transfusion centre
NBTS	National blood transfusion service
NPHLS	National Public Health Laboratory Services
OD	Optical density
Path	Pathology
PCR	Polymerase chain reaction
QA	Quality assurance
RBTC	Regional Blood Transfusion Centre
RhD	Rhesus D
Rpm	Revolutions per minute
SPSS	Statistical Package for Social Sciences
STD	Sexually transmitted disease
TT-CMV	Transfusion Transmitted cytomegalovirus
TTI	Transfusion transmissible infections
TMB	Tri-methyl benzoate
UK	United Kingdom
Ul (ul)	Microlitres
U/ml	Units per millilitre
UON	University Of Nairobi
USA	United States of America
VDRL	Venereal Disease Research Laboratory
WBC	White blood cells
WHA	World Health Assembly
WHO	World Health Organisation

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ABSTRACT

Background

Cytomegalovirus (CMV) is known to be associated with significant morbidity and mortality following homologous blood transfusion in neonates and other immune compromised persons. Documentation of the status of antibodies to cytomegalovirus in the blood donor pool in our setting is vital to the understanding of the potential likelihood of transmission through donor blood and for determining the best transfusion practices to prevent transfusion-acquired CMV infection. There is little information available with regards to the level of Cytomegalovirus antibodies in donor blood at the National Blood Transfusion Centre in Nairobi.

Objective

To determine the prevalence of CMV infection among blood donors at the National Blood Transfusion Centre (NBTC).

Design and Setting

A cross sectional study conducted at the National Blood Transfusion Centre, Nairobi and the University of Nairobi immunology laboratory.

Methods

Four hundred eligible blood donors, as defined by the NBTS criteria, were studied. Demographic data (age, gender, marital status, level of formal education, geographical area of residence and monthly income) was obtained by direct interviews. Documentation of laboratory results for Human Immunodeficiency Virus (HIV), syphilis, hepatitis B and hepatitis C was done from donor registers at the NBTC. Enzyme-linked immunosorbent assay (ELISA) tests for anti-CMV IgG and IgM antibodies were carried out at the UON immunology laboratory.

Data handling

The data collected and that generated from the laboratories were entered in pre-designed study questionnaires. This data was then entered into a computer data base from which spreadsheets were generated and transferred to the SPSS (version 15) statistical software for analysis. Summary of the statistics was determined during the analysis and presented as proportions and percentages in the form of tables and graphs.

Results

A total of 400 blood donors were recruited into the study out of whom 57.9% were male and 42.1% were female. The age group pattern showed that: 42.5% were in the 16 to 20 years age bracket, 24.3% in the 21-25, 14.4% in the 26-30 and 17.2% were above 30 years of age. The unmarried participants constituted 78.5% whereas 20.5% were married and 0.8% were divorced. The educational level profile was as follows; only primary school education 5.4%, secondary school 54.8%, college 33.9% and university 5.9%. Their monthly incomes were; 31.1% less than Kshs 5,000, 66.1% between 5,000 and 50,000 and 2.9% between 50,000 and 100,000. Of all the participants 60.5%, were sexually active while 39.5% recorded that they were not.

There were 97.0% and 3% of the studied participants who tested positive and negative respectively for anti-CMV IgG antibodies. Those who tested positive for anti-CMV IgM were 3.5% while 96.5% tested negative. All the participants who tested positive for anti-CMV IgM antibodies also tested positive for anti-CMV IgG antibodies. Female blood donors showed a higher prevalence of anti-CMV IgG than male donors ($p=0.016$). There was however no statistically significant difference in the prevalence of IgM between the genders ($p=0.955$). The study did not show any statistically significant difference in sero-prevalence (of both IgG and IgM) with age, marital status, education level, income or occupation.

Those who tested positive for HIV were 1.3% while 98.7% tested negative. There were 0.3% participants who were positive for syphilis and 99.7% who were negative. Hepatitis B positive participants were 2.3% and 97.7% were negative. Those positive for Hepatitis C were 1.0% while 99.0% were negative.

Conclusions

This study shows that the prevalence of anti-CMV antibodies in blood donors at the NBTC was 97% and 3.5% for IgG and IgM respectively. Female blood donors showed a higher prevalence of anti-CMV IgG compared to the male donors. The study does not demonstrate any significant influence of age, marital status, education or even the sexual status on the prevalence of anti-CMV antibodies. Infection with other transfusion transmissible infections does not show any significant relationship with infection with CMV.

Recommendations

It is important to screen donor blood for anti-CMV IgM especially that which is intended for transfusion to patients at the risk of severe morbidity from CMV disease. Further studies will be necessary to determine the reasons for the difference in the sero-prevalence of IgG between female and male donors as well as to determine the age of seroconversion of (and therefore the earliest exposure to) CMV. It is also recommended that the high risk groups who have been transfused be evaluated to determine if they may have acquired transfusion-transmitted CMV.

INTRODUCTION

The transfusion of blood is an age old therapeutic procedure used as a life saving intervention in all clinical disciplines[1]. Blood transfusion, like all medical interventions, though generally safe, is not entirely free of risks to the recipient. Some of the risks may be life threatening, especially if it is not performed according to the set norms, standards and guidelines[2].

One of the biggest challenges to blood safety particularly in the developing world is accessing safe and adequate supplies of blood and blood products. Only 30% of the world's blood supply is available to the countries with low human development index in spite of the fact that 80% of the world's population live in these countries[1]. Moreover, these same countries are fraught with enormous disease burdens including HIV/AIDS and malarial infection, which often require blood transfusion in the course of their management. Some of these conditions like HIV infection limit the choice of donors to the patient.

The AIDS epidemic update from UNAIDS/WHO of December 2007 states that sub-Saharan Africa (which includes Kenya) has more than 60% of all the people living with HIV yet it constitutes just over 10% of the worlds population. The number of HIV-infected individuals was 22.5 million by the end of 2007 and an estimated 2.5million people in the region became newly infected in 2007 alone[3]

Besides the HIV/AIDS pandemic, the African region is faced by many other health problems for which management requires transfusion of blood. These include malaria, malnutrition, helminthiasis, and obstetric complications among others. Malaria infection in expectant mothers and the resultant anaemia are important causes of low and very low birth weights in neonates. Most of these conditions lead to anaemia as a complication. Anaemia is one of the commonest reasons for transfusion. Indeed, a study done in Kenya by the MOH, JICA and KEMRI involving 43 hospitals found that anaemia and ante- and post partum haemorrhage were among the three leading reasons for blood transfusion, accounting for 67.4% (16,816 units) and 7.6 % (1835 units) respectively[4].

Kenya, like many other developing countries in the world, has a large population of people at risk of infection with TTIs. 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 for infections (HIV, hepatitis and syphilis) to include other TTI like CMV. This is especially important when the blood and blood components are intended for high-risk groups.

Cytomegalovirus is a double stranded DNA virus that is found throughout the world in all geographical locations and socio-economic groups. It infects between 50% and 85% of all adults in the United States by the age of 40 years[5]. The rate of seropositivity increases with age and decreasing socio-economic status[5-, 7].

Cytomegalovirus transmission requires repeated or prolonged intimate exposure [8]. Some of the documented methods of transmission include sexual contact and blood transfusion. [4] The major risk groups for infection are to the foetus during pregnancy, people who work with children, the immunocompromised and newborn babies[9, 10].

Infection with CMV has been recognized as an important cause of morbidity and mortality in immunocompromised patients who include organ transplant recipients, patients undergoing hemodialysis, cancer patients, patients on immunosuppressive drugs, and HIV-infected patients[2,3,6]. CMV infection also poses serious health problems in infants, particularly in very low birth weight neonates (below 1500g), where the consequences of such infection can be severe or even fatal. Infections in the neonate can be congenital or acquired. Congenital infections occur in 0.4-2.3% of live births according to a study done by Demmler[6, 11, 12]. One of the ways in which the expectant mother can acquire CMV is through transfusion of blood and blood products.

The neonate may also acquire CMV while in utero by transplacental transfusion with infected blood components. Postnatal infection in the neonate can be through the ordinary blood transfusion or through exchange transfusion[5, 11]. Because of these risks, exposing these patients to CMV should be minimized. Whenever possible, patients without CMV infection should be given organs and/or blood products that are free of the virus[5, 13, 14].

For most healthy persons who acquire CMV after birth, there are few symptoms[3, 4, 15]. Some individuals develop infectious mononucleosis- like illness, with prolonged fever, and occasionally a mild hepatitis[16-18]. However, for the vast majority of immunocompetent individuals, CMV infection does not pose a serious health problem [19-20, 21].

Infection with CMV results in shedding of infectious viral particles into plasma and body fluids[22, 23]. The presence of the virus triggers the body to produce antibodies, which are initially IgM and later IgG[24-26]. The presence of anti-CMV IgM antibodies is an important marker of active CMV infection and these persons can easily transmit the infection to blood product recipients if their blood is used for transfusion[27].

Screening of donor blood for CMV antibodies (particularly IgM) is therefore of paramount importance in the prevention of transfusion-acquired CMV infection. This is even more so when the blood is to be used in patients at risk of severe CMV disease[28]. There has been no demonstrable clinical benefit to providing products of reduced CMV risk to sero-positive patients[29]. Such patients are already at risk of reactivation disease. Although second-strain infections may occur, the clinical significance of these has not been demonstrated[28].

Diagnosis of CMV infection can be made by a variety of tests[30]. These include serology for CMV antibodies[30, 31]. The most commonly employed serological tests for diagnosis of CMV are the Enzyme Immunoassays (EIAs), particularly ELISA (enzyme-linked immunosorbent assay). ELISA, especially the newer generation kits, tests show a high degree of sensitivity and specificity[32]. They are also faster and cheaper to run compared to the other methods available for the diagnosis of CMV[33, 34].

This study was designed to establish the prevalence of CMV infection among the blood donors at the NBTC.

LITERATURE REVIEW

Blood transfusion is the procedure of transferring blood or blood-products from one individual, termed the donor, into the circulation system of another individual, termed the recipient[7]. It is an age old, crosscutting therapeutic procedure used as a life saving intervention in all clinical disciplines and an indispensable practice in the health systems of all nations. Transfusion of blood saves millions of lives each year around the world particularly among vulnerable groups such as children and women of child-bearing age. Formal recognition of blood transfusion as an essential part of patient care by World Health Organisation (WHO) started more than 30 years ago with the recommendation that all WHO member states should base national transfusion services on non-remunerated volunteer blood donors. This was adopted as the resolution WHA28.72 (1975) by the World Health Assembly and was based on findings that donors not paid for their donations have the lowest risk of transfusion-transmitted viruses. [35]

Though a life-saving procedure, when blood transfusion is not performed in accordance with set norms, standards and guidelines, there are potential risks of spreading transfusion transmissible infections such as HIV/AIDS, Hepatitis B, Hepatitis C, and cytomegalovirus, among others; and causing transfusion reactions that could be life threatening[1, 2, 3]. The Regional Committee for Africa, during its fourteenth session in September 1994, noted with great concern that only 10 out of 46 Member States of the Region could guarantee the safety of blood transfusion in their health care settings[35]. The provision of adequate and safe blood is vital in the efforts being made to achieve the three Health Targets of the United Nations Millennium Development Goals, i.e. reduce child mortality, improve maternal health and combat HIV/AIDS, malaria and other diseases[3].

To reduce the risk of disease transmission donor blood is screened for most of these infections. In developed countries, donor blood is screened for HIV (1 and 2), hepatitis (B and C), HTLV (1 and 2), syphilis, West Nile virus and CMV[2, 3]. Blood screening for CMV is mainly done in non-directed blood donation and blood intended for transfusion to those at high risk of severe or even fatal CMV disease[7]. In Kenya, donated blood is screened for syphilis, HIV (1 and 2) and hepatitis (B and C).

In some geographical regions diseases caused by certain other microorganisms like bacteria, viruses, and parasites, such as babesiosis, Chagas disease, malaria, Lyme disease and others can also be transmitted by blood and blood product transfusions. Screening of potential donors with questions about health status and travel ensures that such cases are extremely rare[8].

Cytomegalovirus

Cytomegalovirus is an enveloped virus that belongs to the genus of Herpes viruses[3]; in humans the species is known as Human herpes virus 5 (HHV-5). It has double strand DNA, four species of mRNA, a protein capsid, and a lipoprotein envelope. Like other herpes viruses, CMV demonstrates icosahedral symmetry, replicates in the cell nucleus and can cause a lytic and productive or a latent infection[6, 8]. It belongs to the *Betaherpesvirinae* subfamily of *Herpesviridae*. The name cytomegalovirus means a "very big cell virus"[7].

Cytomegalovirus can be distinguished from other herpes viruses by certain biological properties such as, host range and type of cytopathology induced[7]. Viral replication is associated with the production of large intranuclear inclusions and smaller intracytoplasmic inclusions. The virus seems to replicate in a variety of cell types in vivo (including those of endothelial, epithelial, mesenchymal, hematopoietic and neuronal lineages)[8]; in tissue culture it grows preferentially in fibroblasts. Although there is little evidence that CMV is oncogenic in vivo, the virus does transform fibroblasts in rare instances, and genomic transformation fragments have been identified[6].

Epidemiological aspects

Cytomegalovirus has a worldwide distribution[6, 8]. Serologic surveys in the United States and Great Britain have shown that 40-60% of adults of middle or upper socio-economic status have antibodies to CMV. The seropositivity rate is approximately 80% for adults in the lower socio-economic status[6]. Other studies show that 58.9% of individuals aged 60 and over are infected with CMV; this number rises to 90.8% of individuals aged 80 and over[7, 8].

