

1. Introduction

1.1. Background

Human cytomegalovirus (HCMV) is a member of herpesviridae family, subfamily beta-herpesvirinae, with human CMV and other animal species. The genome is of double-stranded linear DNA, the virus capsid is icosahedral symmetry and has envelope (Murray *et al.*, 2007).

The virus can be transmitted via saliva, sexual contact, placental transfer, breast feeding, blood transfusion and solid-organ transplantation (Bowden., 1991).

The virus has the ability to persist in the host in a latent state after primary infection (Emery., 2001), severe impairment of the body's immune system by medication or disease consistently reactivates the virus from the latent or dormant state (CDC., 2005), so it is an important pathogen in immunocompromised patients (Alford and Britt., 1996).

Now CMV is believed to be the most common pathogens of man. Most of CMV infections remain asymptomatic. The outcome is usually fatal in rarely cases (Bhatia and Ichhpujani., 2008).

Several types of viral infections have been associated with increased risk of diabetes mellitus (Jaeckel *et al.*, 2002). The researchers found that the CMV seropositivity is significantly associated with various indicators of glucose regulation and

therefore CMV infection might be a risk factor for the development of type 2 diabetes in the elderly (Chen *et al.*, 2012).

1.2. Rationale

Infection with CMV is known to play a role in type 1 diabetes, but there is a paucity of information on its role in diabetes mellitus type 2 (Roberts and Cech., 2005). CMV seropositivity was only slightly higher in patients with DM (Michiel *et al.*, 2013).

According to the list of human diseases associated with infectious pathogens; diabetes mellitus type 2 is associated with cytomegalovirus (Roberts and Cech., 2005), and according to a study from the Netherlands, CMV is common among older people with Type 2 diabetes (Maria., 2012).

A recent study showed that in the above 85 year olds CMV seropositivity was higher in patients with DM, unfortunately no other pathogens were determined in that study (Chen *et al.*, 2012).

There are two interesting theories about CMV and type 2 diabetes mellitus. The first one is that the virus may affect the pancreas, the gland that makes the sugar-processing hormone, insulin. The other is that diabetes also may affect the immune system, making CMV infection more likely (Maria., 2012). This theories shows only a link but not cause.

1.3. Objectives

1.3.1. General objective

To detect the frequency of cytomegalovirus IgG among type 2 diabetic patients.

1.3.2. Specific objectives

1. To detect specific CMV IgG among type 2 diabetic patients.
2. To see the relationship between age, gender, family history of diabetes, history of blood transfusion, level of education and CMV IgG seropositivity among type 2 diabetic patients hospital admission.

2. Literature Review

2.1. Cytomegalovirus

Cytomegalovirus belongs to the herpesviruses family. The name of herpes viruses came from the Greek word *herpein*, meaning to creep. More than 100 herpes viruses had been isolated from a range of hosts that includes mammals, birds, reptiles, amphibians and mollusks (Carter and Saunders., 2007).

Latency is the property shared by all herpesviruses which allows them to persist indefinitely in their host after a primary or secondary infection (Pastoret *et al.*, 1984), which can be reactivated from time to time, especially if the host becomes immunocompromised. Both primary and reactivated herpes virus infections can either be asymptomatic or can result in disease of varying severity. The outcome depends on the interplay between the particular virus and its host and especially the immune status of the host (Murray *et al.*, 2007).

2.1.1. History

The characteristic cytomegalic cells of CMV disease were first noted by Ribbert in 1881 in the kidney and parotid glands of a syphilitic neonate, and confirmed by Jesionek and Kiolemenoglu in 1904. They interpreted these cells as protozoa (Ho., 2008). In 1950 the name "generalized cytomegalic inclusion disease (CID)" was suggested (Wyatt *et al.*, 1950). In 1956 the virus was isolated by three independent groups, those of

Smith, Weller and Rowe (Smith., 1956, Rowe *et al.*, 1956, Weller *et al.*, 1957).

