بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Seroprevalenceof Cytomegalovirus Infection among Blood Donors in Khartoum State

الانتشار المصلي للإصابة بالفيروس المضخم للخلايا بين متبرعي الدم في مدينة الخرطوم

A dissertation submitted in partialfulfillment of requirements of M.Sc in medical laboratory science (Microbiology)

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قال تعالى :

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (١) خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ (٢) اقْرَأْ وَرَبُّكَ الْأَكْرَمُ (٣) الَّذِي عَلَّمَ بِالْقَلَمِ (٤) عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ (٥)

صدق الله العظيم

سورة العلق الآيات 1-5

DEDICATION

To my parents

Family

Teachers

Colleagues

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First of all, my gratitude to ALMIGHTY ALLAH for helping me to complete this work properly. Thanks must be extended to my supervisor Dr.Yousif Fadlalla for this close supervision , valuable advices, and stimulating suggestions . his pleasant personality made it easy for us to do this work together.

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ABSTRACT

This study was conducted during the period from March to May 2014. The aim of this study was to determine the seroprevalence of Human Cytomegalovirus (HCMV) among blood donors attending Blood Bank department at National Public health Laboratory and Blood Bank department at Khartoum Teaching Hospital.

A total of 100 subjects (n = 100) were included in the study. The blood donors ages range from 15 to60and from both genders. From the study participants, serum sample were obtained and personal and clinical data were collected , sera were tested for CMV IgM and IgG antibody using Enzyme Linked Immuno Sorbent Assay (ELISA).

Out of the 100 blood donors tested, 73 % and 19 % were CMV IgG and CMV IgM positive, respectively, subjects were divided in two age groups (<30 years and ≥ 30 years) in order to evaluate the effect of age on CMV seropositivity from the above finding we concluded that, there was no significant difference ($p > 0.05$) between the two age groups on CMV IgG and IgM seropositivity .

Also we concluded that there was high percentage of human cytomegalovirus HCMV among blood donors, and there was no significance difference ($p > 0.05$) between both history of previous surgical operation and blood transfusion and CMV seropositivity . Since about19 % of blood donors are seropositive for CMV IgM , it would be very useful to screen blood donors for CMV to identify the CMV- seronegative blood donors, and maintain an inventory of them for use as donors, in which recent studies revealed that the virus can transmit even after using universal leucoreduced blood product.

ملخص الاطروحة

أجريت هذه الدراسة في ولاية الخرطوم في الفترة الزمنية بين مارس ومايو 2014 و كان الهدف الرئيسي الكشف عن الفيروس المضخم للخلايا عند متبرعي الدم ببنك الدم في المعمل القومي الصحي National Public health Laboratory **وبنك الدم فيمستشفى الخرطوم التعليمي .**

شملت هذه الدراسة 100 شخصا من متبرعي الدم , وتراوحت أعمار المشاركين في الدراسة من 15 **الى** 60**.سنة . وشملت الدراسة الجنسين . تم جمع عينات من المصل من المشاركين بالدراسة وتم جمع المعلومات الشخصية والطبية عن طريق الإستبيان بعد موافقتهم الشفوية ومن ثم أخذت العينات و فحصت لمعرفة احتوائها على أجسام مضادة من النمط IgG وIgM للفيروس المضخم الخلايا باستخدام اختبار الاليزا .**

من مجموع 100 شخصا من متبرعي الدم 73%كانت لديهم اجسام مضادة من النمط IgG و19% كانت لديهم أجسام مضادة من النمط IgM وتم تقسيم أعمار المشاركين الى مجموعتين أقل من ثلاثين عام وأكبر من ثلاثين عام خلصت الدراسة الى انه لا توجد فروق ذات دلالة احصائية (P > o.o5) بين مجموعتي أعمار المشاركين في الدراسة و معدل ايجابية CMV IgG و CMV IgM.

من النتائج أعلاه خلصت الدراسة الى ان هناك نسب عالية من الفيروس المضخم للخلايا عند متبرعي الدم كما خلصت الدراسة على أنه لا توجد فروق ذات إحصائية (P> 0.05) بين العمليات الجراحيةالسابقة ونقل الدم المصاحب لهذه العمليات الجراحية ومعدلات ايجابية / CMV IgG IgM ، وحيث أن معدل النتائج الايجابية كان 19% فمن المهم جدا اختبار عينات متبرعي الدم للفيروس المضخم للخلايا وتحديد قائمة بأسماء المتبرعين وذلك للحد من انتقال الفيروس للمرضى حيث ان الدراسات الحديثة اثبتت امكانية انتقال الفيروس بالرغم من استخدام مكونات الدم المنزوع منه معظم الكريات البيض.

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ABBREVIATIONS

AIDS Acquired immunodeficiency syndrome

CMV Cytomegalovirus

COVCut - off - value

CPECytopathic effect

EIAEnzyme Immunoassay

ELISAEnzyme Linked Immunosorbent Assay

HIV Human Immunodeficiency virus

PCR Polymerase Chain Reaction

pp65 Phosphoprotein 65

SCT Stem cell transplantation

SOTSolid – organ transplantation

SPSS Statistical Package of Social Science

TT-CMV Transfusion Transmitted cytomegalovirus

TLRs Toll-like receptors

C H A P T E R O N E

1. Introduction

1.1. Background

Transfusion Transmitted Disease (TTD) is a major challenge to the transfusion services all over the world. The presence of viruses in blood cells or plasma of asymptomatic donors is the major risk factor for transmitting infectious agents through blood transfusion. Other transfusion transmitted agents include bacteria, mycoplasma, and even protozoa. The main viruses associated with transfusion related infections are hepatitis viruses, retroviruses, and cytomegalovirus (CMV). The risk of transmitting hepatitis viruses, human retroviruses, such as human immunodeficiency virus (HIV) and human T-lymphotropic viruses types I and II has been reduced drastically by screening for the corresponding antibodies before transfusion (Preiksaitis , 2003).

However, CMV, a member of the human herpes family viruses, transmission is through blood component, transfusions is an important cause of concern world-wide. This is because the majority of adults have serological evidence of previous infections by these viruses. CMV is an ubiquitous agent, and seropositivity rates in the adult population over 40 years of age worldwide are 60 to 100%, possibly due to transmission through breastfeeding, sexual contact and spread from children (Schmaltz *et al;*2005). Like most other herpesviruses, they remain latent in the host after primary infection and persist for lifelong in the organism. Nevertheless, these viruses can be reactivated in immunosuppressed individuals leading to critical outcomes such as interstitial pneumonitis, hepatitis, retinitis, and encephalitis. Active infection results in serious morbidity and mortality, and many consider CMV to be one of the most serious pathogens affecting immunosuppressed

individuals and an important contribution to intrauterine infection. In one study, the relative risk of CMV disease transmission via blood components from seropositive donors was 0.4% to 12%. Transfusion-transmitted CMV (TT-CMV) is a significant cause of morbidity and mortality in immunosuppressed patients (Schmaltz *et al* ; 2005).

The widespread prevalence of CMV seropositivity makes it difficult for some blood banks to maintain sufficient CMV seronegative blood components for patients at risk for CMV disease. Therefore, the most effective way to minimize the risk of CMV transmission in high-risk recipients would be to administer CMV seronegative blood products or leucoreduced blood products. Otherwise, a way out would be to determine the frequency of primary CMV infection among seronegative blood donors (High , 2005).

1.2. Rationale

CMV diseases predominantly occur as an opportunistic infection in patients with severe immunosuppression, such as cancer and renal failure patients, and rarely occur in immunocompetent patients. bloodtransfusion is major cause of concern world wide because of high prevalence of Cytomegalovirus infection among adult in Ghana and Sudan.A study was conducted in military hospital in Accra , Ghana ,(Hecker *et al;* 2004). the seroprevalence of antibodies specific for CMV was tested using CMV IgG/IgM particle agglutination test kit and ELISA , of the 264 blood donors, 18 were negative and 246 were positive for CMV IgG antibodies, giving an overall CMV prevalence rate of 93.2%. None of the 264 blood donors were positive for IgM (Kuhn, 2006),

1.3. Objectives

1.3.1 General Objective

To investigate the seroprevalence of Cytomegalovirus among blood donors attendinding Khartoum Teaching Hospital and National public Health Laboratory blood bank department .

1.3.2. Specific Objectives

1- To detect CMV IgG and IgM antibodies among blood donors at Khartoum teaching hospital .
2- To study the effect age on CMV IgG and IgM seropositivity .
3- To study the effect of history of previous surgical operation and blood transfusion on CMV IgG and IgM seropositivity.

C H A P T E R T W O

2. LITERATURE REVIEW

2.1. History

CMV was first noticed by Ribber in 1881 , when he saw large "protozoan-like"cells in the kidney of still born infant, the first report of the visualization of CMV by electron microscopyappeared in 1953, when cytomegalic inclusion cells from an infant pancrease were viewed and particles were observed in both the cytoplasmand the clear hallo around the inclusions. The virus was cultured for the first time in 1956 by Rowe, Smith and Weller, all of whom worked independently (Bhatia and Ichhpujani , 2008) .