It is estimated that about 1% of newborns in the United States are infected with CMV, and the percentage is higher in many less developed countries[8, 37]. However in most developing countries prevalence data for CMV infection is not available.

Modes of Transmission

Cytomegalovirus is not readily spread by casual contact but requires repeated or prolonged intimate exposure for transmission[7, 8]. In late adolescence and young adulthood, CMV is often transmitted sexually and asymptomatic viral carriage in semen and cervical secretions is common[15]. CMV antibody is present in nearly 100% of female prostitutes and sexually active homosexual men. Sexually active adults may harbor several strains of CMV simultaneously[8]. Transfusion of whole blood and/ or certain blood products containing viable leucocytes may also transmit CMV with frequency of 0.14 to 10% per unit transfused [38]. In Kenya, most of the transfusions done use whole blood, according to a study done by the MOH, JICA and KEMRI in 2002[5].

Transmission by blood transfusion

Transfusion of blood can lead to CMV infection in the recipient by three mechanisms; as transfusion-transmissible CMV (TT-CMV), as reactivated CMV infection occurring when a seropositive recipient experiences reactivation of latent CMV following blood transfusion from a seronegative donor (possibly due to immunomodulatory interactions between MHC mismatched leucocytes of the donor and recipient), and as CMV super infection (second strain infection) when a seropositive recipient contracts a new strain of CMV from an infectious blood component.[8] These three mechanisms appear to occur with similar frequencies. TT-CMV is more clinically important as the recipient lacks immunologic memory[8].

Although TT-CMV produces a primary CMV infection, in the immunocompetent transfusion recipient it is of no more clinical significance than the community acquired CMV infection[7, 8]. Furthermore, the risk of TT-CMV is very low in these patients. The likelihood of CMV transmission by transfusion (transmission is only through cellular blood components[28, 38]) of unscreened blood is higher in immuno-deficient patients, with observed transmission rates of 5 to 13% in low-birth-weight infants, 20% in seronegative recipients of seropositive solid organs and 25-65% in seronegative recipients of seropositive bone marrow transplants[28]. Other studies suggest that 13-37% of

immunocompromised patients will contract TT-CMV from transfusion of unscreened and unfiltered cellular blood components[8].

Clinical presentation

For most healthy individuals who acquire CMV after birth there are few or no symptoms[7]. Some persons with symptoms experience infectious mononucleosis-like illness with prolonged fever, and a mild hepatitis, with sore throat being common. When an individual becomes infected, the virus latently persists in the body for the person's life and can exhaust the immune system at old age, increasing risk of mortality from other diseases[8]. Recurrent disease rarely occurs unless the person's immune system is suppressed due to therapeutic drugs or disease[7]. Rarely CMV infection may be fatal in the immuno-competent hosts; and some of those who survive may have recurrent episodes of fever and malaise that are sometimes associated with autonomic nervous system dysfunction[6]. But generally, for the vast majority of people, CMV infection has no serious consequences.

Risk groups for CMV infection

Cytomegalovirus infection is important in certain high-risk groups. Some of the documented risk factors for infection include; low socio-economic status, low education levels, the unmarried, low income levels, and increased parity on women[10]. The major areas of risk of infection may include risks to the fetus during pregnancy, people who work with children, the immunocompromised patients, such as organ transplant recipients and persons infected with human immunodeficiency virus (HIV). In HIV infection, CMV disease is an AIDS defining infection, indicating that the T-cell count has dropped to significantly low levels, and to the newborn babies[15].

Cytomegalovirus infection in pregnancy and neonates

The incidence of primary CMV infection in pregnant women varies widely dependent on geographical region and socioeconomic status for example in the United States varies from 1% to 3%[5]. Other studies show that 0.7 - 4.1% of susceptible women acquire CMV infection during pregnancy[39, 40]. Healthy pregnant women are not at special risk for disease from CMV infection. When infected with CMV, most women have no symptoms

and a few have the disease resembling infectious mononucleosis. The developing fetuses are at risk for congenital CMV disease.

Cytomegalovirus remains the most important cause of congenital viral infection in the United States and occurs in 0.4-2.3 % of live4 births[9]. Infants who are infected by their mothers before birth, run the risk of two potential problems: First generalized infection may occur in the infant, and symptoms may range from moderate hepatosplenomegaly, with jaundice, to fatal illness. Studies have shown that 67% of infants with congenital CMV infection develop hepatosplenomegaly, jaundice, petechiae, and thrombocytopenia[41]. With supportive treatment most infants with CMV disease usually survive. However, from 80% to 90% will have complications within the first few years of life that may include hearing loss, vision impairment, and varying degrees of mental retardation[7].

Secondly, another 5% to 10% of infants who have subclinical infection at birth will subsequently develop varying degrees of hearing and mental or coordination problems. Neurologic abnormalities like seizures and hypotonia are common and microcephaly occurs in 75% of infants. Unilateral or bilateral sensorineural hearing loss that may be mild to profound develops in about 30% of infants with symptoms at birth, and hearing deteriorates in >50%[37]. Dental defects can be found in about 40% of survivors of neonatal symptomatic disease[42].

These complications, however appear to be almost exclusively associated with women who previously have not been infected with CMV and who are having their first infection with the virus during pregnancy[39]. This may come about due to transfusion with unscreened (infected) blood. Even in these cases, two-thirds of the infants will not become infected, and only 10% to 15% of the remaining third will have symptoms at the time of birth. There appears to be little risk of CMV-related complications for women who have been infected at least six months prior to conception[40]. For this group, which makes up 50% to 80% of the women of child-bearing age, the rate of newborn CMV infection is 1%, and these infants appear to have no significant illness or abnormalities[37].

In summary, during a pregnancy if a woman who has never had CMV infection becomes infected with CMV, there is a potential risk that after birth the infant may have serious CMV-related complications, the most common of which are associated with hearing loss, visual impairment, or diminished mental and motor capabilities. On the other hand, infants and children who acquire CMV after birth have few, if any, symptoms or complications.

The virus can also be transmitted to the infant at delivery from contact with genital secretions or later in infancy through breast milk. These infections usually result in little or no clinical illness in the infant.

Low birth weight and premature infants are at particular risk of severe or even fatal CMV disease. This is mainly because they are likely to be transfused for anemia, anemia of the premature infant, or even undergo exchange transfusion (for neonatal jaundice)[40, 43].

Cytomegalovirus in the immunocompromised

Primary CMV infection in the immunocompromised patient can cause serious disease. In patients with a depressed immune system, CMV-related disease may be much more aggressive[7, 8]. CMV hepatitis may cause fulminant liver failure. Other specific disease entities recognized in these patients are cytomegalovirus retinitis (inflammation of the retina, characterized by a "pizza pie appearance" on ophthalmoscopy which is an important cause of blindness in this group) and cytomegalovirus colitis[6]. Other manifestations of CMV disease in the immuno-compromised individuals include CMV pneumonia (seen in about 20% of bone marrow recipients and is associated with 84-88% case fatality), pneumonitis and rarely meningo-encephalitis[44, 45].

The groups of immunocompromised patients in whom infection with CMV is a major cause of disease and death, include organ transplant recipients, patients undergoing hemodialysis, cancer patients, patients receiving immunosuppressive drugs, and HIV-infected patients. Because of the risks involved, exposing immunosuppressed patients to outside sources of CMV should be minimized. Whenever possible, patients without CMV infection should be given organs and/or blood products that are free of the virus[7, 8].

Fatal CMV infection is associated with persistent viraemia and involvent of multiple organ systems[18]. Progressive pulmonary infiltrates, pancytopenia and hyperamylasaemia are features seen in terminal CMV disease[6]. Extensive adrenal necrosis with CMV inclusions has also been documented, at autopsy[4].

Laboratory diagnosis of CMV

Most infections with CMV are not diagnosed because the virus usually produces few or no symptoms and tends to reactivate intermittently without symptoms[46]. Diagnosis of CMV infection can be made by a variety of tests. These include serology for CMV antibodies, histologic recognition of cytomegalic inclusion bodies, immunostaining of histologic specimens, in situ hybridisation for histopathological identification of infected cells in tissue, cytomegalic endothelial cells in the blood of immunocompromised patients with disseminated CMV, viral isolation, antigenaemia assay and amplification techniques (PCR amplification and other amplification techniques)[15].

Following primary CMV infection or reactivation of latent virus, the body reacts by production of antibodies[17, 18]. These are initially IgM antibodies and their presence in blood usually indicates an active infection. CMV-specific IgM antibodies are produced within three to five days of the acute infection but become detectable in blood (by ELISA) after a patient has been clinically ill for a week or more. Some patients may, however, continue to produce CMV-specific IgM antibodies for six to nine months following a primary infection[9]. Immunoglobulin G (IgG) antibodies appear 10-14 days later and persist in the body for the lifetime of that individual. The presence, in blood, of CMV-specific IgG antibodies implies exposure to CMV but not necessarily active CMV infection[19].

A variety of serologic assays that detect these antibodies to CMV, have been developed and are widely available from commercial laboratories. They detect increases in titres of antibody to CMV antigens[51]and are useful in determining if an individual is having an active infection or just previous exposure. An increased antibody level may not be detectable up to four weeks after primary infection, and titres often remain high after primary infection. Therefore, paired samples (usually two weeks apart) are preferred to single sample antibody determinations. Detection of CMV-specific IgM is useful in the diagnosis of recent or active infection but circulating rheumatoid factors may result in

occasional false positive IgM test results[51, 52]. Diagnosis of active CMV infection by ELISA for CMV-specific IgM antibodies has been shown to be superior and practical[53]. Detection of CMV-specific IgG antibodies in blood is an indicator of previous exposure to CMV (while IgM antibodies are associated with active CMV infection) [51, 54, 55].

During CMV infection, active viral replication results in shedding of infectious virions into plasma and body fluids, including saliva, tears, breast milk, urine, stool and semen. Isolation of the virus or detection of CMV antigens from appropriate specimens is the preferred diagnostic approach[18]. Virus excretion is readily detected by culture of specimens obtained from urine, throat swabs, bronchial lavages and tissue on human fibroblast monolayers.

When viral titres are high, as is frequently the case in congenital disseminated infection or in AIDS patients, cytopathic effects may be detected in a few days[8]. Cytopathic effects may, however, take several weeks to appear especially when the viral titres are low. Isolation of virus from saliva or urine does not provide proof of acute infection since virus excretion from these sites may continue for months or even years after illness. Detection of CMV viraemia is a better predictor of acute infection[6].

Detection of CMV antigens in peripheral blood leucocytes or of CMV DNA in blood or tissues may hasten the diagnosis of CMV in certain populations, like the organ recipient transplants and persons with AIDS[47, 49, 51]. These assays may yield positive results several days earlier than culture methods. The most sensitive way to detect CMV in blood or other fluids is by amplification of CMV DNA by PCR. PCR detection of CMV DNA in blood may predict the risk of disease progression, and PCR detection of CMV in CSF is useful in the diagnosis of CMV encephalitis or polyradiculopathy[48]. Qualitative and quantitative PCR testing for CMV allows physicians to monitor the viral load of CMV-infected patients[47, 48].

Viral culture and PCR amplification techniques are very sensitive and specific but they are expensive and require special equipment and specially trained personnel to perform. They also have a long turn around time and may delay decision making especially when blood transfusion is required for emergencies[8]. ELISA tests, on the other hand, have a high level of sensitivity and specificity (up to 99% especially with the newer generation kits) [8]. They are generally much cheaper and easy to perform. They employ equipment

and know-how that is available in all transfusion centres in Kenya and are therefore easy to integrate into the system that already exists[33, 34].

Prevention: importance of screening donor blood for CMV

There are prophylactic measures that are useful in the prevention of CMV infection in patients at high risk. The use of blood from seronegative donors or of blood that has been frozen, thawed and deglycerolised greatly decreases the rate of transfusion-associated transmission of CMV[23]. Additionally matching of organ or bone marrow transplants by CMV serology, with exclusive use of organs from seronegative donors in seronegative recipients, reduces the rates of primary infection following transplantation[6].

The risk of CMV transmission to high risk individuals can be greatly reduced by restricting their transfusions to blood products obtained from seronegative donors[21]. Multiple studies, including prospective randomized controlled trials, have demonstrated that exclusive use of CMV seronegative units can decrease the incidence of TT-CMV, compared with the use of unscreened units[8]. Breakthrough TT-CMV may occur in cases where the earlier generation serology assays have been used or when the donor was in the window period at the time of blood donation[8].

Detection of anti-CMV antibodies of the IgM class (early during the course of infection) is important for prevention of TT-CMV[8]. One study showed that patients who received components with detectable IgM anti-CMV antibodies had a TT-CMV rate of 8.8% , compared with a rate of 0.3% among those who received blood without anti-CMV IgM ($P<0.001$)[8]. In the same study, only 1 of 163 neonates who received seropositive but IgM negative developed TT-CMV. Screening of donor blood for CMV is, therefore, very important in providing safe blood for those patients who are at risk of severe CMV infection[21].

Alternative to screening blood donations for CMV antibodies, studies have demonstrated that transmission of CMV can be greatly reduced by processes that remove the majority (two to three logs) of WBCs from blood products[22]. Most investigators have observed virtually no transmission of CMV by leuco-reduced blood components. A randomized clinical trial study comparing the risk of CMV transmission through transfusion of leucoreduced compared to seronegative blood products showed no statistically significant

difference in transmission rates between the two groups (2.4 and 1.3 % respectively). It has therefore been concluded that it is acceptable to use either[27].