2.1.2. Classification

Human cytomegalovirus (HCMV) or human herpesvirus 5 (HHV-5) belongs to the viral family known as herpesviridae or herpesviruses (Ryan and Ray., 2004). Within herpesviridae, HCMV belongs to the beta-herpesvirinae subfamily, which also includes cytomegaloviruses from other mammals (Koichi *et al.*, 2007).

Human cytomegalovirus (HCMV) is the type of species of the genus cytomegalovirus, and its name is derived from the enlargement of the cells (cyto=cell, mega=large) infected by the virus. Herpes virus 6(HV-6) and herpes virus 7 (HV-7) are now classified with CMV among the beta-herpesviruses (Brooks *et al.*, 2010).

2.1.3. Structure of the virus

The general structure of the virus seen in (Figure A). Cytomegalovirus characterized by slow growing, cytomegalic (Cytopathic effect, CPE). Which from its name, and enlargement of cell with acidophilic inclusion bodies in the nuclei that resemble (owl's eye) (Figure B). It is the genus megalovirus that its official name HHV-5 (Brooks *et al.*, 2010).

Compared to other human herpesviruses, HCMV is largest, with genome of 235 kb encoding 165 genes (Davinson *et al.*, 2003).

Cytomegalovirus is composed of large DNA genome (240 kpb), which is double strand, linear and in form of toroid. In spite of

its genetically different strains, it is being in human population. The DNA genome is surrounded by a protein coat that gives icosahedral symmetry with 160 capsomeres. Its nucleocapsid is surrounded by an envelope that derived from nuclear membrane of the infected cell, measured 150-200 nm and contain glycoprotein spikes 8 nm act as Fc receptor for nonspecific binding (Brooks *et al.*, 2010).

Between the envelope and capsid, found an amorphous layer called the tegument. The naked virion measures 100 nm contains one of the immediate early stronger enhancers due to concentration of binding sites for cellular transcription factors (Figure A). Herpesviruses encode an array of virus-specific enzymes involved in nucleic acid metabolism, DNA synthesis and protein regulation (DNA polymerase-thymidine kinase-protein kinase (Brooks *et al.*, 2010).

The tegument compartment contains the majority of the virion protein, with the most abundant tegument protein being the lower matrix phosphoprotein 65 (pp 65), also termed unique long 83, UL83 (Varnum *et al.*, 2004).

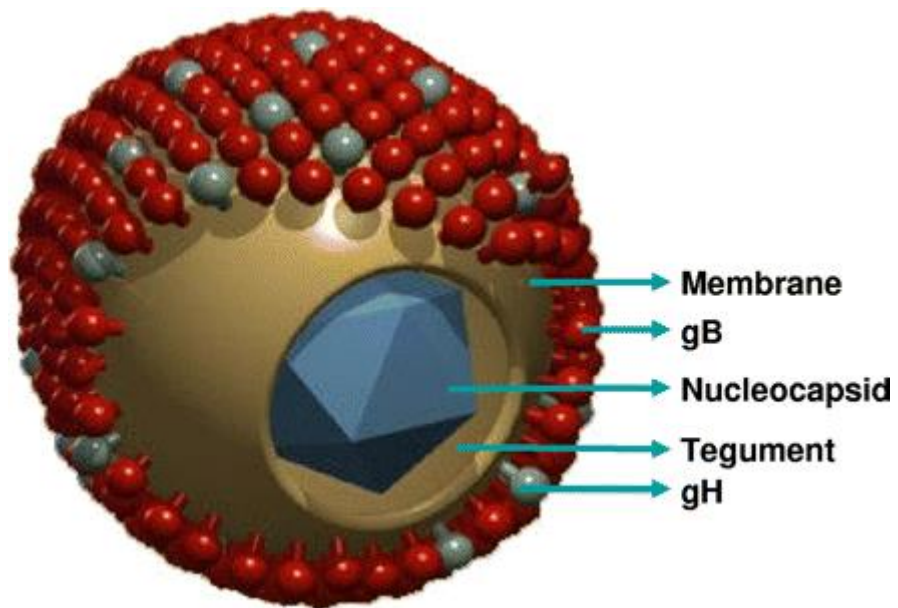


Fig A: Virtual three-dimensional model of HCMV showing various components of the virus (Tania and Rajiv., 2009).