2.2. Classification

Cytomegalovirus formarly designated as human herpesvirus 5 (HHV-5) by the International Committee on Taxonomy of Viruses, is a member of the family Herpesviridae , and it is classified in the subfamily Betaherpesvirinae. Human CMV (HCMV) is the type species of the genus cytomegalovirus, and its name is derived from the enlargement of cells (cyto=cell, mega= large) infected by the virus. Its classification as a herpesvirus is based on its tendency to infect mononuclear cells and lymphocytes - and on its molecular phylogenetic relationship to other herpesviruses.Its classification as a betaherpesvirus is based on its long replication cycle, cytopathology, and restricted host range, which are all characteristic of the betaherpesviruses, (Todd and Wills , 2006).

2.3. Structure of the virus

Compared to other human herpesvirus , HCMV is the largest , with a genome of 235 kb encoding 165 gene (Davison *et al;* 2003), the virion consists of double stranded linear DNA core in an icosahedral nucleocapsid,

enveloped by a proteinacous matrix (the tegument). These components are enclosed in a lipid bilayer envelope that contains a number of viral glycoproteins , these include glycoprotein B (gB), gH, gL, gM, gN and gO, Mature virions range in diameter from 200 to 300 nanometer (Crough and Khanna, 2009) (Fig.2.1).

The tegument compartment contain the majority of the virion proteins, with the most abundant tegument protein being the lower matrix phosphoprotein 65 (pp 65), also termed Unique Longe 83(UL83) , the function of the tegument proteins can be separated in to two classes : (i) protein that play a structural role and are important for the assembly of the virions and the disassembly of the particle during entry and (ii) protein which modulate the host cell response to infection (Moncarski *et al* ; 2007) .

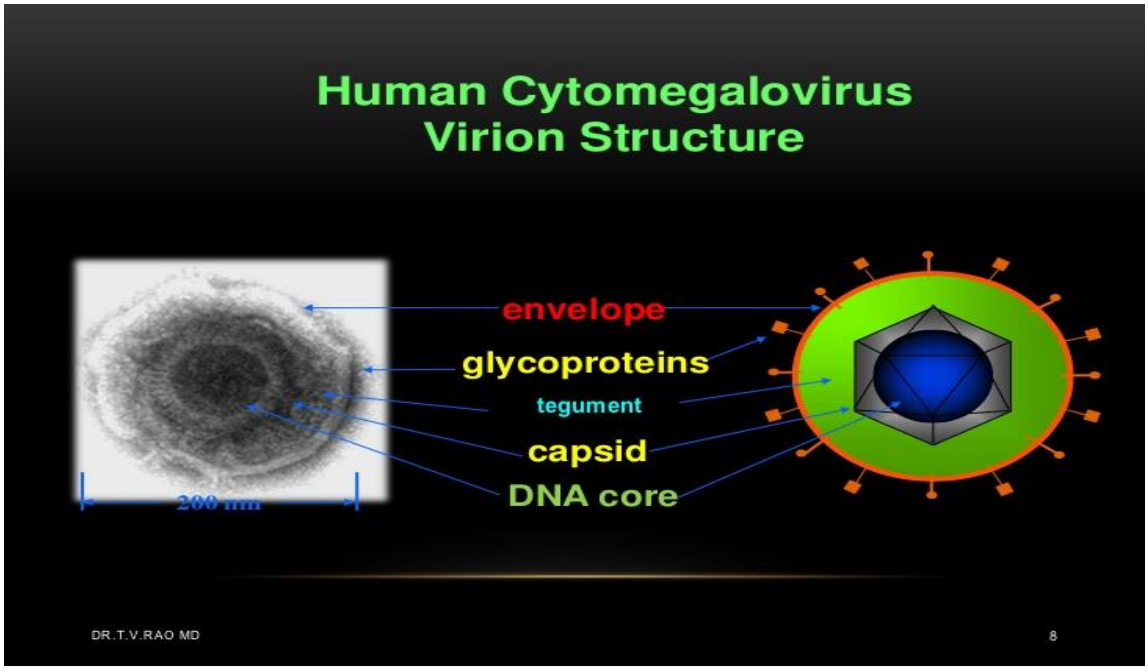


Figure 2.1. CMV virion structure

2.4. Properties of CMV

Cytomegalovirus has the largest genetic content of the human herpesvirus. It's DNA genome (240 kbp) is significantly larger than that of herpes simplex virus. Only a few of many proteins encoded by the virus (over 200) have been characterized. One, a cell surface glycoprotein, act as Fc receptors that can non specifically bind the Fc portion of immunoglobulins. This may help infected cells evade immune elimination by providing a protective coating of irrelevant host immunoglobulins. The major immediate early promoter - enhancer of CMV is one of the strongest known enhancers, due to the concentration of binding sites for cellular transcription factors. It is used experimentally to support high - level expression of foreign genes.

Many genetically different strains of CMV are circulating in the human population. The strains are sufficiently related antigenically, however , so that strain differences are probably not important determinants in human disease (Brooks *et al* ; 2010) .

Cytomegalovirus is very species - specific and cell type-specific . All attempts to infect animals with HCMV have failed. A number of animal CMV exists, all of them species - specific . HCMV replicates in vitro only in human fibroblasts, although the virus is often isolated from epithelial cells of the host. CMV replicate very slowly in cultured cells, with growth proceeding more slowly than that of HSV or varicella zoster virus. Very little virus becomes cell-free , infection spreads primarly cell - to - cell. It may take several weeks for an entire monolayer of cultured cells to become involved (Brooks *et al* ; 2010)

CMV produces a characteristic cytopathic effect , perinuclear cytoplasmic inclusion form in addition to the intranuclear inclusion typical of herpesviruses, multinucleated cells are seen, many affected cell become

greatly enlarged, inclusion-bearing cytomegalic cells can be found in samples from infected individuals (Brook *et al*, 2010).

2.5. Replication of CMV

Cytomegalovirus attaches to cell surface at the site of the receptor for fibroblast growth factor. After entry into the cell, the virion is uncoated and the genome DNA enter the nucleus. Within the nucleus, the incoming genome DNA changes its configuration from linear to circular. Early virus messenger RNA (mRNA) is transcribed by host cell RNA polymerase and then translated into early, non structural proteins in the cytoplasm. The viral DNA polymerase replicates the genome DNA, and late protein synthesis begins. Then the virion assembly occur in the nucleus. The virion obtain its envelope by budding through the nuclear membrane and exits the cell via tubules or vacuoles that communicate with the exterior (Levinson, 2010) .

2.6. Transmission

HCMV can be transmitted via saliva, sexual contact, placental transfer, breast feeding, blood transfusion, solid organ transplantation (SOT), or hematopoietic stem cell transplantation (SCT) (Sia and Patel, 2000). Day care centers are a significant source of CMV infection. Children less than three years of age with postnatally acquired CMV infection have been demonstrated to excrete CMV in their urine and saliva for 6 to 42 months (Nyholm and Scheiss, 2010), CMV transmission in infant breaset fed by seropositive women shedding virus in their breast milk has been reported to be 58 % to 69 % (Nyholm and Schleiss, 2010) .

2.6.1. Blood donation

Blood transfusions typically use sources of blood: one's own (autologous transfusion), or someone else's (allogeneic or homologous transfusion). The latter is much more common than the former. Using another's blood must first start with donation of blood. Blood is most commonly donated as whole blood intravenously and collecting it with an anticoagulant. In developed countries, donations are usually anonymous to the recipient, but products in a blood bank are always individually traceable through the whole cycle of donation, testing, separation into components, storage, and administration to the recipient. This enables management and investigation of any suspected transfusion related disease transmission or transfusion reaction. In developing countries the donor is sometimes specifically recruited by or for the recipient, typically a family member, and the donation occurs immediately before the transfusion.

All donated blood is tested for infections. The current protocol tests donated blood for HIV-1, HIV-2, , HTLV-2, Hepatitis B, Hepatitis C, Syphilis*(Treponema pallidum)*, Chagas disease*(Trypanosoma cruzi*), and West Nile Virus. In addition, platelet products are also tested for bacterial infections due to its higher inclination for contamination due to storage at room temperature. Presence of Cytomegalovirus (CMV) is also tested because of risk to certain immunocompromised recipients if given, such as those with organ transplant or HIV. However, not all blood is tested for CMV because only a certain amount of CMV-negative blood needs to be available to supply patient needs. Other than positivity for CMV, any products tested positive for infections are not used, (Wales,*et al*; 2001).

2.6.2. Neonatal Transfusion

Cytomegalovirus is a special case in donor testing in that many donors will test positive for it. The virus is not a hazard to a healthy recipient, but it can harm infant and other recipients with weak immune systems.The safety of donating blood during pregnancy has not been studied thoroughly, and pregnant women are usually deferred, (Klein *et al* ; 2010).

2.6.3. Leucoreduction Transfusion

Leucocytes are not often used because of the difficulties in obtaining sufficient quantities and because of their short life span in vivo. Donor antibody screening is clearly effective in reducing CMV infection. The use of leukocyte filtered blood products is an adjunct when seronegative products are not available. In addition to donor screening, leukocyte filtration is best considered as a transfusion management practice; not as a replacement for donor screening, (Squizzato, 2007).