It is generally recommended that cellular products with a reduced risk of transmitted CMV (antibody screened or leuco-reduced) be used for patients at high risk of severe primary CMV disease (as described above) [24, 25]. Products with reduced CMV risk are often provided also for seronegative patients who are likely to be treated with transplantation in the future to reduce their risk of CMV reactivation disease[29].

There has been no demonstrable clinical benefit to providing products of reduced CMV risk to sero-positive patients. Such patients are already at risk of reactivation disease. Although second-strain infections may occur, the clinical significance of these has not been demonstrated[8, 28].

Both live attenuated and CMV subunit vaccines have been evaluated but have not been approved for clinical use. CMV immune globulin has been reported to reduce the rates of CMV associated syndromes among seronegative renal transplant recipients; this has not been proved with bone marrow transplant recipients. Prophylactic acyclovir or valacyclovir may reduce rates of CMV infection and disease in certain seronegative renal transplant recipients, though neither is effective in the treatment of active CMV disease[7, 49].

Treatment of CMV infection

Ganciclovir is a guanosine derivative that has considerable activity against CMV. It is a selective inhibitor of CMV DNA polymerase and clinical studies have indicated response rates of 70-90% among patients with AIDS given ganciclovir for treatment of CMV retinitis or colitis. In bone marrow transplant recipients with CMV pneumonia, ganciclovir (in combination with CMV immune globulin) elicits 50-70% clinical response[7, 8, 56].

In view of the fact that vaccines are not available and the results of treatment of CMV disease are still not satisfactory, it is recommended that these high risk groups be transfused with CMV negative blood and blood products[57, 58, 59, 60].

In many patients with AIDS, persistently low CD4+ cell counts and CMV disease, clinical and virologic relapses occur promptly if treatment with ganciclovir is discontinued. Resistance to ganciclovir is common among patients treated for more than three months and is usually related to mutation in the CMV UL97 gene[7, 56].

Treatment with ganciclovir consists of a 14-21 day induction phase at 5mg/kg intravenously twice daily followed by a prolonged maintenance course, twice weekly for the parenteral preparation or daily for the oral preparation. Other drugs that have been shown to be active against CMV include forscanet and cidofovir. These are however not preferred for use due to the considerable toxicities and the inability for most patients to tolerate them[7].

RATIONALE AND STUDY OBJECTIVES

Rationale

Cytomegalovirus infection has been shown to be present throughout the world in all geographical locations and socio-economic groups. Infection with this virus is known to cause serious morbidity and even mortality in certain high risk groups of patients in whom infection may progress to severe, and often disseminated, disease. These patients are among the most commonly transfused individuals and include the immunosuppressed, those on therapy for malignant diseases, expectant mothers, low birth weight neonates and recipients of organ and haemopoietic cell transplants among others.

Blood transfusion is a life saving intervention and cytomegalovirus as a transfusion transmissible infection is recognised globally. Transfusion acquired cytomegalovirus infection is a well documented complication of blood transfusion whose impact on patient care has been appreciated in many centres especially in the developed countries. These countries have since introduced the screening of donor blood for CMV especially that which is intended for use in the high risk groups.

To date, there has not been any satisfactorily effective treatment for CMV disease though many anti-viral drugs have been tried and are being used. Vaccines against CMV are not available. This therefore means that the best available preventive measure is to prevent CMV infection in the first place. Use of reduced CMV risk blood products is the best available method of preventing transfusion acquired CMV infection which can be done by use of blood products obtained from seronegative blood donors.

In Kenya, donor blood is not screened for CMV and the prevalence of CMV among blood donors is not known. It is important that policy regarding the best transfusion practices for preventing transfusion acquired CMV be developed to boost blood safety in the country. To be able to do this, knowledge on the prevalence of CMV among blood donors is imperative. This study serves to bridge the knowledge gap by providing the much-needed data on the sero-prevalence of CMV infection among blood donors.

Research Question

What is the prevalence of CMV infection among blood donors at the NBTC?

Objectives

General objective

To determine CMV seroprevalence among blood donors at the NBTC.

Specific objectives

1. To determine the proportion of blood donors who have been exposed to CMV at the NBTC.
2. To determine the proportion of blood donors who have active CMV disease at the NBTC.
3. To correlate the social demographic characteristics of blood donors with active CMV infection at the NBTC.
4. To correlate exposure to and infection with CMV with that of other TTI (HIV, syphilis and hepatitis).

MATERIALS AND METHODS

Study design and target population

Design

This was a cross-sectional study carried out at the National Blood Transfusion Centre (NBTC) and the Immunology Unit, University of Nairobi.

Target population

The study targeted individuals who were available to donate blood at the various NBTC blood collection sessions. These blood donors included students from the various institutions like secondary schools and colleges within Nairobi province. They also included the general public recruited in towns, hotels and churches in the areas covered by the NBTC (appendix 1).

Case definition

The study participants were either male or female aged between 16 and 65 yrs. They weighed 50kgs and above and had a haemoglobin level of 12.5g/dl (determined using the copper sulphate method, appendix 2) and above. They consented to donate blood and have the blood tested as required by the NBTC. They were screened using the donor screening card (appendix 3) and gave an informed consent to take part in the study (appendix 4). They also had other vital information captured in the study questionnaire (appendix 5).

Inclusion / exclusion criteria

Inclusion

- All individuals available for blood donation who qualified to donate blood as per the guidelines set by the NBTC (appendix 2) were eligible for the study.
- They also had to give an informed consent to voluntarily take part in the study.

Exclusion

- All individuals who did not qualify as blood donors as per the guidelines set by the NBTC (appendix 2) were excluded from the study.
- All blood donors who declined to give consent (to take part in the study) were excluded from the study.

Sampling and sample size

The sample size taken was 400 donors. This was arrived at using the descriptive study sample size formula: The tests run to determine the prevalence of CMV infection among the blood donors were qualitative; such that each donor was either positive or negative. This meant that each donor stood a 50% chance of having CMV infection. Using 50% and rounding off the number to the nearest 100, 400 donors were recruited.

Sample size = $z^2 \times p(1-p) / C^2$

Where: z^2 is the alpha and beta errors

P is the prevalence

1-p is 1- prevalence

C^2 is the confidence limit

1.96x1.96x0.5x0.5/0.05x0.05

=384

Approximately 400 participants.

It was estimated that approximately 100 units of blood were collected at the various sessions organised by the NBTC each day, Monday through to Friday, during the study period .This implied that about 500 units were collected each week and around 2000 each month. The study was carried out over one month, in which approximately 2000 units were collected from the same number of donors. Four hundred donors were required for the study and thus every 5^{th} donor was recruited.

Recruitment of study participants and the process of consenting

The study participants were recruited from the potential blood donors at the various blood donation exercises organised by the NBTC (appendix 1 and 2). To take part in the study, the potential donors were briefed on the importance of the study and the need to give an informed consent. They were also informed the advantages and disadvantages of taking part in the study as well as how they may receive their test results on their CMV status (appendix 4). Their socio-demographic data and their test results were recorded in the study questionnaires (appendix 5).

Laboratory procedures

Specimen collection, transport and storage

Approximately 3 ml of blood was drawn from the cubital vein of each study participant using a sterile needle and syringe and collected into clean well labelled plain vacutainer tubes. The samples were placed in cool boxes at the venue of blood donation and transported in the same cool boxes to the University of Nairobi, Immunology Laboratory where they were tested for CMV-specific IgG and IgM antibodies.

Once in the laboratory, the specimens were separated and the serum transferred into cryo-vials, which were labelled with the same unique number. The samples were stored at 4°C overnight and testing was done the next morning, in batches.

Assay procedure

The samples and kits were retrieved from the refrigerators. The reagents, samples and controls were then brought to room temperature. The tests for anti-CMV IgG and IgM were carried out as per the kit manufacturer's instructions (appendix 6). The calibrators and controls were run in duplicates. Below is a summary of the key steps.

Test for IgG

Diluted serum specimens (1:100) were incubated for 20 minutes to allow specific antibodies to CMV to bind to the antigen-coated wells. After washing away unbound antibodies and other serum constituents, CMV specific IgG was detected using rabbit anti-human IgG conjugated to horseradish peroxidase. After 20 minutes incubation, unbound conjugate was removed by washing, and TMB enzyme substrate added for 10 minutes. A blue colour developed if antibodies to CMV were present. Addition of stop solution gave a yellow colour and the optical densities of calibrators, controls and the samples were measured using a microplate reader (detailed assay procedure, see appendix 6).

Test for IgM

Test sera were diluted (1:100) with the sample diluent. Anti-human IgG was added to the sample diluent to eliminate the possibility of interference by antigen-specific IgG and rheumatoid factor, if present. Diluted serum or plasma specimens were incubated for 20 minutes to allow specific antibodies to CMV to bind to the antigen-coated wells. After washing away unbound antibodies and other serum constituents, CMV specific IgM was detected using rabbit anti-human IgM conjugated to horse radish peroxidase. After 20 minutes incubation, unbound conjugate was removed by washing, and TMB enzyme substrate added for 10 minutes. Addition of stop solution gave a yellow colour and the optical densities of calibrators, controls and sample were measured using a microplate reader (detailed assay procedure, see appendix 6).

The optical densities obtained were used to calculate the results: the mean optical densities for the calibrators, the negative controls and the positive controls were calculated. The CMV IgM and IgG indices were calculated by dividing the mean values of each sample by calibrator mean value.

The interpretation of the results was based on the CMV IgG and IgM indices: a negative result was taken as having a CMV IgG or IgM index less than 0.90 and a positive result as having an index of equal to or greater than 1.00. An index between 0.91-0.99 was taken as equivocal and these samples were re-tested.

Quality assurance

Stringent measures in quality assurance were followed to ensure the results obtained were valid and a true representation of the CMV infection status of the tested donors. These are as outlined below (appendix 7). Similar quality assurance measures were followed at the NBTC laboratories in the testing for HIV, syphilis, hepatitis B and C.

Ethical considerations

The study was carried out after approval had been obtained from the KNH ERC (see appendix 11). Clearance was obtained from the MOH through the NBTC to carry out the study at the NBTC (see appendix 10).

Participants in the study were recruited from individuals eligible to donate blood as per the NBTC requirements, which included giving informed consent to donate blood and the blood thus donated be tested as required by the NBTC (appendix 1, 2 and 3).

All participants in the study were informed that their participation in the study was totally voluntary and no remuneration was offered. They gave informed consent before being recruited into the study. This was by signing a consent form which briefly defined the study and its importance. It also explained to the participant the benefits and risks of taking part in the study (see appendix 4).

All the information obtained from the donors was treated confidentially. All those participants who tested positive for anti-CMV IgM were referred for further management and follow-up through the NBTC (appendix 10)

Data management

Demographic data on the participants' age, gender, marital status, income, residence and level of formal education was obtained by direct interviews. Laboratory results for HIV, syphilis, hepatitis B and hepatitis C were obtained from donor registers at the NBTC. ELISA tests for anti-CMV IgG and IgM were carried out.

The data collected and that generated from the laboratories were entered in pre-designed study questionnaires. This data was then entered into a computer data base from which spreadsheets were generated and transferred to the SPSS (version 15) statistical software for analysis. Summary of the statistics was determined during the analysis and presented as proportions and percentages in the form of tables and graphs.

When relating variables to each other, multivariate analysis was done. Chi-square test and student t-test were employed to detect any significant correlation between different variables. A p value of <0.05 was considered to yield a statistically significant result. Relative risk was also calculated.

RESULTS

Socio-demographic characteristics of the study participants

There were a total of 395 participants recruited into the study. Of the 395, 228 (57.8%) were male and 167 (42.2%) were male. Majority, 169 (42.8%), of them were aged between 16 and 20years. The mean age was 24.17 years with a standard deviation of 7.710. The median was 22 years and the mode was 19 years (47 blood donors, 11.9%). The youngest donor was 16 years old and the oldest was 54 years. Most of the participants were unmarried, 310 (78.5%), had been educated up to secondary school, 215 (29.1%) and earned between Kshs 5,000 and 50,000 per month, 115 (60.8%). (Table 1)

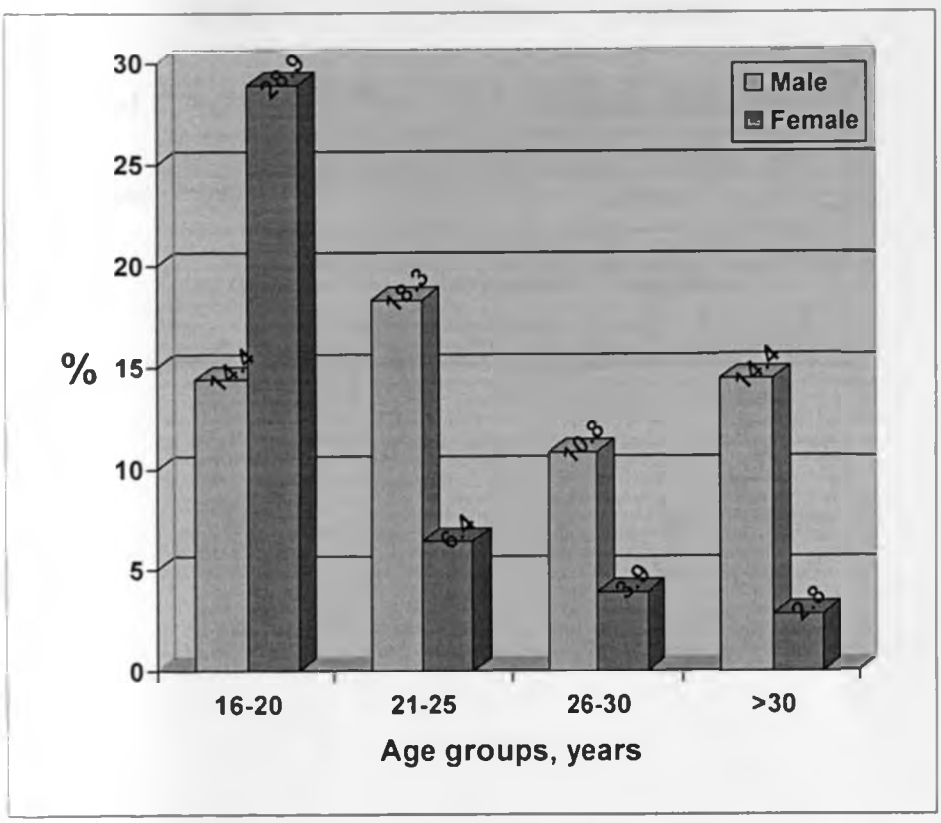
Table 1: Socio-demographic characteristics of the study participants, n = 395