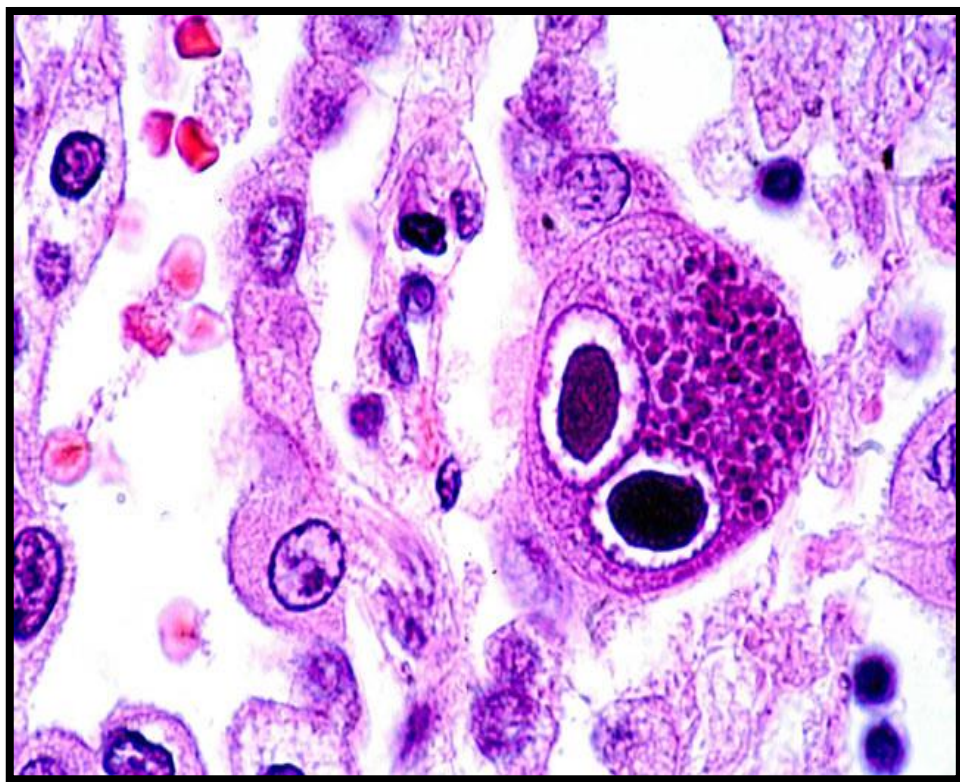


Fig B: CPE of the virus (owl's eye) (Brooks *et al.*, 2010).

2.1.4. Properties of the virus

Cytomegalovirus has the largest genetic content of the human herpesviruses, its DNA genome (240 kpb) is significantly larger than of herpes simplex virus. Only a few of the many proteins encoded by the virus (over 200) have been characterized. One, a cell surface glycoprotein, acts as Fc receptors that can non-specifically bind the Fc portion of the 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 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 strains 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 replicates 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 primarily cell-to-cell. It may take several weeks for an entire monolayer of cultured cells to become involved (Brooks *et al.*, 2010).

Cytomegalovirus produces a characteristic cytopathic effect. Perinuclear cytoplasmic inclusion form in addition to the intranuclear inclusion is typical of herpesviruses. Multinucleated cells are seen. Many affected cells become greatly enlarged. Inclusion-bearing cytomegalic cells can be found in samples from infected individuals (Brooks *et al.*, 2010).

2.1.5. Replication of the virus

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, nonstructural 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 exist the cell via tubules or vacuoles that communicate with the exterior (Levinson., 2010).

2.1.6. Transmission

HCMV can be transmitted via saliva, sexual contact, placental transfer, breast feeding, blood transfusion, solid organ transplantation (SOT) (Sia and Patel., 2000). Day care centers are a significant source of CMV infection (Nyholm and Schleiss., 2010).