2.7. Epidemiology

HCMV is highly species-specific, with humans being the only host. Furthermore CMV has been found in every human population tested. The prevalence of infection is greater in developing countries and among lower socioeconomic groups of developed countries. Overall , the seroprevalence of infection varies between 65 % to 90 % among middle age adult in the USA, where primary CMV infection during pregnancy occurs in 2% of women of child bearing age who are of lower socioeconomic background (Nassetta *et al*; 2009).

Crowded living conditions, poor sanitation , sexual practices and increased exposure to infant and children all contribute to increasing rates of infection

and a higher seroprevalence. Virus can be isolated from urine , saliva , cervical and vaginal secretions , semen , breast milk , tears , blood products and transplantated organs (Bowden, 1991). The risk factor for maternal acquisition of CMV during pregnancy is frequent and prolonged contact with a child less than three years of age , this occurs among women employed in child care centers (Adler, 2011).

2.8. Pathogenesis

Cytomegalovirus is an enveloped double stranded DNA virus. On this envelope, there are molecules called glycoproteins. These molecules, especailly glycoprotein B are determinants of humoral immunity , that they can cause a humoral immune response. Proteins in the viral tegument, the proteinaceous layer between the capsid and the envelope, elicit cellular responses to CMV. Specifically the abundant molecule phosphoprotein 65 is the molecule targeted by cytotoxic T cells in the cellular response. Although the host has the ability to defend itself against a CMV invasion, CMV has the ability to evade the host immunity, and in this way, it can cause infection in the host. CMV has genes that interfere with the antigen presentation of infected cells, thus limiting the immune response to it. Therefore, it can remain alive for a long time in a host, (Riley, 1997).

2.8.1. Infection in immunocompetent hosts

There are three manifestations of CMV disease in humans . The first is acute acquired CMV. This is when a healthy person becomes infected with CMV. Often the person will present no symptoms. If symptoms are present, they are similar to those of mononucleosis, and include fever, fatigue, and muscle

pain. These symptoms can last up to three weeks. Other clinical abnormalities have been associated with CMV infection in normal hosts, including Gullian-barre syndrome, meningoencephalitis , hemolytic anemia, and thrombocytopenia , after this initial infection, the virus has the ability to remain dormant in the host (Moncarski *et al;*2007).

2.8.2. Infection in immunocompromised patient

The second type of CMV disease occurs after this initial infection, and is a reactivation of the latent CMV, in which CMV is a serious opportunistic infection in immunocompromised individual. If a person becomes immunocompromised, for example someone with HIV, someone who received an organ or bone marrow transplant, or even someone under a lot of stress, the virus can flare up again. If this occurs, the symptoms can be more serious and include pneumonia, hepatitis which may cause fulminant liver failure, encephalitis, myelitis, colitis, retinitis (inflammation of the retina), and neuropathy.

2.8.3. Congenital and neonatal infection

The third type of CMV disease is congenital or perinatal CMV. This is when a woman who is infected with CMV (even if she doesn't have any symptoms) passes it to her child. In congenital CMV, a pregnant woman passes the disease through the placenta to the fetus. In perinatal CMV, a mother passes CMV to her child through nursing. Severity ranges from a simple fever of unknown origin, to enlarged liver and/or spleen, and even motor/mental retardation. The spread of the virus is not associated with food or water. It is generally spread through close contact with bodily fluids of an infected person. When the host is infected, CMV DNA can be detected with

polymerase chain reaction (PCR) in all the different cell lineages and organ systems in the body. Upon initial infection, CMV infects the epithelial cells of the salivary gland , resulting in a persistent infection and viral shedding (Todd and Wills, 2006) .

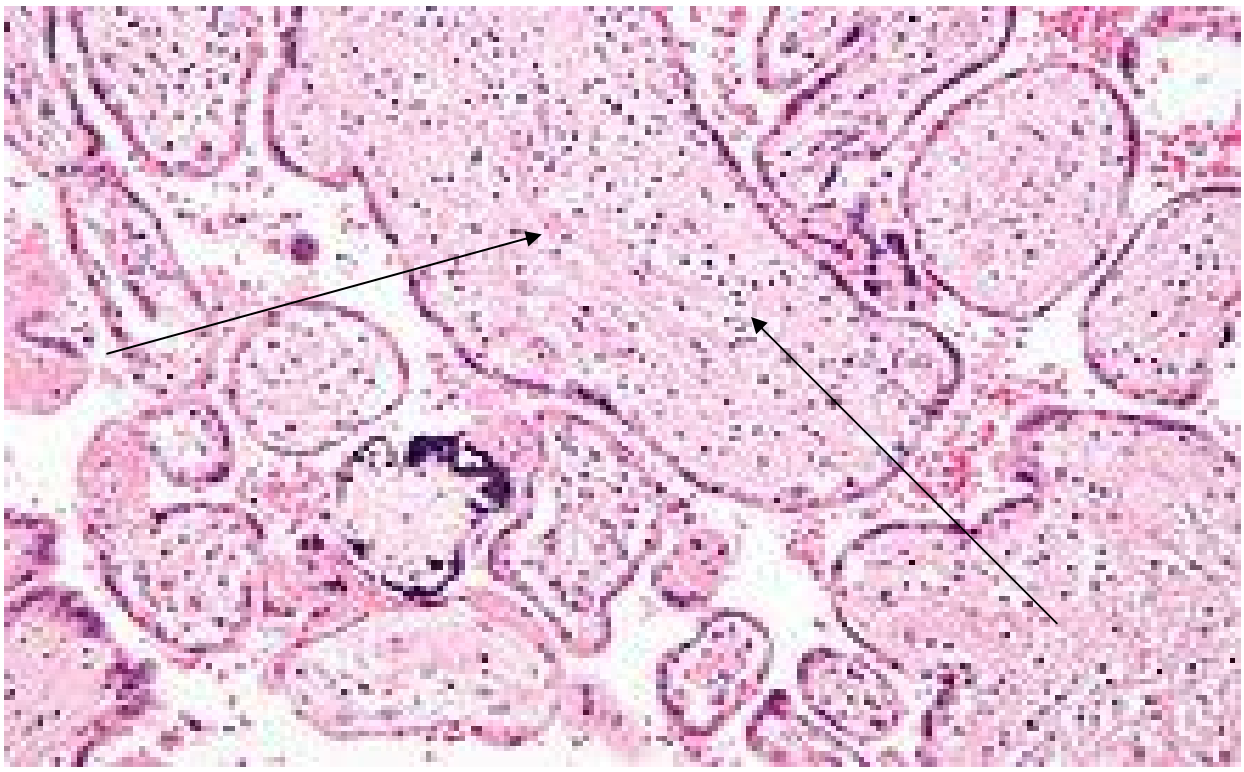


Figure 2.2. Micrograph of placental infection (characteristic viral inclusion bodies small pink globules H&E stain).

2.8.4. Infection in transfusion recipient

The problem of transfusion-transmitted cytomegalovirus (CMV) infection differs from that for other transfusion-transmitted infections in that only patients who are immunocompromised require CMV-free blood or components, (TT-CMV) is a significant cause of morbidity and mortality in immune-suppressed patients, including premature low-birth weigh infants

(<150g) born to CMV-seronegative mothers and HIV-AIDS patients (Albrecht and Rapp, 1973).

2.8.5. Complication of TT – CMV

The complication of TT- CMV of fever is the syndrome , with a typical lymphocyte was initially observed in patient who had undergone cardiac surgery using pump oxygenators, and the fresh blood used was suspected as the source of virus, the volume of blood transfused, the age of the blood, the antibody status of the patient and the mechanical damage to cells by the pump all may contribute to the possibility of the patient showing a CMV antibody response, but the relative importance of each factor is difficult to define, Cytomegalovirus (CMV) infection complicating heart surgery in connection with extracorporeal circulation, transfusion of fresh blood and medication of corticosteroids, the clinical syndrome was different from that reported in a small number of cases with post-transfusion CMV complications. A longstanding fever with involvement in the infectious process of lungs, also possibly heart, liver, and spleen, dominated the clinical picture, the use of CMV-seronegative blood products has been the 'gold standard' method of preventingTT- CMV infection, (Albrecht and Rapp ,1973).

2.8.6. Post transfusion and Organ Transplant

The pathogenesis of CMV infection following transfusion or organ transplantation is not clear. The major questions are whether this represents a primary or a reactivated infection , and if the latter, if the source of the virus is the recipient, the blood of the blood donor, there is direct association

between the number of units of blood transfused and CMV antibody conversion, (Alexander ,1967).

2.8.7. Post transplant

Cytomegalovirus infection following organ transplantation may be associated with a variety of clinical feature or may be completely a symptomatic. The source of the virus is unknown, it may be introduced with blood or in the donor organ or be endogenous in origin. there is some suggestion that the patient who acquires the primary CMV infection following allograft reception and immunosuppressive therapy has viremia and signs of illness, (Armstrong*et al* ; 1971).