Characteristic	n (%)
Sex	
Male	228 (57.8)
Female	167 (42.2)
Age group	
16-20	169 (42.8)
21-25	97 (24.5)
26-30	59 (14.9)
>30	70 (17.7)
Marital status	
Single	310 (78.5)
Married	82 (20.7)
Divorced	3 (0.8)
Education level	
Primary	23 (5.8)
Secondary	215 (54.4)
College	133 (33.7)
University	24 (6.1)
Monthly income	
<5,000	54 (13.7)
5,000-50,000	115(29.1)
50,000-100,000	5 (1.3)
Sexual status	
Active	239 (60.5)
Not active	156 (39.5)

Age-sex distribution of the study participants

Of the 395 study participants, 228(57.8%) were male donors and 167 (42.2%) were female donors. Of the female donors, 114 (68.7%) were aged between 16 and 20 years, 25 (15.3%) between 21 and 25 years, 15 (9.2%) and 12 (6.7%) between 26 and 30 years and above 30 years respectively. Among the male donors, 54 (24.9%) were aged between 16 and 20 years, 72 (31.5%) between 21 and 25 years and 42 (18.7%) and 54 (24.9%) for the 26-30 and over 30 years age groups. (Figure 1).

Figure 1: Age-sex distribution of the study participants (n = 395)



Distribution of the marital status of the study participants by gender

Majority of the participants were unmarried; 153 (67.1%) male and 157 (94.6%) female. There were 74 (32.5%) married male participants and 7 (4.2%) married female participants. (Table 2)

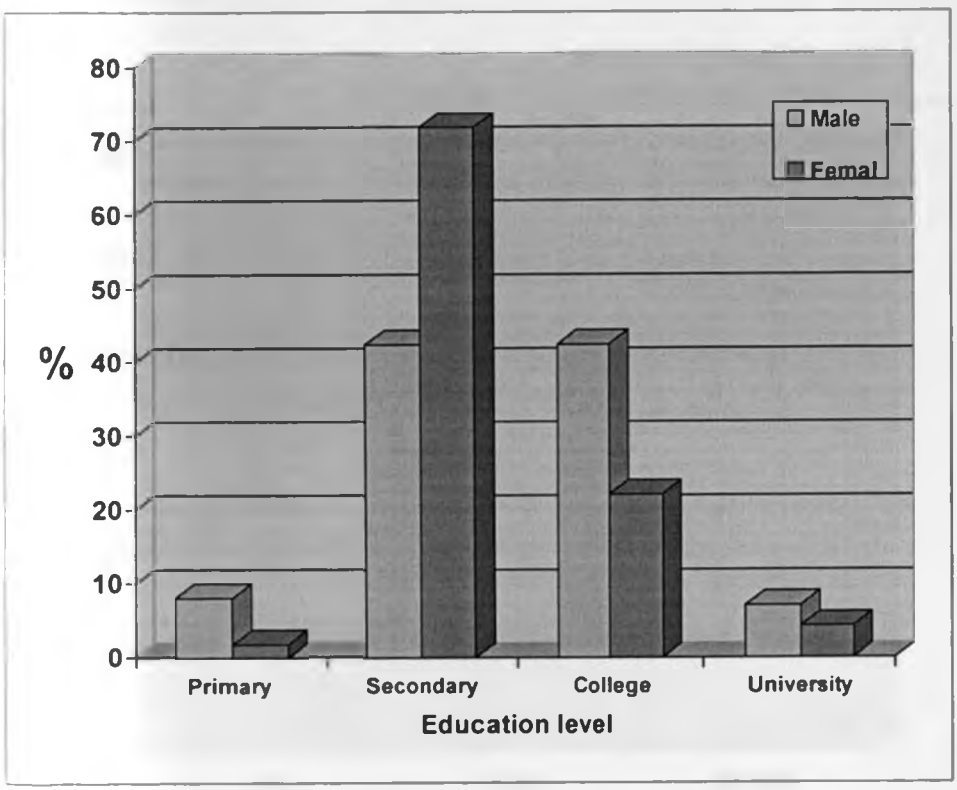
Table 2: Gender-marital status distribution of the study participants (n=395)

Marital status	Gender		Total
	Male Number (%)	Female Number (%)	
Single	153 (67.1)	157 (94.6)	**310 (78.5)**
Married	74 (32.5)	7 (4.2)	**81 (20.6)**
Divorced	1 (0.4)	3 (1.2)	**4 (0.9)**
Total	**228 (100)**	**167 (100)**	**395 (100)**

Formal education distribution by gender of the study participants

Most, 117 (71.8%), of the female participants had received formal education up to secondary school level. For the male participants, 95 (42.4%) had attained secondary education and the same number had attained college education. (Figure 2)

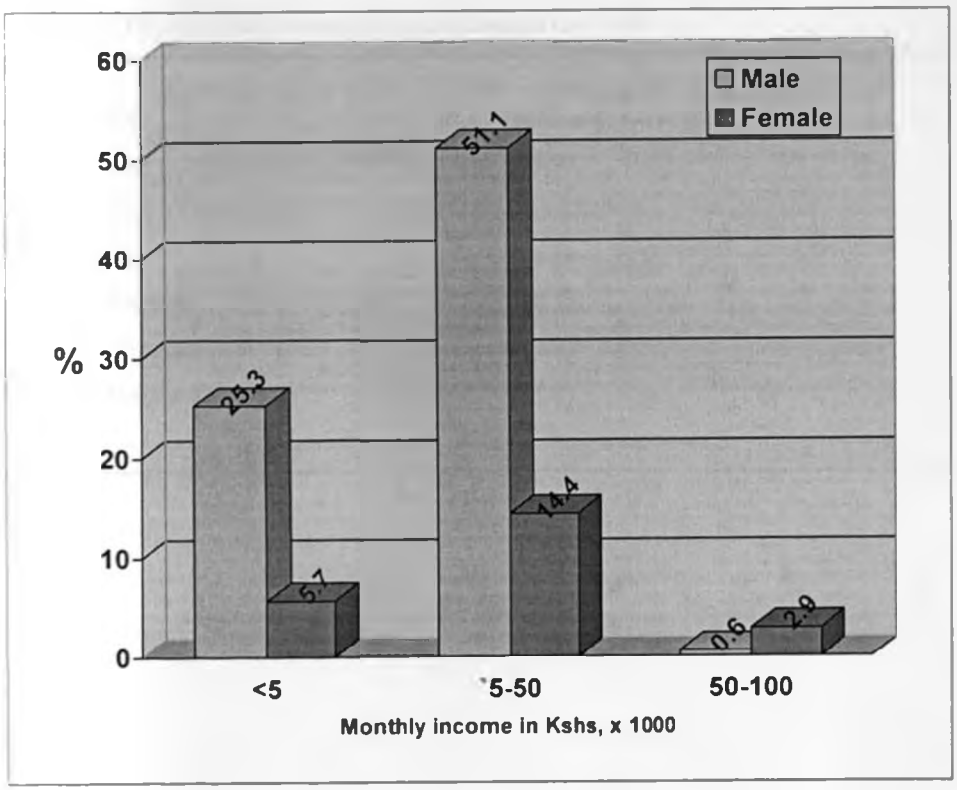
Figure 2: Gender-formal education distribution (n = 395)



Income per month in Kshs of the male and the female participants

Of the 174 participants who were in employment, majority of them earned between Kshs 5,000 and 50,000 per month for both males, 89 (51.1%), and females, 25 (14.4%). There was one (0.6%) male participant earning >Kshs 50,000 and 5 (2.9%) female earning the same amount. (Figure 3)

Figure 3: Monthly income in Kshs by gender (n = 395)



Transfusion transmissible infections (TTI) prevalence among the study participants

There were 5 (1.3%) participants who were HIV positive whereas 390 (98.7%) were HIV negative. One (0.3%) donor tested positive for syphilis and 394 (99.7%) tested negative. Nine (2.3%) participants were positive for hepatitis B and 386(97.1%) were negative while 4 (1.0%) tested positive for hepatitis C and 391(99%) tested negative. 383 (97%) participants were positive for IgG anti-CMV antibodies and 12 (3%) were negative while 14 (3.5%) tested positive for IgM anti-CMV antibodies and 381 (96.5%) tested negative. (Table 3).

Table 3: TTI prevalence among the participants (n = 395)

TTI	Number positive (percentage)
Anti –CMV IgG	383(97)
Anti – CMV IgM	14(3.5)
HIV	5 (1.3)
Syphilis	1 (0.3)
Hepatitis B	9 (2.3)
Hepatitis C	4 (1.0)

Relationship between anti-CMV IgG positivity and the various demographic variables

A total of 383 (97%) blood participants tested positive for anti-CMV IgG. Out of these, 217(95.2%) were and 166(99.4%) were female. In the 16-20 years age group, 163 (97%) of the participants were positive for anti-CMV IgG. Of those aged 21-25 years, 92 (95.8%) were positive for IgG as were 56 (98.2%) of the 26-30 year olds and 66 (96.9%) of those above 30 years of age.

Anti-CMV IgG positivity in unmarried donors was found in 300 (96.8%) participants, 79 (97.5%) of the married, and 3 (100%) and 1 (100%) of the divorced and separated participants respectively. Of the study participants who had been educated to primary school level, 19 (90.5%) were positive for IgG as were 207 (97.6%), 126 (96.2%) and 23 (100%) of those had received up to secondary, college and university education respectively.

Of the participants earning less than Kshs 5,000 per month, 52 (96.3%) were positive for IgG as were 112 (97.4%) and 5 (100%) of those earning between 5,000 and 50,000 and 50,000 and 100,000 respectively. Of the sexually active participants 219 (97.3%) were positive for IgG as were 141 (95.9%) of those that were not sexually active.

There was a statistically significant difference in the positivity for anti-CMV IgG between the male and the female donors ($p = 0.016$) but none (statistically significant difference) with the other socio-demographic characteristics. (Table 4)

Table 4: Anti-CMV IgG positivity and the various demographic variables

Variable	Number (%)	Total Number (%)	P- value	Relative risk	Confidence interval
Gender					
Male	217 (95.2)	382 (97)	0.016	0.96	0.93-0.99
Female	165 (99.4)				
Age group					
16-20	163 (97.0)				
21-25	92 (95.8)	377 (96.9)	0.867	0.99	0.94-1.03
26-30	56 (98.2)				
>30	66 (97.1)				
Marital status					
Single	300 (96.8)	383 (97.0)	0.251	0.99	0.95-1.03
Married	79 (97.5)				
Divorced	3 (100)				
Education level					
Primary	19 (90.5)				
Secondary	207 (97.6)	375 (96.9)	0.238	0.92	0.82-1.06
College	126 (96.2)				
University	23 (100)				
Monthly income					
<5,000	52 (96.3)				
5'-50,000	112 (97.4)	169 (97.1)	0.856	0.99	0.93-1.05
50'-100,000	5 (100)				
Sexual status					
Active	219 (97.3)	360 (96.8)	0.450	1.01	0.98-1.06
Not active	141 (95.9)				

Anti-CMV IgG antibody positivity and other TTI

All the HIV positive participants were also positive for IgG. The 1(100%) participant who was positive for syphilis was negative for IgG. All the 9(100%) participants who were positive for hepatitis B were also positive for IgG. 3(75%) of the participants who tested positive for hepatitis C also tested positive for IgG. (Table 5)

Table 5: Relationship between anti-CMV IgG and other TTI.