2.1.7. Epidemiology

At least 60% of the US population has been exposed to CMV, with a prevalence of more than 90% in the high risk groups (e.g. homosexual males, diabetes, chronic disease, chemotherapy, newborns) (Akhter and Tood., 2011).

HCMV is highly species-specific, with human 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 developing countries. Overall, the seroprevalence of infection varies between 65% to 90% among middle age adults in the USA (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 transplant organs (Bowden., 1991).

2.1.8. Pathogenesis

The virus first infect the upper respiratory tract then local lymphocyte, circulating lymphocytes then spread the virus to other lymphocytes and monocytes in the spleen and lymph nodes. The virus finally spread to a variety of epithelial cells including those of the salivary glands, kidney tubules, testes and cervix. Infections are usually asymptomatic (sub-clinical) but glandular fever is sometimes seen in young adults. The virus can inhibit T cell responses. The virus elicits both humeral and cell

mediated immunity from the possibility of spread from cell to cell. If suppressed, the virus later reactivates, particular in cases of immunosuppressants. Indeed, infection by the virus can, it self, be immunosuppressive (Hunt., 2010).

2.1.8.1. Infection in immunocompetent hosts

There are many manifestations of CMV disease in humans. One of them 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).

2.1.8.2. Infection in immunocompromised patient

The other 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 (Moncarski *et al.*, 2007).

2.1.9. Laboratory diagnosis

The virus can be diagnosed by electron microscopy, cell culture, biopsy staining, serology and molecular methods (Steve and Daniel., 2002).

2.1.9.1. Collection and transportation of samples

Most useful specimens for isolation are throat washings, urine and blood. CMV can also be isolated from saliva, breast milk, cervical secretions and semen as well as various biopsy materials (Bhatia and Ichhpujani., 2008).

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

2.1.9.2. Direct detection

2.1.9.2.1. Microscopy

Microscopical examination of tissues and culture is useful in diagnosis of CMV infection, although it has limited utility especially in immunocompromised patients (Steve and Daniel., 2002).

2.1.9.2.2. Cell culture technique

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, the virus stays cell-associated (Brooks *et al.*, 2010). Immunofluorescence test or immunoperoxidase staining using specific antisera can be employed to confirm the results of CPE (Bhatia and Ichhupujani., 2008).

Cultural techniques are complicated, need sterile conditions with safety cabinet, special procedures to prepare the sample before inoculation, take long time, need identification method and also need electron microscopy to detect the presence of the virus by production of CPE (Timbury., 1997).

2.1.9.2.3. Histopathology technique

Autopsy and biopsy need histopathology techniques to prepare the sections, staining and immunofluorescent techniques to detect the virus. Sometimes CMV does not produce CPE in tissues so other confirmatory methods are needed (Timbury., 1997, Brooks *et al.*, 2010).

2.1.9.3. Antigen detection

Currently the gold standard quantitative assay is the antigenemia assay for the detection of the HCMV pp65 antigen in leukocytes. Flow cytometry has been applied to the antigenemia assay to make it automated and less subjective (Steve and Daniel., 2002).

2.1.9.4. Serological tests

Serological methods for the diagnosis of human cytomegalovirus (HCMV or CMV) infections are including detection of CMV IgM and IgG. IgG Avidity testing is now a useful serodiagnosis test to differentiate a new infection from a reactivation (Steve and Daniel., 2002). The enzyme-linked immunosorbent assay (or ELISA) is the most commonly available serologic test for measuring antibody to CMV (CDC., 2005).

2.1.9.5. Polymerase chain reaction (PCR)

This is diagnosed to detect replicating virus, not latent genomes. Blood and urine are most commonly tested, PCR assays can provide viral load data, which appears to be important in predicting CMV disease (Brooks *et al.*, 2010).

2.1.9.6. 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 vial are centrifuged at a low speed and placed in an incubator. After 24-48 hours, the tissue culture medium is removed and the cells are stained using a fluorescein-labeled anti CMV antibody. Alternatively, the cells are stained with an antibody against CMV, followed by a fluorescein-labeled anti-immune globulin.