2.8.8. CMV and Malignant Disease

The patient with malignant disease such as lymphoma or leukemia or hodgkins disease may have a sever or protracted illness with CMV infection , and they may suffer from more episode of pneumonitis or fever with rash but not more episode of hepatitis, and there may be other possible syndrome such as encephalitis or ulcerative colitis, (Baron *et al*;1969) .

2.9. Immunity

The innate immune system plays an important role in defence against HCMV and also in priming the adaptive immune response. It is becoming increasingly apparent that HCMV is subject to innate sensing by toll – like receptor (TLRs). The stimulation of TLRs by pathogens such as HCMV activates signal transduction pathways, which induce the secretion of inflammatory cytokines that recruit cells of the innate immune system , and the upregulation of costimulatory molecules such as CD80 and CD86 ,

which are important for activation of adaptive immunity (Boehme and Compton , 2004) .

The establishment of long - lasting immunity in response to a primary HCMV infection , which serves to control subsequent HCMV reactivation in the host , is important for preventing uncontrolled replication and serious HCMV disease .

HCMV is a potent immunogen that trigger strong immune response from all arms of the immune system , while the contribution of antibodies for protection against and control of HCMV has been debated, evidence does support a role for humoral immunity in the effective immune response against HCMV, predominantly in restricting viral dissemination and in limiting the severity of the disease (Boppana and Britt, 1995). The cell-mediated immune response is the predominant mechanism by which HCMV replication is controlled , as with the exception of congenital infection , severe HCMV disease occurs almost exclusively in patient with profound cellular immunodeficiency (Crough and Khanna, 2009) .

2.10. Laboratory diagnosis

Laboratory diagnosis of CMV infection depend on (i) detecting CMV cytopathology, antigen, or DNA in infected tissues (ii) isolating the virus from tissue or secretions (iii) demonstrating seroconversion (Drew, 2004)

2.10.1. Collection of specimen

Most useful specimens for CMV isolation are throat washing and urine. The virus is shed in urine for months after clinical features have subsided. CMV can also be isolated from saliva, breast milk, cervical secretions , blood and semen as well as various biopsy materials (Bhatia and Ichhpujani , 2008).

All specimen should be sent to the laboratory without delay, if delay of more than a few hours is anticipated, then all sample should be sent refrigerated or on wet ice, but under no circumstances should any specimen be frozen at any temperature (Griffiths, 2004) .

2.10.2. Cell Culture

Viral culture of the urine and saliva obtained within the first and two weeks of life continues to be the gold standard for diagnosis of congenitally-infected infants (Ross *et al* ; 2011) , the virus can be recovered most readily from throat washings and urine (Brooks *et al*; 2010). All cultures should be observed at least twice weekly for the typical focal CPE of CMV (Griffiths, 2004), In culture, 2 to 3 weeks are usually needed for the appearance of cytologic changes, consisting of small foci of swollen, translucent cells with large intranuclear inclusion (Figure 2.3), the virus stays cell- associated (Brooks *et al* , 2010). Immunoflurescence test or immunoperoxidase staining using specific antisera can be employed to confirm the results of CPE (Bhatia and Ichhupujani, 2008). Cell culture methods of viral isolation are too slow to be useful in guiding therapy, particularly in immunocompromised patients (Brooks *et al*; 2010).

2.10.3. Shell vial assay

The shell vial assay is performed by adding the clinical specimen to a vial that contains a permissive cell line for CMV. The shell vials are centrifuged at low speed and placed in an incubator, after24-48 hours , the tissue culture medium is removed and the cells are stained using a fluorescein - labeled anti CMV antibody .

The cell are observed using a flurescent microscope, alternatively, the cells are stained with an antibody against CMV, followed by a flurescein - labeled anti-immune globulin. This test has been found to be as sensitive as traditional tissue culture, probably because of the enhancement of the infectivity provided by configuration (Jahan, 2010) .

2.10.4. Cytopathology

Cytologic technique may applied in an attempt to find characteristics intranuclear inclusions in specimens , the microscopic hallmark of CMV infection is the large (25-35µm) cell containing a large basophilic intranuclear inclusion is reffered to as Owls eye, because it is separated from the nuclear membrane by a hallo, these inclusion are seen well with papanicolaou or hematoxylin-eosin stains (Jahan,2010) (Figure 2.4)

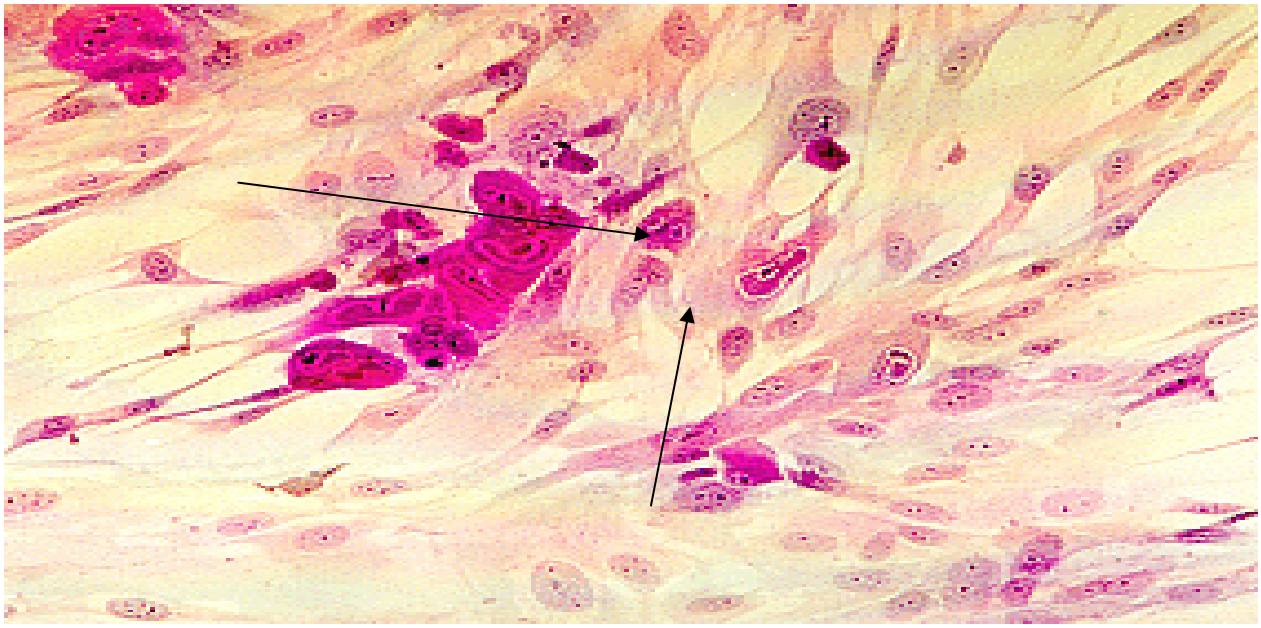


Figure 2.3. CMV Cytopathic effect (intranuclear inclusion bodies in tissue stained with H&E stain).

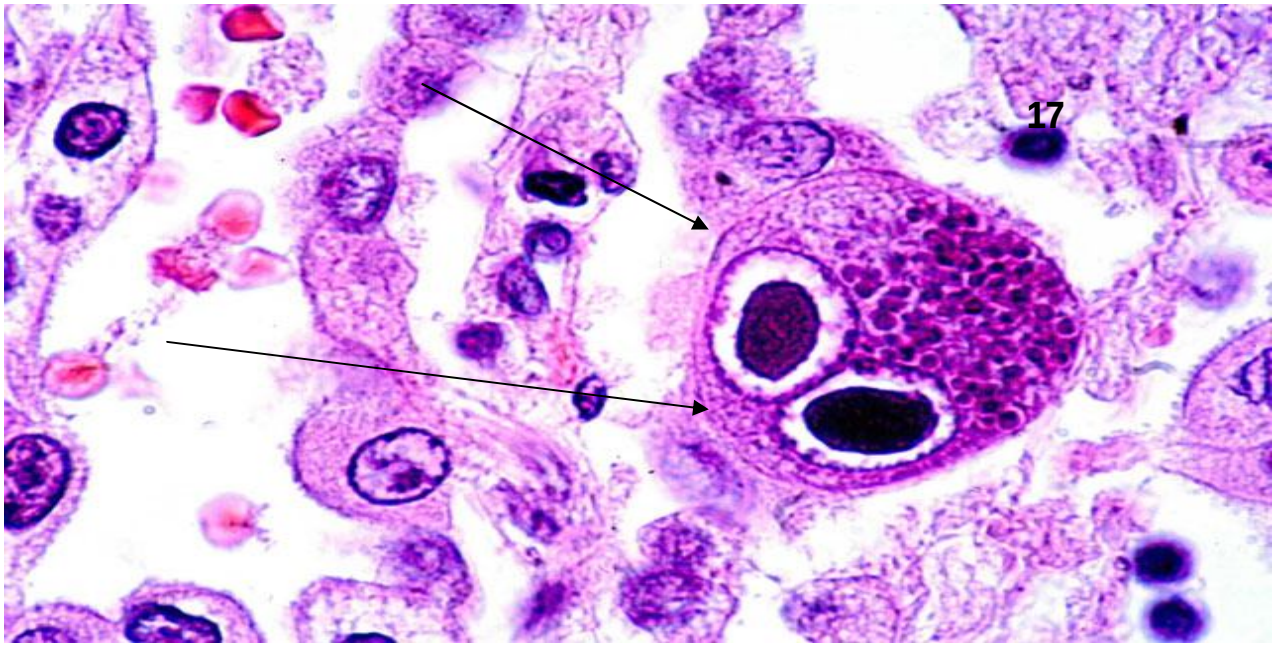


Figure 2.4. characteristic intranuclear Owls eye appearance within kidney-bean shape like nucleus.