TTI	IgG positive Number (%)	P -value
HIV positive	5 (100)	**0.690**
Syphilis positive	0 (0.0)	-
Hep B positive	9 (100)	**0.591**
Hep C positive	3 (75)	**0.010**

Socio-demographic characteristics of the anti-CMV IgM positive participants

Of the 14 (3.5%) donors who tested positive for anti-CMV IgM, 8 (53.8%) were male and 6 (46.2%) were female. Their ages ranged from17 to 40 years with a median age of 27years, mode of 18 years, a mean of 26.79 and standard deviation of 8.514. 11 (71.4%) of these donors were unmarried and 3 (28.6%) were married. Eleven (71.4%) of them lived in Nairobi, 2 (18.1) in Machakos and 1 (9.5%) in Kiambu. Eight (53.8%) of them were educated to secondary school level and 6 (46.2%) to college level. Six were students, 3 worked in the hospitality industry as stewards or stewardesses, one was a clerk, one was a supervisor, 1 was a chef, one was a house-keeper and one was a vendor. Seven (50%) of them earned between Kshs 5,000 and 50,000 per month, one (9.5%) earned less than 5,000 while 6 were students and did not have any income of their own. Eight (58.3%) of the 14 were sexually active and 6 (46.2%) were not. Of the sexually active, 7 (85.7%) were heterosexual and 1 (14.3%) was bi-sexual. (Table 6)

Table 6: Socio-demographic characteristics of the anti-CMV IgM positive study participants (n = 14)

Characteristic	Number (%)
Sex	
Male	8 (53.8)
Female	6 (46.2)
Age	
16-20	5 (35.7)
21-25	2 (14.3)
26-30	52 (14.3)
>30	5 (35.7)
Marital status	
Single	11 (71.4)
Married	3 (28.6)
Education level	
Secondary	8 (53.8)
College	6 (46.2)

Relationship between anti-CMV IgM positivity and the various demographic variables

There were 8 (3.5%) male donors and 6 (3.6%) female donors who were positive for IgM. In the 16-20 years age group, 5 (3.0%)participants were positive for anti-CMV IgM and so were 2 (2.1%) of the 21-25 year olds. Among those aged 26-30, 2 (3.5%) were positive for IgM. For those above 30 years old, 14 (3.6%) were positive for IgM.

IgM positivity was 10 (3.2%) in the unmarried, 4 (4.9%) in the married and zero in the divorced and the separated. None of those with up to primary school education were positive for IgM. Of the secondary school, college and university educated participants, IgM positivity was 7 (3.3%), 5 (3.8%) and zero respectively.

One (1.9%) of the participants earning less than Kshs 5,000 per month was positive for IgM as were 7 (6.1%) and zero of those earning between 5,000 and 50,000 and 50,000 and 100,000 respectively. IgM positivity in the sexually active was 7 (3.1%) and 6 (4.1%) in the non-sexually active.

There was no statistically significant relationship between IgM positivity and any of the socio-demographic characteristics.

Table 7: Anti-CMV IgM positivity and the various demographic variables

Variable	Number (%)	Total Number (%)	P- value	Relative risk	Confidence interval
Gender					
Male	8 (53.8)	14 (3.6)	0.955	0.97	0.34-2.75
Female	6 (46.2)				
Age group					
16-20	5 (35.7)	14 (3.6)	0.310	0.57	0.12-2.23
21-25	2 (14.3)				
26-30	2 (35.7)				
>30	5 (14.3)				
Marital status					
Single	11 (71.4)	14 (3.5)	0.873	0.94	0.27-3.30
Married	3 (28.6)				
Divorced	0 (0.0)				
Education level					
Primary	0 (0.0)	14 (3.1)	0.646	0.83	0.29-2.33
Secondary	8 (53.8)				
College	6 (46.2)				
University	0 (0.0)				
Monthly income					
<5,000	1 (1.9)	8 (4.6)	0.417	0.30	0.04-2.41
5,000-50,000	7 (6.1)				
50'-100,000	0 (0.0)				
Sexual status					
Active	8 (53.8)	13 (3.5)	0.616	0.87	0.31-2.45
Not active	6 (46.2)				

Prevalence of TTI in the IgM positive study participants and the relationship between anti-CMV IgM antibody positivity and other TTI

All the 14 (100%) tested negative for HIV, 1(7.1%) tested positive for syphilis, none of the 14 (0%) tested positive for hepatitis B while, 2 (14.3%) tested positive for hepatitis C. All the 14 (100%) tested positive for CMV IgG. There was no statistically significant relationship between positivity for anti-CMV IgM and other TTI. (Table 7)

Table 8: TTI prevalence in the anti-CMV IgM positive study participants (n = 14)

TTI	*Number (percentage)*	P value
HIV	0 (0)	0.666
Syphilis	1 (7.1)	-
Hepatitis B	0 (0)	0.561
Hepatitis C	2 (14.3)	-
Anti-CMV IgG	14 (100)	0.896

DISCUSSION

A preponderance of the blood donors were male and this reflects on the situation of blood donors in general (at the NBTC) as the sampling method used applied equally to male and female donors. This is despite the fact that there are more females than males in the general population in Kenya[61]. This may be explained by the fact that women are more likely not to qualify as blood donors because of a low haemoglobin level as compared to the male donors who tend to have higher haemoglobin levels. The lower haemoglobin level in women is due to blood loss during menstruation and increased iron requirement during pregnancy and lactation. At the same time, pregnancy and lactation is an exclusion criterion for blood donation and only applies to the female donors (appendix 2).

Majority of the blood donors were in the 16-20 years age bracket. This is due to the fact that most of the blood donation exercises target secondary schools whose students are in this age group. It is also observed (in this study) that even where blood donation exercises are not carried out in schools, students still form the majority of blood donors. For the same reasons (that secondary school students are the majority of blood donors), most of the blood donors are single (unmarried), and are educated up to secondary school level.

The prevalence of anti-CMV IgG antibodies among the blood donors recruited into this study was 97%. This means that 97% of the donors who participated in this study had been exposed to CMV at some point earlier in their lives. This indicates a rather high rate of exposure to CMV and compares well with what may be expected for a developing country given that the western countries show prevalence of up to 80% and the rates are expected to be higher with decreasing socio-economic status[7]. The prevalence of anti-CMV IgM was 3.5% in blood donors at the NBTC indicating that this number of donors had active CMV infection and could potentially transmit this infection to transfusion recipients.

A similar study conducted on voluntary blood donors in Delhi, India and published in December 2002 showed that 95% of the donors were positive for anti-CMV IgG while none of the 200 donors tested positive for anti-CMV IgM antibodies[54]. Other studies done in the same country in the 1970s showed prevalence rates in blood donors ranging from 84-100%. These figures correlate with the figures obtained in this study for the Kenyan

situation. The two countries enjoy more or less the same socio-economic conditions, developing countries[54].

These figures however contrast with those obtained from the western, more developed, countries where the sero prevalence of anti-CMV antibodies in blood donors is reported to range between 38 and 75%. Other studies shave shown the sero-prevalence to be 40% in industrialized countries and up to 100% in the less developed countries. Japan and Hong Kong are however reported to have prevalence rates of over 90%[21].

The high prevalence rates in Kenya indicate the endemicity of the infection which could be related to socio-economic and environmental conditions.

The female donors showed a higher prevalence of CMV exposure (as evidenced by a positive test for anti-CMV IgG antibodies) at 99.4% compared to the male donors at 95.2% (p=0.016, odds ratio=8.36). This has also been shown in other studies (that the seroprevalence of CMV is higher in females than in males)[54, 59]. This may possibly be explained by the methods of transmission especially via the sexual route as it has been shown in studies that increased parity in women is associated with higher rates of infection and transmission of the virus. Other studies have shown CMV antibodies to be present in up to 100% of female prostitutes[8]. However, this study did not demonstrate any statistically significant difference in exposure to CMV between the sexually active and those who were not sexually active (p=0.450).

There was no statistically significant difference in the prevalence of IgM between the genders (p=0.955).

The study did not demonstrate any statistically significant difference in sero-prevalence (of both IgG and IgM) with marital status (p=0.251), education level (p=0.238), income (p=0.856) or occupation (p=0.873). This differs from the western studies which show that the same factors affect the prevalence of CMV infection in the general population. This could possibly be due to early acquisition of CMV in the Kenyan population resulting in high prevalence of CMV even in the younger individuals.

Like the study in India where there was no demonstrable statistically significant variation in sero-prevalence with age[54], this study did not demonstrate any statistically significant variation on sero-prevalence with age (p= 0.867). In the studies done in the west, there was a statistically significant increased sero-positivity with increasing age (p=0.021)[7]. This may possibly be due to earlier acquisition of CMV infection in Kenya and India as compared to the western countries, leading to higher prevalence even in the young adults[54].

Of the 14 (3.5%) donors who tested positive for anti-CMV IgM, most were males (57.1%) and lived in Nairobi (71.4%). This is possibly because there were more male than female donors and that the blood donors were drawn from within the administrative boundaries of Nairobi province. There was no demonstrable statistically significant relationship between anti-CMV IgM positivity and HIV (p=0.666) and hepatitis B (p=0.561). There was a significant relationship between anti-CMV IgM positivity and syphilis (p=0.000) and hepatitis C (p=0.000). This may be explained by similar routes of transmission, especially for hepatitis C.

There was no significant correlation between anti-CMV IgG positivity and positivity for HIV (p=0.690) and Hepatitis B (0.591). This is possibly because CMV can be transmitted through many ways including close person to person contact, sexual intercourse, blood transfusion, saliva, urine and faeces among others. There however was a correlation between positivity for CMV and that for syphilis (p=0.000) and hepatitis C (p=0.010). This may be explained by the shared routes of transmission between the two viral infections including community acquired Hepatitis C and vertical transmission.

It is important to note that while this study seems to suggest that there is a statistically significant relationship between CMV exposure (IgG) and infection (IgM), with syphilis and hepatitis C, the numbers of the donors who tested positive for the two conditions were very few (one for syphilis and three for hepatitis C). This study may therefore not have established this relationship conclusively.

In this study, 1.3% of the donors tested positive for HIV, 2.3% positive for hepatitis B, 0.3% positive for syphilis and 1% for hepatitis C. These figures correlate with the national average from the NBTC (appendix 11) which showed a prevalence of 1.55% (2006) and 1.36% (2007) for HIV; 2.88% (2006) and 2.60 %(2007) for hepatitis B; 0.49 % (2006) and 0.30 % (2007) for syphilis; and 1.05 % (2006) and 1.01 % (2007) for hepatitis C. A similar study done in India showed that 3% of the donors were positive for hepatitis B (HBsAg), 1% positive for HIV, 2% positive for hepatitis C and 4.5% positive for syphilis[54]. These figures differ from those from the USA, according to the American Association of Blood Banks (AABB), which show prevalence of 1 in 2,000,000, 0.02-0.04%, 0.21% for HIV, hepatitis B and hepatitis C respectively among blood donors. Some studies on syphilis did not find any positive donors while others showed a total of 516 for the period 1995 to 2000 (22 primary, 81secondary and 413 tertiary)[62] .

This study could not differentiate primary infection from re-activated disease. This may be done by other tests like estimation of IgG avidity index, where a low avidity index would indicate early primary infection and a high index would indicate re-activation or re-infection. For the purposes of transfusion, this may not be necessary as both primary and re-activated CMV disease is transfusion transmissible.

Detection of anti-CMV antibodies of the IgM class (early during the course of infection) is important for prevention of TT-CMV[8]. One study showed that patients who received components with detectable IgM anti-CMV antibodies had a TT-CMV rate of 8.8% , compared with a rate of 0.3% among those who received blood without anti-CMV IgM ($P<0.001$)[8]. In the same study, only 1 of 163 neonates who received seropositive but IgM negative blood developed TT-CMV[8].

The American association of blood banks recommends the use of CMV sero-negative blood for those patients who are risk of the severe or even effects of CMV disease[62]. These are the infants, and particularly very low birth weight neonates (below 1500g), and the immunocompromised patients who include organ transplant recipients, patients undergoing hemodialysis, cancer patients, patients on immunosuppressive drugs, and HIV-infected patients in whom the consequences of such infection can be severe or even fatal.[4,14,15] This is possible, even, in Kenya. This is because, as the studies show, it is mainly the IgM positive blood that is more likely to transmit CMV[8], only 3.5% of the blood donors were positive for anti-CMV IgM.

This is also supported by the fact that it is not all patients requiring blood will need CMV negative blood as studies have shown that there is no demonstrable benefit of giving CMV negative blood to individuals who are sero-positive for CMV. These patients are already at risk of re-activated CMV disease[29]. In the Kenyan population, 97% of the individuals aged between 16 and 65 years have been exposed to CMV, according to this study. In this age bracket, there will be only 3% to provide CMV negative blood for. In the overall population this number is expected to be larger because of the infants and young children who may fall within the category of individuals at risk of CMV disease. It will be important to determine the prevalence of CMV in those patients who require CMV negative blood so as to be able to assess if there will be adequate supplies of safe blood for them.

Further to this, patients who require blood transfusion and are not at risk of CMV disease will not necessary be transfused with IgM negative blood. This is because studies have shown that these individuals rarely get clinical manifestation of the infection[4, 17, 18]. This will check wastage of blood at the blood banks, which are already struggling with inadequate supplies.

The cost implications of screening donor blood for anti-CMV IgM is not any different from that for other TTI since the kits cost roughly the same amount and the technology employed is the same as that used for the screening for the other TTI. This means there will be no extra expenses in buying new equipment and training of personnel. In fact, it will be much cheaper to screen for CMV since only blood intended for the high risk groups will be screened, who are less than 3% in the 16-65 year bracket.

Other (CMV) reduced risk products may also be availed for use in these patients. These include leuco-reduced products, saline washed red blood cells (RBCs), frozen deglycerolised RBCs among others.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. This study shows that the prevalence of anti-CMV antibodies in blood donors at the NBTC was 97% and 3.5% for IgG and IgM respectively.
2. Female blood donors showed a higher prevalence of anti-CMV IgG compared to the male donors.
3. In this setting, the NBTC, there is no significant influence of age, marital status, education or even the sexual status on the prevalence of anti-CMV antibodies.
4. Infection with other transfusion transmissible infections does not show any significant relationship with infection with CMV.

Recommendations

1. Donor blood intended for transfusion to individuals at risk for severe CMV disease should be screened for anti-CMV IgM as these are likely to transmit the virus to these high risk groups.