This test has been found to be as sensitive as traditional tissue culture, probably because of these enhancement of infectivity provided by centrifugation (Jahan., 2010).

2.1.10. Treatment

Antiviral agents for CMV infections are available but serious side effects limit their use to life-or slight-threatening complications (Ogilvie., 2007).

Ganciclovir is an acyclic 2-deoxyguanosine analogue for the management of CMV. It is available in oral and parenteral formulations. Oral ganciclovir is poorly absorbed, with a bioavailability of only 5%. Management of active CMV disease is therefore with intravenous ganciclovir or its oral valyprodrug ganciclovir (Nichols and Boeckh., 2000).

Intravenous ganciclovir is used as first-line treatment of CMV disease in bone marrow and solid organ transplant recipients. Reversible bone marrow suppression is the most common adverse effect of ganciclovir (Eid *et al.*, 2008).

Foscarnet is approved for the treatment of CMV retinitis in patients with AIDS. It has been used to treat other CMV disease in immunocompromised patients, especially those unable to tolerate ganciclovir and those infected with ganciclovir-resistant virus (Razonable., 2011).

Cidofovir is a nucleoside analogue used for treatment of CMV other herpesviruses and other DNA viral infection. The major clinical indication for cidofovir is the treatment of CMV retinitis in patients with AIDS (Razonable., 2011).

2.1.11. Prevention

Prevention strategies are classified as primary, secondary and tertiary prevention. Primary prevention strategies try to avoid an infection and are mostly accomplished by precautions against exposition to the virus, i.e. hygiene measures and change of behavior. Secondary prevention strategies allow identifying infected patients at an early stage, with the aim of stopping progression of infection and disease. In the case of symptomatic disease, tertiary prevention strategies try to prevent the development of severe sequel after infection (Nigro and Jeon., 2009).

2.2. Diabetes mellitus

Diabetes mellitus (DM) is defined as a chronic disease that occurs when the pancreas is deficient of insulin or does not produce enough insulin, or alternatively when the body can not effectively use the produced insulin. Insulin is a hormone that regulates blood glucose concentration. Hyperglycaemia, or raised blood glucose levels, is a common effect of uncontrolled diabetes and which over time leads to serious damage to many systems of the body, especially the nerves, eyes, kidneys and blood vessels (WHO., 2006).

Diabetes mellitus is classified into four broad categories: type 1, type 2, gestational diabetes and other specific types (Lawrence *et al.*, 2008).

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. This type can be further classified

as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which beta cell loss is a T-cell-mediated autoimmune attack (Rother., 2007). Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed "juvenile diabetes" because a majority of these diabetes cases were in children (Dorner *et al.*, 1977).

Type 2 diabetes mellitus is characterized by insulin-resistance, which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 diabetes is the most common type. In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose by the liver (Lawrence *et al.*, 2008).

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2-5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. About 20-50% of affected women develop type 2 diabetes later in life (Lawrence *et al.*, 2008).

According to WHO classification; other specific types of diabetes include genetic defects of beta-cell function, genetic defects in insulin action, diseases of the exocrine pancreas, endocrinopathies, drug- or chemical-induced infections, uncommon forms of immune-mediated diabetes and other genetic syndromes sometimes associated with diabetes (WHO., 1999).

The major two clinical classes of DM are type 1 and type 2 diabetes; which had the same complications. For both type 1 and type 2 diabetes, clinical course and prognosis of the disease are linked to the glycaemic control and duration of diabetes (WHO., 1994). Macrovascular diseases caused by diabetes are the predominant causes of mortality and morbidity in diabetes (Tuomilehto and Rastenyte., 1997), including coronary heart disease (CHD), peripheral vascular disease (PVD) and cerebrovascular disease (Venkat *et al.*, 2000). Microvascular complications of diabetes consist of neuropathy, retinopathy and nephropathy. Limb amputations, infections and ulceration are common complications of diabetes that could be prevented through foot care programs. Acute metabolic complications include diabetic ketoacidosis, hypoglycemia and hyperosmolar non-ketotic coma (Amos *et al.*, 1997).