2.10.5. Serology

Enzyme immunoassay (EIA) in which multiple specimens can be handled daily at a relatively low cost. Kits that detect CMV IgG are available from a number of commercial sources. The kits are easy to use , and the manufactures have provided detailed instructions (Hodinka, 2007). The development of robotics technology has led to the commercial availability of both fully automated and semi-automated EIA instruments, including sample dispensers, diluters, washers, and spectrophotometers with complete computer programming and generation of written reports (Hodinka, 2007).

Many types of assays can detect CMV IgG antibodies, indicative of past infection (and the potential to undergo reactivation). Detection of viral IgM antibodies suggest a current infection (Brooks *et al*; 2010). Antibody avidity, which is an indirect measure of the tightness of antibody binding to its target antigen, increases in the first weeks after a primary infection, low avidity IgG antibodies to CMV persist for up to 20 weeks after primary CMV infection, these low avidity antibodies are then replaced by high avidity antibodies . Currently, the combination of the presence of CMV IgM antibodies and low avidity CMV IgG antibodies along with maternal or fatal symptoms are used for the diagnosis of a primary maternal infection, (Alder, 2011) .

Complement fixation tests are adequate for showing seroconversion after primary infection in competent hosts. To screen for seropositive status a more sensitive assay such as enzyme immune-assay for CMV IgG or total antibody or latex agglutination assay, is appropriate. these test can be done urgently for donor- recipient assessment (Ogilvie, 2007). CMV IgM is found after primary or secondary infection, but it may not be possible to detect IgM in the neonate or immunocomromised patient, serologic assay are thus

not informative for immunocompromisedpatients. Furthermore, serologic techniques cannot distinguish stain difference among clinical isolates (Ogilvie, 2007).

2.10.6. Polymerase Chain Reaction

PCR assay have replaced virus isolation for routine detection of CMV infections. The PCR assays are designed to detect replicating virus, not latent viral genomes. Blood and urine are most commonly tested, PCR assays can can provide viral load data, which appears to be important in predicting CMV disease (Brooks *et al*, 2010).

Diagnosis of acute maternal CMV infection by the presence of IgM and low avidity IgG require confirmation of fetal infection , which is typically performed using of PCR assays for CMV on amniotic fluid. PCR assays of dried blood spots from newborn have been shown to lack sufficient sensitivity for the identification of most neonate with congenital CMV infection for universal screening purpose . However , saliva PCR assays are currently being assessed as a useful screening method for congenital CMV infection .

2.11 . Treatment

Cytomegalovirus Immune Globulin Intravenous (Human) (CMV-IGIV) is an immunoglobulin G (IgG) containing a standardized amount of antibody to Cytomegalovirus (CMV). It may be used for the prophylaxis of cytomegalovirus disease associated with transplantation of kidney, lung, liver, pancreas, and heart, alone or in combination with an antiviral agent. It has been shown to: (i) reduce the risk of CMV-related disease and death in some of the highest-risk transplant patients, (ii) provide a measurable long-

term survival benefit (iii) produce minimal treatment-related side effects and adverse events.

Ganciclovir (Cytovene) treatment is used for patients with depressed immunity who have either sight-related or life-threatening illnesses. Valganciclovir (Valcyte) is an antiviral drug that is also effective and is given orally. The therapeutic effectiveness is frequently compromised by the emergence of drug-resistant virus isolates. A variety of amino acid changes in the UL97 protein kinase and the viral DNA polymerase have been reported to cause drug resistance. Foscarnet or cidofovir are only given to patients with CMV resistant to ganciclovir, because foscarnet has notable nephrotoxicity, resulting in increased or decreased Ca^{2+} or P, and decreased Mg^{2+}, (Gilbert and Boivin , 2005).

2.12. Prevention and control

Some preventive action is undertaken by way of screening organ donors and recipients to avoid, where possible, a seronegative recipient from receiving an organ from a seropositive donor, this has been shown to reduce morbidity and mortality significantly in all forms of allogeneic transplant, blood donor screening to select seronegative units for support of seronegative patients in transplant programmes is important but not always available. Seriously compromised donors must receive screened blood from CMV seronegative donors or as routin now in the UK leucocyte - depleted blood. However, a more recent study has demonstrated that TT-CMV does occur even after leucoreduction, that CMV-seronegative blood products may thus be superior to leucoreduced blood product (Cohen , 2000).

C H A P T E R T H R E E

3. MATERIALS AND METHODS

3.1. Study design

Descriptive cross sectional study conducted at Khartoum Teaching Hospital and blood bank of National Public Health Laboratory.

3.2. Study area

Blood donors living in Khartoum attending blood bank department at Khartoum Teaching Hospital and National Public Health Laboratory.

3.3. Study duration

The study was carried out during the period from March to May, (2014).

3.4. Sample collection

A total of one hundred blood specimens (n = 100) were collected from blood donors, and investigated for CMV – specific immunoglobulin IgM and IgG . The age of all blood donors tested ranged from 15 and 60 years. The blood samples were collected under aseptic conditions, allowed to clot, centrifuged at 3000 rpm for 5 minutes , and sera were collected in sterile containers and stored at -80 ° C until tested .

3.5. Sampling Technique

The study was based on non - probability convenience sampling technique during attendance of blood donors to Khartoum Teaching Hospital and National Public Health Laboratory blood bank department.

3.6. Data collection

Personal and clinical data were collected by direct interviewing questionnaire from each subject (Appendix).

3.7. Ethical consideration

Permission to carry out the study was obtained from the College of Graduate Studies, Sudan University of Science & Technology. All blood donors examined were informed for the purpose of the study before collection of the samples and verbal consent was taken from them.

3.8. Laboratory work

The enzyme linked immunosorbent assay (ELISA) was used to detect the specific HCMV IgG and IgM antibodies .

3.8.1. ELISA for detection of CMV IgG antibodies

3.8.1.1. Principle

The HUMAN CMV IgG ELISA is based on the classical ELISA technique. The microtiter strip wells as solid phase are coated with cell culture derived CMV antigens (CMV Ag). In the first incubation step corresponding specific antibodies (CMV-IgG-Ab) present in patient specimens or control bind to the antigens at the solid phase . At the end of the incubation unbound components are washed out .

For the second incubation step anti IgG conjugate (anti - human IgG antibodies, peroxidase conjugate) is added which binds specifically to IgG antibodies resulting in the formation of typical immunocomplexes . After a second washing step to remove unbound conjugate, TMB/Substrate is added

(step 3). A blue color develops changing to yellow after stopping the reaction. The intensity of the color is directly proportional to the CMV IgG Ab concentration in the specimen. The absorbance of controls and specimens are determined by using ELISA microplate reader (humaReader). Results for patient samples are obtained by comparison with cut – off value

3.8.1.2. Procedure

Commercial ELISA Kits (HUMAN Diagnostic Co.Ltd.Germany) were used as described by the manufacturers. All reagents and samples were brought to room temperature before beginning the procedure . the serum sample was diluted (1:100) with dilution buffer IgG (DIL-G) and washing solution diluted (1:20) with fresh deionised water, other reagents were ready for use . Briefly, 100 µl of the negative control (NC), 100µl of the positive control (PC) , both were in duplicate form , and 100 µl of diluted samples were incubated in microtiter strip wells coated with CMV antigen at room temperature for 30 minutes. the wells were washed 4 times by washing solution using automatic washer to remove unbound components. Then 100 µl of anti-IgG conjugate were added to each well and incubated at room temperature for 30 minutes. After another washing step (5 times) to remove excess conjugate, an enzyme substrate reagent (TMB Substrate) was added (100 µl / well) and the plate was incubate for 15 minutes . The blue color changed to yellow after adding the stop solution (sulphuric acid - 100 µl). The optical density (OD) in a microplate reader was read within 30 minutes at 450 nm .

3.8.1.3. Calculation of control values and cut-off

Mean absorbance values of NC (MNC) and mean absorbance value of PC (MPC) were calculated . The cut-off value was then calculated following this equation:

Cut-off value COV = MNC + (0.2× MPC)

The test run was validated according to the manufacturer's criteria for validity as below :

1- Ab substrate blank < 0.150
2- MNC < 0.250
3- MPC > 0.750

3.8.1.4. Interpretation of the results

Samples with the absorbance > COV were considered as anti - CMV- IgG - Ab positive, while sample with absorbance < COV were considered as anti-CMV-IgG-Ab-negative.