2. Further studies on the relationship between CMV sero-positivity and the socio-demographic characteristics of blood donors should be carried out. These should include studies to establish the reasons for the difference in sero-prevalence between the male and the female donors.

3. Population studies to determine the age of seroconversion of CMV will be important to determine the age of exposure to CMV in our population as well as determine the methods of transmission that play the most significant role in the same population.

4. Sero-prevalence of CMV in patients at risk of severe CMV disease need to be studied to enable the policy makers plan for availability of CMV negative blood to these patient groups.

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APPENDICES

APPENDIX I

THE KENYA TRANSFUSION SERVICE (KTS) AND THE NBTC

The Blood Transfusion service in Kenya is a clearly identifiable unit in the Ministry of Health within the National Public Health Laboratory Service (NPHLS). It has a centrally coordinated management structure with a National blood transfusion centre (NBTC) and a network of regional transfusion centres located in Nakuru, Kisumu, Mombasa and Embu. Satellite hospital blood banks operate under the auspices of the NBTC where those hospitals do not have easy access to the regional BTS. The NBTC mainly organises blood donation exercises within Nairobi province (that is within the administrative boundaries).

The Kenya Transfusion Service has a central board of management with a medical director as the chief executive of the service. The service also has a technical advisor who hails from the laboratory technology fraternity and is charged with the responsibility of overseeing the technical operations of the service. Other members of staff in the service include; laboratory technicians and technologists, nurses, administrators, clerks, counsellors, and accountants among others.

The National blood transfusion centre (NBTC) is the headquarter of the Kenya transfusion service and is located in close proximity to the Kenyatta National Hospital (KNH), the national referral hospital and teaching hospital for the University of Nairobi's School of Medicine. It is situated approximately 1.5km off Ngong Road along Hospital Road behind KNH. In its vicinity are the offices of the NPHLS, KEMRI's centre for Public Health Research, NLTP and NASCOP.

The country gets its blood supply from voluntary, non-remunerated blood donors who have been recruited from low-risk populations, as defined by the NBTC, and who satisfy the criteria for selection and retention as blood donors. The NBTC serves the following functions as well;

1. Setting the amount to be collected from each donor and the interval between collections.
2. Developing a national register of regular and safe blood donors.
3. Keeping and maintaining donor records in a retrievable manner to allow for easy look back procedures.

APPENDIX 2

DONOR RECRUITMENT AND THE PROCESS OF BLOOD DONATION

Blood donation involves several phases that include creating awareness to the community, donor screening at the venue of blood donation, and the actual blood collection. Blood donation exercises are carried by a team from the BTS (regional or NBTC for Nairobi) that comprises a nurse, clerks, laboratory technicians and a driver. Once the blood is collected it is transported under appropriate conditions to the NBTC laboratories.

The first phase involves creating awareness. This is done at the community level where the NBTC staff educate the public on the importance of donating blood, the requirements for donating blood (including age and health) and the places/ locations where one can donate blood. The potential donors are also educated on the advantage of donating blood which includes reduction in the risk of heart disease for men and stimulation of the generation of red blood cells. In patients prone to iron overload (e.g. due to hemochromatosis), blood donation prevents the accumulation of iron. This information is given at schools, colleges, and churches, through the media or even using billboards.

In the days before donating blood, one is advised to prepare themselves for a procedure that can temporarily weaken the body. It is recommended that potential donors drink extra water and fluids before donating. It may be advisable to avoid caffeinated beverages before donation. Eating well is also important.

At the venue of the blood donation exercise, the same information is given again both at a group level (where it applies like schools and colleges) and individually. Clarifications are also offered on any issues that may not have been clear.

The next phase involves donor screening where specific data on the potential donor is obtained and recorded on a donor-screening card. This is done to determine the potential donor's eligibility to donate blood. The individuals have their weight, blood pressure and haemoglobin level taken and recorded. The donors' body weight is taken using an ordinary weighing scale and the blood pressure done using a sphygmomanometer.

There are several methods that can be used to estimate haemoglobin level and the common ones include:

- Hematocrit: done in some places and requires a centrifuge. 38% is the required level for most blood donors in the US, though autologous donors are accepted with levels as low as 33%. Failing this cutoff is the highest cause of donors not being eligible to donate, as many premenopausal women have lower iron levels.
- Colorimetric hemoglobin test using a hemoglobin photometer: a machine-read result from a chemical reaction on a testing strip.
- Copper sulfate screening test ("float test"): measures the specific gravity of the donor's blood by placing a drop into a copper sulfate solution. The solution is calibrated so that a hemoglobin concentration of in >12.5 g/dl (for donation) sinks. This is the method used at the NBTC.

The potential donors are then subjected to a questionnaire that captures the following information:

- Age
- Sex
- Marital status
- Family name
- Other names
- Institution
- Occupation
- Contact number / E-mail
- If student, name of school
- Postal address

Other information that is captured includes if they have had:

- Transfusion in the last one year
- Donated in the last four months
- Major operation in the last two years
- Dental work in the last one week
- Body piercing in the last one year
- Currently under medication or unwell

- Multiple sexual partners (self)
- Not sure about the sexual partner
- Sexual contact with suspicious partner
- Sexually transmitted infection in the last one year
- Pregnant or breastfeeding
- Tested positive for HIV, HBV, HCV or VDRL

The potential donor should also indicate if they suffer from diseases like:

- Anaemia or bleeding disorder
- Diabetes
- Heart diseases
- Stomach ulcers
- High blood pressure
- Jaundice

The potential donor is also required to give consent to donate blood. This data is captured with the assistance of a nurse who is always part of the team and is charged with responsibilities like measurement of weight, height and blood pressure as well as translating any medical terms that may not be easily understood. The team also includes a laboratory technologist whose main duty is in the estimation of haemoglobin level. This information is recorded in the donor screening card.

Those individuals who would qualify to donate blood are those with the following characteristics:

- Consent to donate blood and have their blood tested for the various infections as required by the NBTS
- Are aged between 16 and 65 years.
- Are not pregnant or breastfeeding, for the female donors.
- Have not had blood transfusion in the last one year
- Have not donated blood in the last four months
- Have not had major surgery in the last two years
- Have not had dental work in the last one week
- Have not had body piercing in the last one year
- Are not currently under medication or are unwell

- Do not have multiple sexual partners
- Are sure about their sexual partners
- Have not had sexual contact with a suspicious spouse
- Have not had an STD in the last one year
- Have not tested positive for HIV, HBV, HCV or VDRL
- Do not have anaemia or a bleeding disorder
- Do not have jaundice, diabetes, epilepsy, heart disease, hypertension or stomach ulcers

The individuals who have the characteristics listed below will not qualify to donate blood. These individuals are usually deferred for some while or indefinitely depending on the reason for their being deferred. For example a menstruating lady whose haemoglobin level falls below the cut-off level of 12.5g/dl is deferred only for a short while and will be eligible to donate as soon as her haemoglobin level is at or above the cut-off. On the contrary, an individual who uses intravenous illicit drugs or has tested positive for HIV or hepatitis B will be deferred indefinitely. The individuals who do not qualify to donate blood will be all males or females available to donate blood who:

- Decline to donate blood and have their blood tested for the various infections as required by the NBTS
- Are aged below 16 or above 65 years.
- Are pregnant or breastfeeding, for the female donors.
- Have had blood transfusion in the last one year
- Have donated blood in the last four months
- Have had major surgery in the last two years
- Have had dental work in the last one week
- Have had body piercing in the last one year
- Are currently under medication or are unwell
- Have multiple sexual partners
- Not sure about their sexual partners
- Have had sexual contact with a suspicious spouse
- Have had an STD in the last one year
- Have tested positive for HIV, HBV, HCV or VDRL
- Have anaemia or a bleeding disorder
- Have jaundice, diabetes, epilepsy, heart disease, hypertension or stomach ulcers.

The potential donor is the issued with a unique number which will identify her/him and all blood products obtained from her/him. This number is entered in the donor screening card, the blood donor venue register, the blood bag into which the blood unit will be collected as well as the vacutainer tubes that will be used to collect samples for serology and blood grouping.

The other information entered into the blood donor venue register includes:

- RBTC code
- Venue site
- District
- Date
- Team leader
- Mobiliser
- Laboratory results entered by, and date
- Donor reference number
- Name of donor Identity of donor
- Date of birth
- Sex
- Number of previous donation
- ABO and Rh (D) blood group
- Pack type
- Laboratory report (blood group and screening results) Donor status
- Donation discards with reasons
- Specifications of blood bag used

All potential donors who have successfully gone through these two phases are now ready to go through the third phase, which is the actual blood donation. During blood donation, the donor lies supine on a couch and loosens all tight clothing. A tourniquet is applied to the upper arm and the donor is encouraged to press an inflated ball that is placed on the palm.

The cubital vein is identified and the cubital fossa is swapped with surgical spirit and allowed to dry. A blood collection set is used to collect blood and this comprises a correctly labelled blood bag (with a large bore needle to avoid haemolysis) and two labelled plain vacutainer tubes. This information contained in the blood collection set should tally with that on the donor card (donor reference number).

A needle is introduced into the cubital vein and with gentle squeezing of the ball in the hand; a unit of blood is collected (400-500 mls) into a special blood bag that contains an anti-coagulant (citrate phosphate dextrose adenine, CPD-A). Once the required volume has been obtained, the needle is removed from the vein and a light sterile dressing applied to the site of venepuncture, for a few minutes till the bleeding stops. A scale is used to weigh the donated blood and thereby determine that the required volume has been donated.

This procedure applies for whole blood donations. Rather than donating whole blood, a donor sometimes has the option to donate only some blood components while retaining others. This process, known as apharesis, is more involved, time consuming, and requires more specialized equipment. Apharesis procedures are not done at the NBTC. Autologous blood donations are more likely to be done at a hospital than at the NBTC.

Approximately 5-7mls of blood are then drawn from the same needle into vacutainer tubes and these are destined to the NBTC laboratory for testing (blood grouping, HIV, syphilis, hepatitis). The blood units and the samples are then transferred into cool boxes for (short-term) storage and transportation to the NBTC. In some countries, testing for TTI is done at the venue of blood donation. Most of these tests used on blood donors are designed to sacrifice specificity for sensitivity, so false positives are not unusual. Blood donors are often told to seek medical care for an actual diagnosis when they test positive for a disease in screening, since these tests are designed for the safety of the recipient, not necessarily for diagnostic purposes. Donors are typically excluded based on the screening test, but may be reinstated depending on the results of more accurate confirmatory testing.

The blood donors are encouraged to rest for about five minutes on the couch after donating blood. They are then offered a half-litre soft drink and then allowed to leave at their own pleasure. Any emergencies that may arise are handled by the nurse. These include continued bleeding at the site of venepuncture and fainting attacks. Complications after donating blood are generally very rare and blood donors are encouraged to seek medical advice should they experience any unusual symptoms on leaving the venue of blood donation.

Once at the NBTC, the blood units are stored at 2-8^{0}C and the blood samples are taken to the laboratory where they are aliquoted and tested for TTI, blood grouping is done as well. The second copy of the donor venue register is completed. The information captured in this register includes:

- Donation reference number.
- Pack weight (g).
- Pack type.
- Laboratory screening results.
- ABO and Rh (D) result.
- Laboratory report Donation status.
- Donation analysis.
- Serology discards.
- Product discards.
- Specifications of reagents / kits used.

NB: Please see attached copies of these forms (registers).

The results of the screening tests are available to the donors who are also issued with blood donor certificates (which indicate their blood groups). The donors undergo counselling before and after receiving the results. Those who test positive for the various TTI are then referred to KNH for further management and follow-up.

APPENDIX 3

DONOR SCREENING CARD

The process of donor screening usually involves answering a set of questions to determine the suitability of the individual as a blood donor. The information is entered into a donor record card and includes:

- Donation number.
- ABO group.
- Sex.
- Marital status.
- Family name.
- Other names.
- Institution.
- Occupation.
- Contact number/E-mail.
- If student, name of school.
- Postal address.
- Weight.
- Hb.
- Bp.
- No of previous donations.
- If accepted.
- If no reasons.
- Pilot No.
- "I confirm the information I have given above is true".
- "I understand my blood will be tested as required by NBTC".

Donor questionnaire

The potential donor is also expected to indicate if they have had:

- Transfusion in the last one year.
- Donated in the last four months.
- Major operation in the last two years.
- Dental work in the last one week.
- Body piercing in the last one year.

- Currently under medication or unwell.
- Multiple sexual partners (self).
- Not sure about the sexual partner.
- Sexual contact with suspicious partner.
- Sexually transmitted infection in the last one year.
- Pregnant or breastfeeding.
- Tested positive for HIV, HBV, HCV or VDRL.

They should also indicate if they suffer from any of the following diseases:

- Anaemia or bleeding disorder.
- Diabetes.
- Heart diseases.
- Stomach ulcers.
- High blood pressure.
- Jaundice.

Other information to be entered into the card includes the date, the centre and the tube number.

NB: please see attached copies of the donor screening card

APPENDIX 4

STUDY PARTICIPATION CONSENT FORM

PREVALENCE OF CMV INFECTION AMONG BLOOD DONORS AT THE NBTC

Study No. .....

General information

This is a study looking at the proportion of blood donors who have been exposed to CMV and the proportion that has active CMV disease. CMV is a virus that infects many people in many parts of the world and is transmitted in many ways, one of which includes receiving donated blood. CMV infection does not cause serious health problems to most people. However, some groups of patients can get very sick or even die when they get infected with the CMV and it is important to protect them by giving them blood that is free of CMV.