3.8.2. ELISA for detection of CMV IgM antibodies

3.8.2.1. Principle

The HUMAN CMV IgM ELISA is based on the classical ELISA technique. The microtiter strip wells as solid phase were coated with cell culture derived CMV antigens (CMV Ag). In the first incubation step corresponding specific antibodies (CMV-IgM-Ab) present in patient specimens or controls bind to the antigens at the solid phase , the sample dilution buffer contains anti human IgG to prevent Rheumatoid Factor (RF) interference and competition for specific IgG present in the specimen .

At the end of the incubation, unbound components are washed out. for the second incubation step anti IgM conjugate (anti-human IgM antibodies, peroxidase conjugate) is added which binds specifically to IgG antibodies resulting in the formation of typical immunocomplexes after a second washing step to remove excess conjugate, TMB /Substrate is added (step 3). A blue color develops changing to yellow after stopping the reaction. the

intensity of the color is directly proportional to the CMV –IgM-Ab concentration in the specimen.

The absorbance of control and specimen is determined by using ELISA microplate reader (humanReader). Result for patient samples are obtained by comparison with cut-off value.

3.8.2.2. Procedure

Commercial ELISA Kits (HUMAN Diagnostic Co.Ltd.Germany) were used as described by the manufacturers. All reagents and samples were brought to room temperature before beginning the procedure. The serum sample was diluted (1:100) with dilution buffer IgM (DIL-M) and washing solution diluted (1:20) with fresh deionised water, other reagents were ready for use. All the steps for the procedure used above for the detection of the CMV IgG, were followed step by step for detection of CMV IgM.

3.8.2.3. Calculation of control values and cut-off

Mean absorbance values of NC (MNC) and mean absorbance value of PC (MPC) were calculated. The cut-off value was then calculated following this equation: Cut-off value COV = MNC + (0.1× MPC)

The test run was validated according to the manufacturer's criteria for validity as below :

1- Ab substrate blank < 0.150

2- MNC < 0.250

3- MPC > 0.400

3.8.2.4. Interpretation of the results

Sample with the absorbance > COV were considered as anti - CMV -IgM - Ab positive, while samples with absorbance < COV were as anti-CMV- IgM - Ab- negative.

3.9. Data analysis

The statistical package of social science (SPSS) was used for statistical analysis. Significance of difference was determined using Chi-square test. Statistical significance was set at $P < 0.05$. Figures were performed by using Microsoft Office and Excel software program.

C H A P T E R F O U R

4. RESULTS

4.1. Detection of CMV IgG among blood donors:

Out of the 100 blood donors tested, 73 subjects (73 %) were CMV IgG positive, while 27 subjects (27 %) were negative for CMV IgG, Figure (4.1).

4.2. Detection of CMV IgM among blood donors:

Out of the 100 blood donors tested, 19 subject (19 %) were CMV IgM positive, while 81 subject (81 %) were negative for CMV IgM , Figure (4.2).

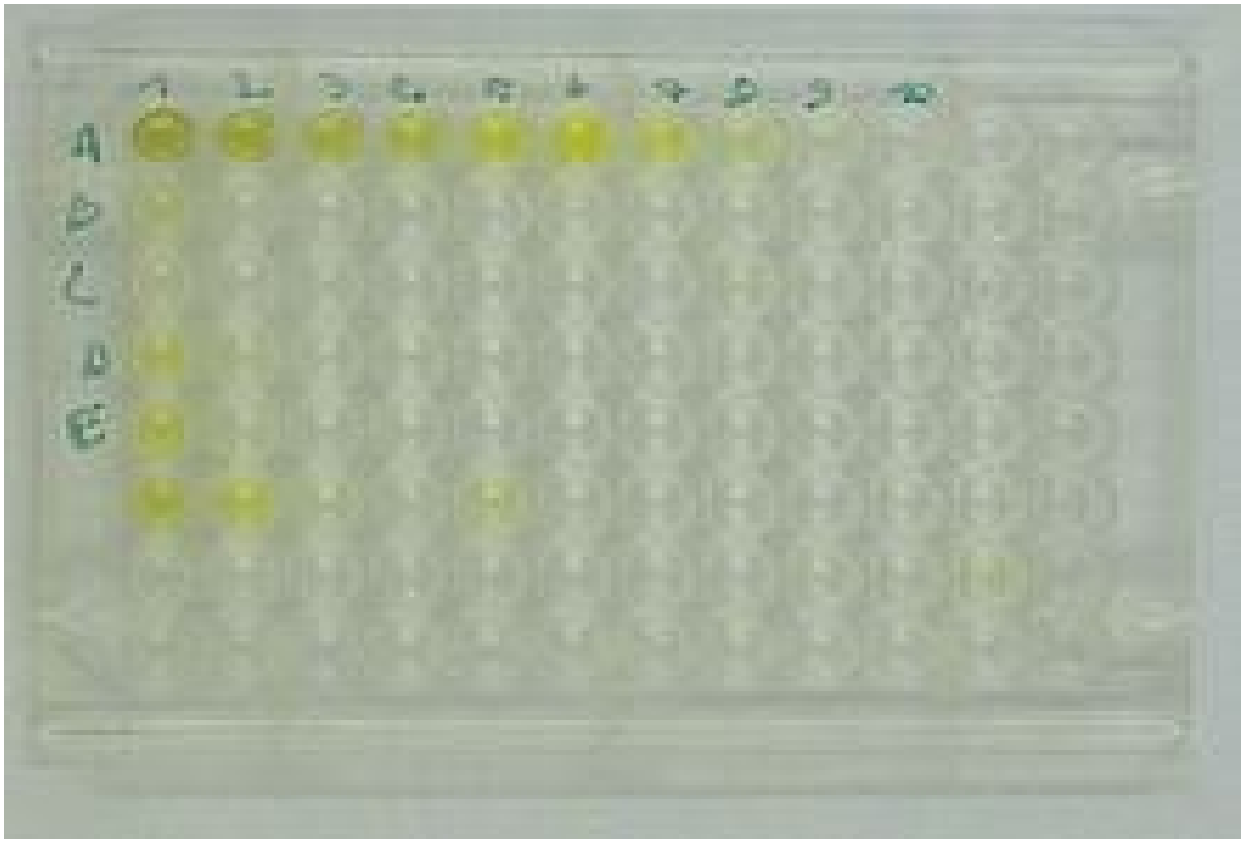


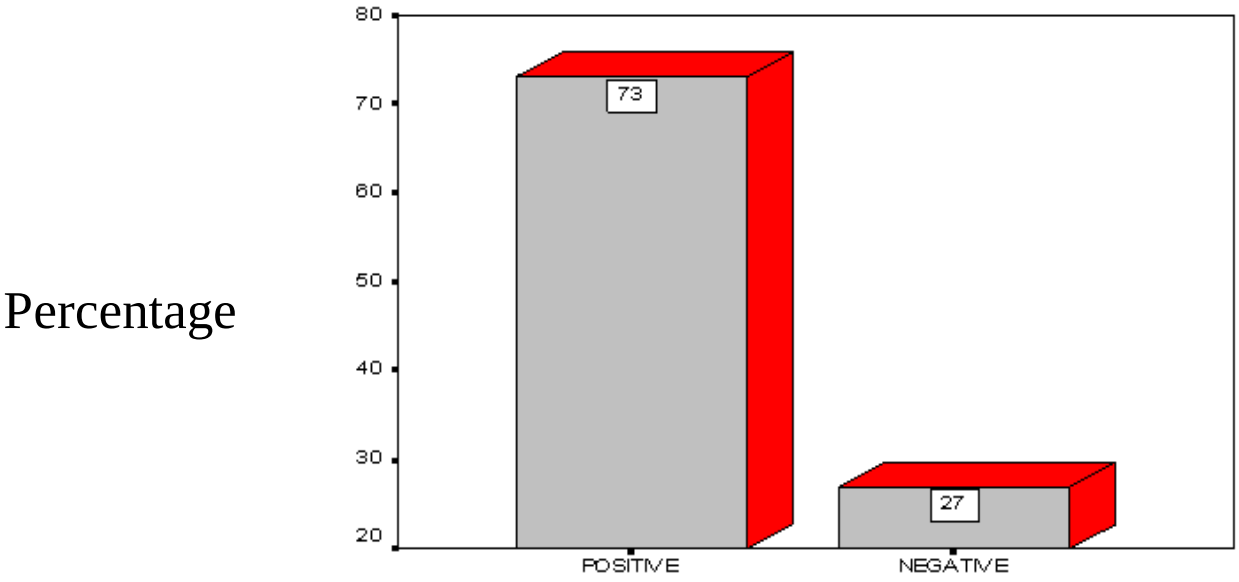
Figure 4.1 ELISA micro titter plate

4.3. The effect of age on CMV IgG and IgM seropositivity among blood donors:

The mean age of subject was 30 years, subjects were divided in two age groups (< 30 years and ≥ 30 years) in order to evaluate the effect of age on CMV seropositivity , while there was no significant difference (***p***>0.05) between the two age groups on CMV IgG / IgM seropositivity .

4.4. The Effect ofprevious surgical operation and bloodtransfusion on CMV IgG and IgM seropositivity among blood donors:

No significant difference (*p*> 0.05) was found between both history of previous surgical operation and blood transfusion and CMV seropositivity.



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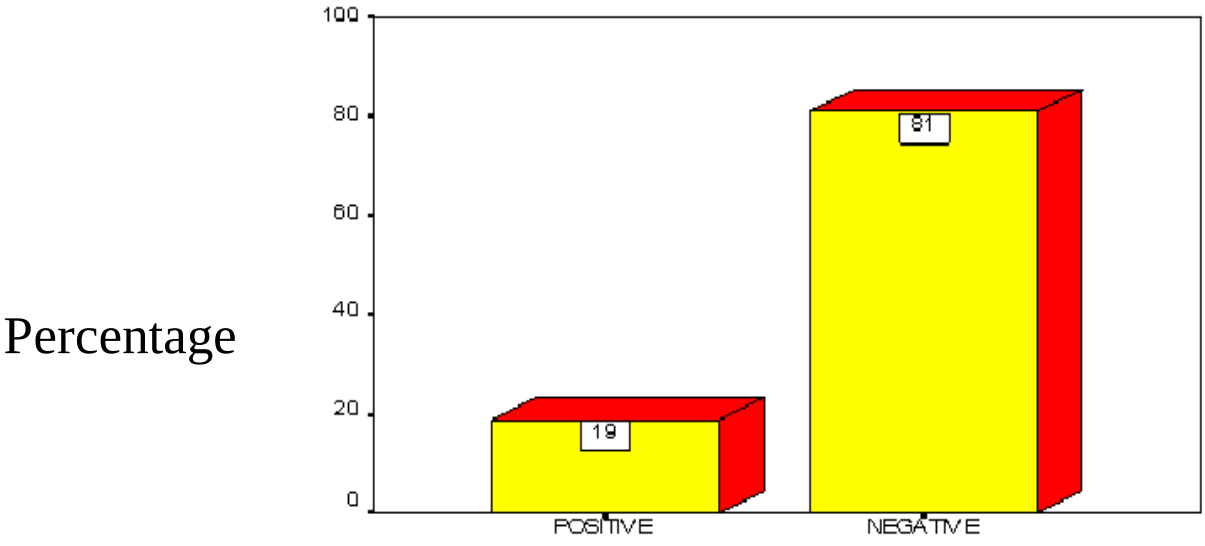
IgG

Figure 4.2 Detection of CMV IgG positive subjects among the blood donors.



IgM

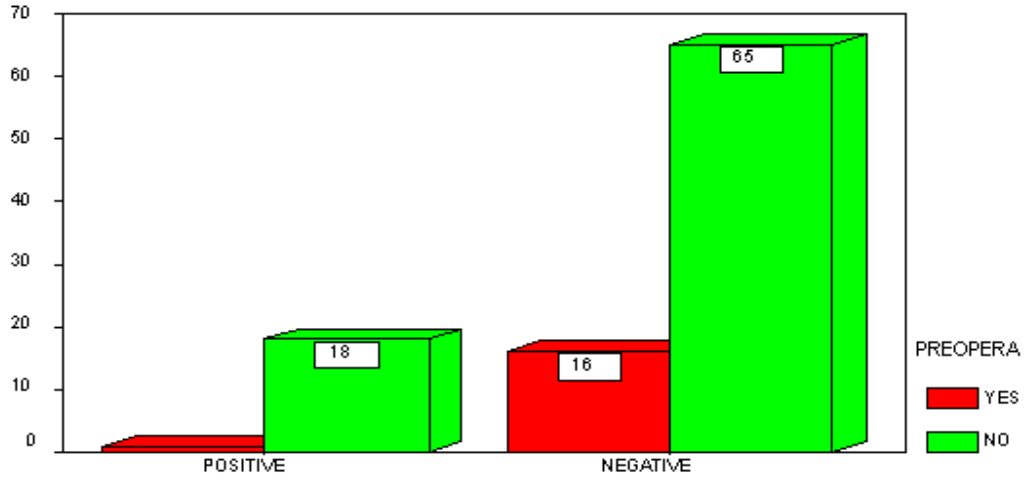
Figure 4.3 Detection of CMV IgM positive subject among the blood donors .

Table 4.1 The effect of age on CMV IgG and IgM seropositivity among the blood donors.

Serological marker (Anti – CMV antibodies)			Age groups (years)	
			< 30 yrs	**≥ 30 yrs**
			Test group	**Test group**
Anti CMV IgG	**Positive**	**Frequency %**	**38** **38 %**	**35** **35 %**
	Negative	**Frequency %**	**14** **14 %**	**13** **13 %**
Anti CMV IgM	**Positive**	**Frequency %**	**10** **10 %**	**9** **9 %**
	Negative	**Frequency %**	**41** **41 %**	**40** **40 %**

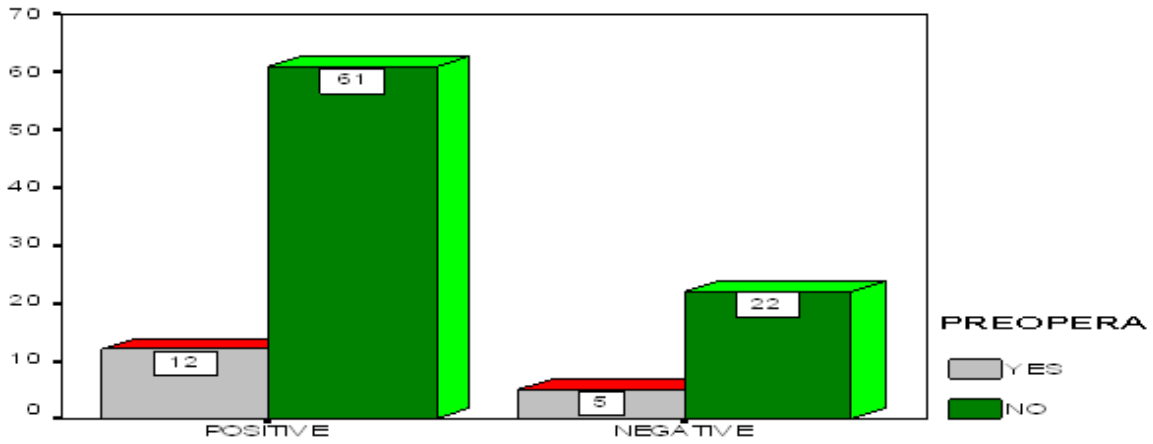
Table 4.2 The effect of previous surgical operation and bloodtransfusion on CMV IgG and IgM seropositivity among the blood donors.

Serological marker (Anti- CMV Antibodies)			Previous surgical operation and blood transfusion	
			YES	**No**
			Test group	**Test group**
Anti CMV IgG	**Positive**	**Frequency %**	**12** **12 %**	**61** **61 %**
	Negative	**Frequency %**	**5** **5 %**	**22** **22 %**
Anti CMV IgM	**Positive**	**Frequency %**	**1** **1 %**	**18** **18 %**
	Negative	**Frequency %**	**16** **16 %**	**65** **65 %**



IgM

Figure 4.4. The Effect of previous surgical operation and blood transfusion on CMV IgM seropositivity.



IgG

Figure 4.5. The Effect of previous surgical operation and blood transfusion on CMV IgG seropositivity.

C H A P T E R F I V E

5. Discussion

5.1. Discussion

This study was conducted on 100 blood donors , and revealed that the CMV seroprevalence among blood donors in blood bank of National Public Health Laboratory and blood bank of Khartoum Teaching Hospital was 73 % and 19 % for CMV IgG and CMV IgM , respectively.

The positive cases were detected among blood donors using ELISA , and our results are nearly in accordance with that conducted in military hospital in Accra , Ghana, (Hecker *et al;* 2004). The detection of CMV indicated that the blood donors had previously been infected with CMV , After CMV infection , IgG remains in the body for life and protects considerably against the next infections . Thus negative results of CMV IgG test means that the blood donors have not been infected with CMV (Bagheri *et al*; 2012).

The Seroprevalence of CMV IgG observed in this study was similar to the result obtained by Kasim *et al;* (2008). The previous CMV infection observed in this study was similar to that reported in other developing communities but higher than in the developed communities obtained by Barbara and Tegtmeier, (2007). This may be attributed to the low socioeconomic status and poor hygienic practice which might play an important role in increasing the rate of CMV infection.

In the present study, the rate of positive CMV IgM was 19% among the blood donors, which may reflected an active recent infection or reactivation of the virus (Farrel *et al;* 1999). this finding was higher than that of (Kasim *et al*; 2008) who reported the rate of positive CMV IgM as 11%.

The blood donors comprised largely of male donors making sex comparisons statistically undesirable, subject were divided in to two age groups (< 30 years and ≥ 30 years) in order to evaluate the effect of age on CMV IgM / IgG seropositivity and concluded that there was no significant difference (P > 0.05) between the two age groups and CMV IgG and IgM seropositivity . also the study revealed that there was no significance difference (P > 0.05) was found between the history of previous surgical operation and blood transfusion and CMV seropositivity .

5.2. Conclusion

It is concluded that CMV IgG and IgM antibodies was highly detected in blood donors and the study revealed that there was no significant assocoiation between age and both history of previous surgical operation (p> 0.05).