By taking part in this study, you will benefit by knowing your CMV status (this information will be availed to you through the NBTC) so that in case you are at risk of severe effects of this disease, you can be referred to a centre where you will receive treatment and follow-up. The risks you will be exposed to are the same as were explained to you when you were recruited as a blood donor.

A small extra volume of blood (about one teaspoonful) will be taken from you and tested at the University Of Nairobi immunology laboratories for antibodies to CMV to determine if you have been exposed to or are having active CMV disease.

Participant declaration

I have understood the purpose of the study as explained to me by .....

I wish to voluntarily take part in the study.

Name .....

Witness .....

Signature .....

Guardian /parent signature (those below 18yrs) .....

Date .....

APPENDIX 5

STUDY QUESTIONNAIRE

PREVALENCE OF CMV INFECTION AMONG BLOOD DONORS AT THE NBTC

Study No. .....

Donor Ref No.....

1. Personal details

1.1 Names .....

1.2 Physical address .....

1.3 Postal address .....

1.4 E-mail address .....

1.5 Tel. number .....

2. Socio-demographic data

Age .....

Sex

☐ 2.2.1 Male

☐ 2.2.2 Female

Marital status

☐ 2.3.1 Single

☐ 2.3.2 Married

☐ 2.3.3 Widowed

☐ 2.3.4 Divorced

☐ 2.3.5 Other.....

2.3 Parity (for female participants) .....

2.4 Residence .....

2.5 Education level (tick as appropriate)

- ☐ 2.5.1 Primary school
- ☐ 2.5.2 Secondary school
- ☐ 2.5.3 College level
- ☐ 2.5.4 University level

2.6 Occupation .....

2.7 Income per month (in KSHs)

- ☐ 2.7.1 < 5,000.00
- ☐ 2.7.2 5,000.00 – 50,000.00
- ☐ 2.7.3 50,000.00 – 100,000.00
- ☐ 2.7.4 >100,000.00

2.8 Are you sexually active (tick as appropriate).

- ☐ 2.8.1 Yes
- ☐ 2.8.2 No

3. Laboratory results

3.1 HIV status

- ☐ 4.1.1. Positive
- ☐ 4.1.2. Negative

3.2. Syphilis test

- ☐ 4.2.1. Positive
- ☐ 4.2.2. Negative

3.3 Hepatitis B

- ☐ 4.3.1. Positive
- ☐ 4.3.2. Negative

3.4 Hepatitis C

- ☐ 4.4.1. Positive
- ☐ 4.4.2. Negative

3.5 CMV

4.5.1. IgG

☐ Positive

☐ Negative

4.5.2. IgM

☐ Positive

☐ Negative

APPENDIX 6

ASSAY PROCEDURE FOR IGG AMD IGM

The assays for IgG and IgM were performed as per the manufacturer's instructions (see the attached copies of the manufacturer's instructions). The results were also interpreted as per the manufacturer's guidelines.

A negative IgM result was taken to indicate no current infection with CMV. Such individuals were presumed to be susceptible to primary infection. A positive result indicated an active (primary or reactivated) infection with CMV. Such individuals were presumed to be at risk of transmitting CMV infection.

A negative result indicated no previous infection with CMV. Such individuals were presumed to be susceptible to primary infection. A positive result was taken to indicate a current or previous infection with CMV.

DRG

DRG® CMV IgG (EIA-1797)

Revised 16 May 2005

RUO in the USA

ENZYME IMMUNOASSAY FOR THE DETECTION OF
IgG ANTIBODIES TO CYTOMEGALOVIRUS (CMV) IN HUMAN SERUM

FOR INVESTIGATIONAL USE ONLY
Store at 2 to 8°C.
Proprietary and Common Names
CMV IgG Enzyme Immunoassay

SUMMARY OF ASSAY PROCEDURE

1. Sample dilution 1:40
5 µl / 200 µl

2. Three incubations at 37°C

Diluted Sample	Enzyme Conjugate	TMB Reagent (One-Step)
100 µl	100 µl	100 µl
30 min.	30 min.	15 min.

3. Stop with 100 µl of acid. Read O.D. at 450 nm

INTENDED USE
The CMV IgG ELISA is intended for use in evaluating a patient's serologic status to cytomegalovirus (CMV) infection.

INTRODUCTION
Cytomegalovirus is a herpes virus and a leading biological factor causing congenital abnormalities and complications among those who receive massive blood transfusions and immunosuppressive therapy. About half the pregnant women who contract a primary infection spread the disease to their fetus. When acquired in-utero, the infection may cause mental retardation, blindness, and/or deafness.
Serological tests for detecting the presence of antibody to CMV can diagnose active or recent infection and provide valuable information regarding the history of previous infection. These tests are also useful in screening blood for transfusions in newborns and immuno-compromised recipients. CMV IgG ELISA is an accurate serologic method to detect CMV IgG antibody for identification of CMV infection.

PRINCIPLE OF THE TEST
Purified CMV antigen is coated on the surface of microwells. Diluted patient serum is added to the wells, and the CMV IgG specific-antibody, if present, binds to the antigen. All unbound materials are washed away. HRP-conjugate is added, which binds to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of CMV IgG-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrators and controls.

Reagents

Materials provided with the kit:

- Microtiter Wells: purified CMV antigen-coated wells (12 x8 wells).
- Enzyme Conjugate Reagent (red color): Red cap, 1 vial (12 ml).
- Sample Diluent (green color): 1 bottle (22 ml).
- Negative Calibrator: 0 IU/ml. Natural cap. (150 µL/vial).
- Cut-off Calibrator: 1.2 IU/ml. Yellow cap. (150 µL/vial).
- CMV IgG index= 1.0
- Positive Calibrator: 6 IU/ml. Red cap. (150 µL/vial).
- Positive Calibrator: 18 IU/ml. Green cap. (150 µL/vial).
- Negative Control: Range stated on label. Blue cap. (150 µL/vial).
- Positive Control: Range stated on label. Purple cap. (150 µL/vial).
- Wash Buffer Concentrate: 1 bottle (50 ml, 20x).
- TMB Reagent (One-Step): 1 vial (11 ml).
- Stop Solution: 1N HCl, Natural cap. 1 vial (11 ml).

Storage of Test Kits and Instrumentation

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

Warning and Precautions

1. Potential biohazardous materials:
2. The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components from different lots should not be mixed.
5. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

Specimen Collection and Preparation

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2-8°C for up to 7 days or frozen for up to 6 months. Avoid repetitive freezing and thawing of serum sample.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-25 °C) before use.

2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of Wash Buffer (1x). Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.

ASSAY PROCEDURE

1. Place the desired number of coated wells into the holder
2. Prepare 1:40 dilution of test samples, Negative Control, Positive Control, and Calibrator by adding 5 µl of the sample to 200 µl of Sample Diluent. Mix well.
3. Dispense 100 µl of diluted sera, Calibrator, and Controls into the appropriate wells. For the reagent blank, dispense 100 µl of Sample Diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well.
4. Incubate at 37°C for 30 minutes.
5. At the end of incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 4 times with diluted Wash Buffer (1x) and then one time with distilled water.
6. Dispense 100 µl of Enzyme Conjugate into each well. Mix gently for 10 seconds.
7. Incubate at 37°C for 30 minutes.
8. Remove Enzyme Conjugate from all wells. Rinse and flick the microtiter wells 4 times with diluted Wash Buffer (1x) and then one time with distilled water.
9. Dispense 100 µl of TMB Reagent into each well. Mix gently for 10 seconds.
10. Incubate at 37°C for 15 minutes.
11. Add 100 µl of Stop Solution (1N HCl) to stop reaction.
12. Mix gently for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
    Note: Make sure there are no air bubbles in well before reading.
13. Read O.D. at 450 nm ***<u>within 15 minutes</u>*** with a microwell reader.

CALCULATION OF RESULTS

1. Calculate the mean of duplicate cut-off calibrator value x_c.
2. Calculate the mean of duplicate positive control (x_p), negative control (x_n) and patient samples (x_s).
3. Calculate the CMV IgG Index of each determination by dividing the mean values of each sample (x) by calibrator mean value, x_c.

Example of typical results: **Note:** These O.D. values are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data.

Cut-off Calibrator CMV IgG Index = 1.0

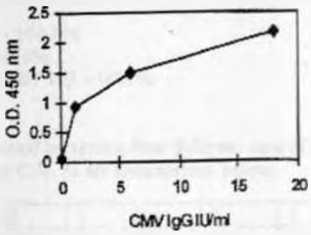
1. Cut-off Calibrator (1.2 IU/ml) O.D. = 0.845, 0.850 — $x_c = 0.848$
2. Negative Control O.D. = 0.154, 0.169 — $x_n = 0.162$
   CMV IgG Index = x_n / x_c = 0.162/0.848 = 0.19
3. Positive Control O.D. = 1.284, 1.255 — $x_p = 1.270$
   CMV IgG Index = x_p / x_c = 1.270/0.848 = 1.50

4. Patient: Sample O.D. = 2.392, 2.243 $x_2 = 2.318$
   CMV IgG Index = $x_2 / x_1 = 2.318/0.848 = 2.73$

QUANTITATIVE DETERMINATION OF CMV IgG

For a quantitative determination of anti-CMV IgG levels of positive specimens in IU/ml, the OD of cut-off and positive calibrators are plotted on the Y-axis of a graph against their corresponding anti-CMV IgG concentrations of 0, 1.2, 6, and 18 IU/ml on the X-axis. The estimates of levels in patient sera are read off the graph using their individual OD values. For example:

CMV IgG (IU/ml)	A_{450}
0	0.056
1.2	0.930
6	1.496
18	2.167



Note: The Standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.
2. If the O.D. value of the Cut-off Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The CMV IgG or Index IU/ml unit for Negative and Positive Control should be in the range stated on the labels.

INTERPRETATION

Negative: CMV IgG Index less than 0.90 is seronegative for IgG antibody to CMV (<1.2 IU/ml)

Equivocal: CMV IgG Index between 0.91-0.99 is equivocal. Sample should be retested.

Positive: CMV IgG Index of 1.00 or greater, or IU value greater than 1.2 is seropositive. It indicates prior exposure to the CMV virus. (>1.2 IU/ml)

Performance Characteristics

I. **Specificity and Sensitivity:**
A total of 199 patient samples were used to evaluate specificity and sensitivity of the test. The CMV IgG ELISA test results were compared to a commercial ELISA kit results:

		Reference CMV IgG ELISA			
		N	E	P	Total
CMV IgG ELISA	N	82(D)	0	0(B)	82
	E	2	0	0	2
	P	2(C)	5	108(A)	115
	Total	86	5	108	199

Sensitivity = A / (A+B) = 108 / 108 = 100.0%
Specificity = D / (C+D) = 82 / 84 = 97.6%
Accuracy = (A+D) / (A+B+C+D) = 190 / 192 = 99.0%

II. **Precision**
The Precision of the assay was evaluated by testing four different sera of 20 replicates on different days or times. The intra-assay and inter-assay C.V. % are summarized below:

Sample	1	2	3	4
Intra-assay	5.1	8.0	2.4	5.1
Inter-assay	7.6	9.7	5.2	9.9

Limitations of the Procedure

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

References

1. Voller, A., J.E. Bidwell, et al. Manual of clinical immunology. Chapter 69. Rose, N. and Friedman, H. eds. Am. Soc. Microbiol. P.506, 1985.

2. Cremer, N.E. Antibodies in serodiagnosis of viral infection. P. 73. In Lennett E.H. ed. Laboratory diagnosis of viral infection. Mercel Dekker, Inc., New York, 1985.
3. Starr, S.E. and H.M. Friedman "Human CMV" Chapter 65. In Manual of Clin. Microbiol., 4th de., Lennett, E.H. et al ed. Am. Soc. Microbiol. Pp. 771-719, 1985

DRG

DRG® CMV IgM (EIA-1796)

Revised 11 May 2005 **RUO** in the USA

Enzyme Immunoassay for the Detection of IgM Antibodies to Cytomegalovirus (CMV) in Human Serum

For Investigational Use Only
Store at 2 to 8°C
Proprietary and Common Names
CMV IgM Enzyme Immunoassay

Summary of Assay Procedure

1. Sample dilution 1:40
   5 µl + 200 µl
2. Three incubations at 37°C

Diluted Sample 100 µl	Enzyme Conjugate 100 µl	TMB Reagent (One-Step) 100 µl
30 min	30 min	15 min.

3. Stop with 100 µl of acid. Read O.D. at 450 nm

Intended Use
The CMV IgM ELISA is intended for use in the detection of IgM antibodies to Cytomegalovirus (CMV) infection in human serum.

Introduction
Cytomegalovirus is a herpes virus and a leading biological factor causing congenital abnormalities and complications among those who receive massive blood transfusions and immunosuppressive therapy. About half the pregnant women who contract a primary infection spread the disease to their fetus. When acquired in-utero, the infection may cause mental retardation, blindness, and or deafness.
Serological tests for detecting the presence of antibody to CMV can diagnose active or recent infection and provide valuable information regarding the history of previous infection. These tests are also useful in screening blood for transfusions in newborns and immuno-compromised recipients. The CMV IgM ELISA is an accurate serologic method to detect CMV IgM antibody for identification of CMV infection.