5.3. Recommendations

1.Screening of CMV IgM is suggested for blood donors particularly for the high risk group such as neonate, immunocompromised patient, and patients with a malignant disease.

2. Blood units must bescreened for anti-IgM CMV or preventive strategies to be implanted to decrease the transmission of CMV in these groups of patients.

3. Blood screened positive for anti-IgM CMV is recommended to be discarded.

4.IgG avidity test should be used to distinguish primary and recurrent infection.

5. PCR is essential for accurate identification of CMV infection.

R E F E R E N C E S

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A P P E N D I C E S

Appendix (1)

Sudan University of Science & Technology

Collage of Graduate studies

كلية علوم المختبرات الطبية

قسم الاحياء الدقيقة

Date…………………………………………………

Questionnaire

Seroprevalence of Cytomegalovirus infection in blood donors

1. Name of donors ……………………….....................................
2. Lab number ………………………………………………………
3. Age………………………………………………………….
4. Gender ……………………………………………………………
5. Previous surgical operation …………………………………….
6. Test required

☐ CMV IgM ☐ CMV IgG

Result :

CMV IgM ……………………………………………………….

CMV IgG ………………………………………………………..

Appendix (3)

CMV IgM

ELISA Test for the Detection of IgM Antibodies to Cytomegalo Virus in Human Serum

Package Size

REF	51103	96 Tests	Complete Test Kit
IVD			

Intended Use

The CMV IgM ELISA is intended for the detection of Immunoglobulin G (IgM) class antibodies to Cytomegalo virus (CMV) in human serum.

CMV infections occur worldwide. About 50% of the general population is seropositive by the third decade of life. CMV is often a sexually transmitted disease, but may also be aquired by blood transfusions or via saliva, feces, urine, or milk.

During pregnacy the fetus may be infected by CMV, and while the majority appear healthy at birth, up to 25% of these asymptomatically infected infants will show developmental disorders later (deafness, mental retardation). In normal adults infection with CMV is usually asymptomatic, but when symptoms do occur, the most common form is mononucleosis. Typically, the patient presents with fever, chills, myalgia, and headache. Often the physician must rely on serological tests to distinguish CMV infection from other infections and clinical syndromes which have similar symptoms.

Principle - Classic EIA -

The HUMAN CMV IgM ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid phase are coated with cell culture derived CMV antigens (CMV Ag). In the first incubation step corresponding specific antibodies (CMV-IgM-Ab) present in patient specimens or controls bind to the antigens at the solid phase. The sample dilution buffer contains anti-human IgG to **prevent rheumatoid factor (RF) interference and competition from specific IgG** present in the specimen.

At the end of the incubation unbound components are washed out. For the second incubation step anti-IgM conjugate (anti-human IgM antibodies, peroxidase conjugated) is added which binds specifically to IgM class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added (Step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the CMV-IgM-Ab concentration in the specimen.

The absorbance of controls and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). Results for patient samples are obtained by comparison with a cut-off value.

Reagents and Contents

MIC	12	**Microtiter Strips** (in 1 strip holder) (Code CMV M) 8-well snap-off strips coated with CMV antigen (cell culture derived)	
NC	2.5 ml	**CMV IgM Negative Control** (green cap) ready for use, human	
PC	2.5 ml	**CMV IgM Positive Control** (red cap) ready for use, human	
DIL-M 5111	100 ml	**Dilution Buffer IgM** (blue cap) ready for use, coloured green Phosphate buffer NaCl Albumin Anti-human-IgG (goat)	 pH 6.5 ± 0.2 10 mmol/l 8 g/l 10 g/l
CON	12 ml	**Anti-IgM Conjugate** (white cap) ready for use, coloured red Anti-human IgM (rabbit), peroxidase-conjugated	
WS 5102	50 ml	**Washing Solution** (white cap) Concentrate for about 1000 ml Tris buffer NaCl	 pH 7.2 ± 0.2 10 mmol/l 8 g/l
SUB 5103	13 ml	**Substrate Reagent** (black cap) ready for use, colourless to bluish 3,3', 5,5'-tetramethylbenzidin (TMB) Hydrogen peroxide	 pH 3.7 ± 0.2 1.2 mmol/ 3 mmol/l
STOP 5104	15 ml	**Stop Solution** (red cap) Sulphuric acid, ready for use	 0.5 mol/l
	2	**Adhesive Strips**	

Preservatives: Total concentration < 0.1%

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

STOP irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

MIC **(Code: CMV M)**

- sealed in an aluminium bag with a desiccant
- must be at room temperature before opening
- unused: return with the desiccant to the zip-lock bag and store in this way at 2...8°C
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.
Reagents not in use should always be stored at 2...8°C.

Notes

The **general purpose reagents** DIL-M 5111, WS 5102, SUB 5103, STOP 5104 are interchangeable between different lots and kits. For IgM **tests** use only IgM dilution buffer DIL-M 5111.

All other reagents are **specific for the individual package lot** and must not be interchanged with other lots. No reagents of other manufacturers should be used along with reagents of this kit.

Working Wash Solution WASH

- dilute WS 5102 1 + 20 with fresh deionised water, e.g. 50 ml WS 5102 + 1000 ml = 1050 ml.
- Stability: **up to 60 days at 15...25°C.**

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 7 days at 2...8°C or longer at -20°C. Freeze **and thaw once only.** Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: MIC - select the required number of Microtiter Strips.

P5: **Run duplicates** for controls. Pipette controls and specimen **on the bottom** in the microwells.

P6: **Always add reagents in the same order and timing to minimise reaction time differences between wells.** This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the controls in the indicated positions at half way time of the series. If more than 1 plate is used; repeat the controls for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading of absorbance.

P8: SUB – incubate in the dark. SUB initiates a kinetic reaction, which is terminated by STOP.

P9: DIL-M – turbidity after addition of the sample has no influence on the results.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove Adhesive Strips, aspirate off the contents into 5% sodium hypochlorite solution and add WASH to each well, aspirate off after 30 sec. soak time and repeat washing 3 resp. 4 times.

W2: In case of automatic washers fill and prime with WASH. Subsequently wash strips 4 resp. 5 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.				
Sample Preparation: Dilute the patient's sera 1 + 100 with DIL-M 5111, e.g. 10 µl serum + 1 ml DIL-M 5111, mix thoroughly (see P9). **Incubate diluted samples at least 5 min. prior to further processing.** Diluted samples can be stored up to 24 h at 2...8°C before testing. Controls are ready for use.				
Step 1	***Well [µl]***			
	A1 Blank	**B1/C1** NC	**D1/E1** PC	**F1...** Sample
NC in duplicate	--	100	--	--
PC in duplicate	--	--	100	--
Diluted samples	--	--	--	100
MIC cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 4 times as described (see W1 - W3)				
WASH	350	350	350	350
Step 2				
CON	--	100	100	100
MIC cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 3 times as described (see W1 - W3)				
WASH	350	350	350	350
Step 3				
SUB 5103	100	100	100	100
Incubate 15 min. at 17...25°C (see P8)				
STOP 5104	100	100	100	100
Mix carefully				
Zero the ELISA microtiter plate reader (HumaReader) using the substrate blank in well A1.				
Measure the absorbance at **450 nm** as soon as possible or **within 30 min.** after terminating of the reaction, using a reference wavelength of 630-690 nm (if available).				

Calculation of Control Values and Cut-off

Mean absorbance values of NC in wells B1 and C1 **(MNC)** and PC in wells D1 and E1 **(MPC)** are calculated according to:

$$MNC = \frac{A_{450}(B1) + A_{450}(C1)}{2}; \quad MPC = \frac{A_{450}(D1) + A_{450}(E1)}{2}$$

Cut-off value **COV = MNC + (0.2 x MPC)**

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

1. **Substrate blank in well A1 < 0.150**
2. **MNC ≤ 0.250**
3. **MPC ≥ 0.400**
4. **MPC : MNC ≥ 3**

Interpretation of Results

A_{450} (patient) ≥ COV + 15%: anti-CMV-IgM-Ab-positive

A_{450} (patient) < COV - 15%: anti-CMV-IgM-Ab-negative

Due to physiological and analytical variations patient results lying 15% above or below the calculated cut-off are equivocal. It is recommended to measure these samples in parallel with a fresh sample taken 7 to 14 days later, each in duplicate. The trend between the specific antibody levels should be used for interpretation, also taking into consideration the specific IgG concentration (HUMAN ELISA IgG), the patient history and additional investigations. Repeatedly reactive or equivocal samples may be subjected to a confirmatory test.

Samples from patients with EBV-induced infectious mononucleosis may give an equivocal or low positive result (sample to cut-off ratio < 1.75), possibly as a result of a reactivation of CMV IgM antibody production caused by EBV-induced B-cell proliferation.

If an ELISA reader is not available a visual interpretation of results is possible:

- The substrate blank in well A1 should appear colourless.
- A specimen can be considered positive if the colour of the sample well is definitely stronger than the colour of the NC wells B1/C1.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-cmvm.pdf or

www.human-de.com/data/gb/vr/el-cmvm.pdf

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

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