Principle of the Test
Purified CMV antigen is coated on the surface of microwells. Diluted patient serum is added to the wells, and the CMV IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away. HRP-conjugate is added, which bonds to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM specific-antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

DRG

DRG² CMV IgM (EIA-1796)

Revised 11 May 2005

RUO in the USA

Reagents

Materials provided with the kit:

- Microtiter Wells: purified *CMV* antigen coated wells (12 x8 wells).
- Enzyme Conjugate Reagent (red color): Red cap 1 vial (12 ml).
- Sample Diluent (blue color): 1 bottle (22 ml).
- Negative Control: Range stated on label. Natural cap. (150 µL/vial).
- Cut-off Calibrator: Yellow cap. *CMV IgM* Index = 1 (150 µL/vial)
- Positive Control: Range stated on label. Red cap (150 µL/vial).
- Wash Buffer Concentrate (20x): 1 bottle (50 ml).
- TMB Reagent (One-Step): 1 vial (11 ml).
- Stop Solution (1N HCl): Natural cap. 1 vial (11 ml).

Storage of Test Kits and Instrumentation

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

Warning and Precautions

1. *Potential biohazardous materials:* The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories," 1984.
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components from different lots should not be mixed.
4. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

Specimen Collection and Preparation

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2-8°C for up to 7 days or frozen for up to 6 months. Avoid repetitive freezing and thawing of serum sample.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-25 °C) before use.
2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of Wash Buffer (1x). Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.

ELISA PROCEDURE

1. Place the desired number of coated wells into the holder.
2. Prepare 1:40 dilution of test samples, Negative Control, Positive Control, and Calibrator by adding 5 µl of the sample to 200 µl of Sample Diluent. Mix well.
3. Dispense 100 µl of diluted sera, Calibrator, and Controls into the appropriate wells. For the reagent blank, dispense 100 µl Sample Diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well.
4. Incubate at 37°C for 30 minutes.
5. At the end of the incubation period, remove liquid from all wells. **Rinse and flick the microtiter wells 4 times with diluted Wash Buffer (1x) and then one time with distilled water.**
6. Dispense 100 µl of Enzyme Conjugate to each well. Mix gently for 10 seconds.
7. Incubate at 37°C for 30 minutes.
8. Remove Enzyme Conjugate from all wells. **Rinse and flick the microtiter wells 4 times with diluted Wash Buffer (1x) and then one time with distilled water.**
9. Dispense 100 µl of TMB Reagent into each well. Mix gently for 10 seconds.
10. Incubate at 37°C for 15 minutes.
11. Add 100 µl of Stop Solution (1N HCl) to stop reaction.
12. Mix gently for 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely.* **Note:** Make sure there are no air bubbles in each well before reading.
13. Read O.D. at 450 nm ***within 15 minutes*** with a microwell reader.

CALCULATION OF RESULTS

1. Calculate the mean of duplicate calibrator value x_c.
2. Calculate the mean of duplicate positive control (x_p), negative control (x_n) and patient samples (x_s).
3. Calculate the CMV IgM Index of each determination by dividing the mean values of each sample (x) by calibrator mean value, x_c.

Example of typical results: Note: The O.D. values are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data.

Cut-off Calibrator CMV IgM Index = 1.0

1. Cut-off Calibrator O.D. = 0.856, 0.830 $x_c = 0.843$

2. Negative Control O.D. = 0.072,0.071 $x_n = 0.072$
   CMV IgM Index = x_n / x_c = 0.072 / 0.843 = 0.09

3. Positive Control O.D. = 1.592, 1.641 $x_p = 1.617$
   CMV IgM Index = x_p / x_c = 1.617 / 0.843 = 1.92

4. Patient sample O.D. = 1.465, 1.411 $x_s = 1.438$
   CMV IgM Index = x_s / x_c = 1.438 / 0.843 = 1.71

Quality Control

The test run may be considered valid provided the following criteria are met:

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.
2. If the O.D. value of the Cut-off Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The CMV IgM Index for Negative and Positive Control should be in the range stated on the Certificate of Analysis.

Interpretation

Negative: CMV IgM Index less than 0.90 is negative for IgM antibody to CMV.
Equivocal: CMV IgM Index between 0.91-0.99 is equivocal. Sample should be retested.
Positive: CMV IgM Index of 1.0 or greater is positive for IgM antibody to CMV.

Performance Characteristics

I. **Specificity and Sensitivity**

A total of 190 patient samples were used to evaluate specificity and sensitivity of the test. The CMV IgM ELISA test results were compared to a commercial ELISA kit results:

		Reference CMV IgM ELISA			
		N	E	P	Total
CMV IgM ELISA	N	125(D)	1	3(B)	129
	E	1	0	2	3
	P	7(C)	0	51(A)	58
	Total	133	1	56	190

Sensitivity = A / (A+B) = 51 / 54 = 94.4%
Specificity = D / (C+D) = 125 / 132 = 94.7%
Accuracy = (A+D) / (A+B+C+D) = 176 / 186 = 94.6%

II. **Precision**

The Precision of the assay was evaluated by testing four different sera of 20 replicates over different days or times. The intra-assay and inter-assay C.V. % are summarized below:

Sample	1	2	3	4
Intra-assay	2.7	4.2	4.4	6.4
Inter-assay	3.9	7.2	7.6	13.5

Limitations of the Procedure

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

REFERENCES

1. Volter, A., J.E. Bidwell, et al. Manual of clinical immunology. Chapter 69. Rose, N. and Friedman, H. eds. Am. Soc. Microbiol. P.506, 1985.
2. Cremer, N.E. Antibodies in serodiagnosis of viral infection. P. 73. In Lennett E.H. ed. Laboratory diagnosis of viral infection. Mercel Dekker, Inc., New York, 1985.
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APPENDIX 7

QUALITY ASSURANCE

Specimen collection

- The specimens used for analysis were venous blood drawn from the cubital vein
- Lipaemic and / or haemolysed samples were not analysed
- The specimens were correctly labelled and the same number was used to identify the sample through all the analytical steps
- The samples were collected into the correct specimen containers

Specimen transport and storage

- The specimens were placed into a cool box immediately upon collection
- The specimens were transported to the laboratory in cool boxes
- Once in the laboratory, the specimens were separated and analysed immediately or stored at 2-8°C for a maximum of five days.

Specimen separation

- After separation, the specimens were placed into correctly labelled tubes
- Measures were taken to avoid contamination
- After separation, the specimens were analysed immediately or stored at 2-8°C for a maximum of five days.

Analytical process

- Care was taken during preparation of the work sheet to ensure that the laboratory numbers assigned were correct and that they corresponded to the correct donors.
- The correct kits were used for the tests
- The kits were verified before use
- The kits were stored at 2-8°C
- Kits used for the study were not expired
- The correct samples were used
- The correct reagents (that were not expired) were used
- Strict care was taken to ensure that only the correct volumes were used
- The correct volumes and dilutions were used.
- Positive and negative controls were used in the assay and they were treated in the same manner as the sample
- Heat and light were avoided in the incubation steps
- The incubation steps were corrected time
- Care was taken to avoid spill-over
- The correct number of washes was done.
- After washing, the wells were dried appropriately

- All the pipettes and equipment used for the conjugate were kept separate from the substrate
- The TMB reagent was added rapidly
- The stop solution was added in the same order as the TMB
- The optical densities were read within 10 minutes of stopping the reaction
- Expected values of the negative and the positive controls were used to ensure validity of the results that were obtained

Post analytical

- Calculations of the cut-off points were carefully done to ensure correct interpretation of results.
- Care was taken to avoid post-transcriptional errors while transferring results from the assigned laboratory numbers to the correct donors and also in recording this information to the correct donor questionnaires.
- Utmost care was also taken during data entry and analysis.

APPENDIX 8

ETHICAL CLEARANCE

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Email KNHplan@Ken.Healthnet.org
19th September 2007

Ref KNH-ERC/ 01/ 4741

Dr Dorothy G Njeru
Dept. of Human Pathology
School of Medicine
University of Nairobi

Dear Dr. Njeru

RESEARCH PROPOSAL: "PREVALENCE OF CYTOMEGALOVIRUS ANTIBODIES IN BLOOD DONORS AT THE NATIONAL BLOOD TRANSFUSION SERVICE" (P230/8/2007)

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** your revised research proposal for the period 19th September 2007 - 18th September 2008

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimen must also be obtained from KNH-ERC for each batch.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

DR. L. MUCHIRI
AG. SECRETARY, KNH-ERC

c.c. Prof. K.M Bhatt, Chairperson, KNH-ERC
The Deputy Director CS, KNH
The Dean, School of Medicine, UON
The Chairman, Dept. of Human Pathology, UON
Supervisors: Prof. W.O. Mwanda, Dept. of Human Pathology, UON
Dr G.W. Kitonyi, Dept. of Human Pathology, UON
Dr. E.C. Njagi, Dept. of Human Pathology, UON

APPENDIX 9

CLEARANCE FROM NBTC

MINISTRY OF HEALTH

Telephone 020-2723569
Fax 020-2723569

Ref No. ST/7/1/7/VOL. I

NATIONAL BLOOD TRANSFUSION
CENTRE (N.B.T.C.)
P.O. BOX 20750
NAIROBI

18th April , 2007

Dr. Dorothy G. Njeru
Department of Human Pathology

Thro

The Chairman
Department of Human pathology,
College of Health, Sciences,
University of Nairobi

RE: PERMISSION TO CARRY OUT STUDY ON CMV AT THE NABTS

This is in reference to your letter of 16th February, 2007 seeking permission to carry out a study on CMV among blood donors and also join the NBTS field staff on blood donation exercises.

This is to let you know that permission is granted for the same We look forward to you sharing the findings of this study as it will contribute immensely to our mission of providing safe blood

DR. JACK NYAMONGO
DIRECTOR - NBTS

I am big hearted
I give blood

GIVE BLOOD TODAY YOU MAY NEED IT TOMMORROW

APPENDIX 10

REFERRAL LETTER

PREVALENCE OF CMV INFECTION AMONG BLOOD DONORS AT THE NBTC

Date.....

Dear .....

(Name of study participant)

I, Dr. D. Njeru, being the principal investigator in the above named study; wish to thank you for voluntarily taking part in this study.

I take this opportunity to advise you to visit the NBTC on any working day during working hours as there are certain aspects of your tests the NBTC team would wish to discuss with you.

Thank you.

Signed .....

APPENDIX 11

TTI PREVALENCE FOR THE YEAR 2006 AND 2007 AT THE NBTC

Table 9: TTI prevalence for the year 2006

Month/ TTI	*HIV*	*HBsAg*	*Syphilis*	*HCV*	*Total number of tests*
	Number (%)	Number (%)	Number (%)	Number (%)	
Jan	58 (1.27)	66 (2.29)	11 (0.38)	21 (0.73)	**4569**
Feb	99 (2.40)	119 (2.90)	21 (0.52)	55 (1.40)	**3328**
Mar	51 (0.50)	117 (2.40)	15 (0.32)	49 (1.00)	**4760**
Apr	52 (1.70)	75 (2.50)	7 (0.23)	6 (0.20)	**1608**
May	33 (1.00)	64 (2.40)	6 (0.20)	40 (1.30)	**2608**
Jun	62 (1.57)	109 (2.77)	7 (0.18)	46 (1.27)	**3138**
Jul	33 (0.90)	69 (2.40)	8 (0.28)	43 (1.50)	**2816**
Aug	43 (1.60)	91 (3.30)	17 (0.62)	35 (1.30)	**2312**
Sep	30 (0.73)	134 (3.20)	12 (0.31)	68 (1.64)	**4164**
Oct	35 (0.65)	74 (2.43)	29 (0.56)	15 (0.29)	**4802**
Nov	50 (1.29)	82 (2.11)	19 (0.49)	15 (0.39)	**2520**
Dec	57 (2.26)	117 (4.63)	38 (1.5)	15 (0.59)	**2183**
Tot /av	**603 (1.55)**	**1117 (2.88)**	**190 (0.49)**	**408 (1.05)**	**38,805**

Key

Tot = total number of tests done that year. This is the total number of individuals who donated blood at the NBTC for that year.

Av = average prevalence of the various TTI for that year.

Table 10: TTI for 2007

Month/ TTI	*HIV*	*HBsAg*	*Syphilis*	*HCV*	*Total number of tests*
	Number (%)	**Number (%)**	**Number (%)**	**Number (%)**	
Jan	38 (1.40)	61 (2.20)	8 (0.30)	14 (0.51)	**2759**
Feb	47 (0.96)	79 (1.57)	8 (0.16)	19 (0.38)	**5018**
Mar	81 (1.76)	87 (1.89)	12 (0.26)	17 (0.37)	**4592**
Apr	27 (1.64)	45 (2.73)	12 (0.73)	9 (0.55)	**1647**
May	32 (0.89)	73 (2.03)	17 (0.47)	18 (0.50)	**3591**
Jun	42 (0.93)	99 (2.19)	7 (0.15)	64 (1.41)	**4526**
Jul	29 (1.23)	58 (2.46)	4 (0.17)	37 (1.57)	**2358**
Aug	32 (1.49)	89 (4.16)	6 (0.28)	22 (1.03)	**2135**
Sep	13 (0.79)	49 (2.98)	6 (0.37)	20 (1.22)	**1642**
Oct	58 (1.49)	126 (3.24)	4 (0.10)	40 (1.03)	**3882**
Nov	30 (1.24)	55 (2.28)	3 (0.12)	22 (0.91)	**2412**
Dec	78 (2.50)	108 (3.50)	16 (0.50)	82 (2.60)	**3122**
Tot /av	**507 (1.36)**	**929 (2.60)**	**101 (0.30)**	**364 (1.01)**	**37,684**

Key

Tot = total number of tests done that year. This is the total number of individuals who donated blood at the NBTC for that year.

Av = average prevalence of the various TTI for that year.