2.2.1. Type 2 diabetes mellitus in Sudan

Until recently diabetes had been considered rare in African countries, probably due to the lack of awareness of diabetes by doctors and compounded by the lack of diagnostic facilities, but in the urban population of Sudan, chronic non-communicable

diseases such as diabetes mellitus and hypertension are now emerging as important health problems (McLarty *et al.*, 1990).

Certain areas of the world have been mapped as type 2 DM red zone including Sudan (Rubeaan., 2010). Type 1 diabetes is rarer in African countries than type 2 (Gill., 2000). The actual number of people with diabetes in Sudan is not known. A small population based study showed that the prevalence of type 2 DM has increased from 8.6% in Khartoum State in 1996, to as high as 19% in a recent house-hold survey (Elbagir *et al.*, 1996).

Recent estimates place the diabetes population at around one million-around 95% of whom have type 2 diabetes. Currently, diabetes in Sudan (and most of Africa) is believed to have one of the highest mortality rates for a non-infectious disease. One study indicated that 10% of adult patient deaths in hospitals were caused by diabetes. Consequently, diabetes is now one of the major health problems in Sudan (Ahmed and Ahmed., 2000).

Type 2 diabetes is associated with an overall age-adjusted mortality that is about twice that of the non-diabetic population and the life expectancy is reduced by 5-10 years (Mandrup., 1998). The prevalence of type 2 DM in the adult population ranges from 0% in Togo to 10.4% in Northern Sudan (Motala *et al.*, 2003).

2.2.2. Role of CMV in type 2 diabetes mellitus

The link between cytomegalovirus and Type 2 diabetes isn't clear-cut (Sarah., 2012). The researchers speculated that cytomegalovirus may predispose people to diabetes by harming cells of the pancreas. The pancreas produces insulin, a hormone

that is critical for getting sugar (glucose) into cells. Type 2 diabetes develops when the body becomes desensitized to insulin, and the pancreas cannot produce enough insulin to compensate. It's also possible that Type 2 diabetes impairs the immune system, and as a result, makes individuals vulnerable to cytomegalovirus infection. It may be that cytomegalovirus infection increases diabetes risk only after years of infection, which could explain why earlier studies in younger adults did not find a link (Rachael., 2012).

Consistent with the possibility of the virus that could influence the pancreatic cells directly; is the report that CMV may infect and reside in pancreatic cells without causing cytopathic effects but nonetheless influencing insulin production directly after repeated reactivations (Lohr and Oldstone., 1990). Additionally, infection of human pancreatic β -cells with CMV induced the release of pro-inflammatory cytokines and increased cellular immunogenicity (Smelt *et al.*, 2012). The indirect effects of CMV could be exerted via infected monocyte production of IL- 1β which induces TNF- α production in human pancreatic duct cells, driving cells into apoptosis and thus compromising β -cell function (Movahedi *et al.*, 2004). Other components of the immune system, influenced by prolonged CMV infection, could hypothetically also contribute to a more pro-inflammatory environment, which is an important feature of type 2 diabetes (Donath and Shoelson., 2011). CMV seropositivity is associated with accumulations of potentially senescent late- differentiated T-cells and elevated numbers of CD4+ and CD8+ effector cells

(Derhovanessian *et al.*, 2011) which are more likely to produce pro-inflammatory cytokines (Almanzar *et al.*, 2005).

2.2.3. Diagnostic criteria for type 2 diabetes mellitus

Diabetes can be diagnosed on any of the following World Health Organization (WHO) criteria:

Fasting plasma glucose (FPG) ≥ 7.0 mmol/l (126 mg/dl), or 75 g oral glucose tolerance test (OGTT) with FPG ≥ 7.0 mmol/l (126 mg/dl) and/or 2 hour plasma glucose ≥ 11.1 mmol/l (200 mg/dl), or glycatedhaemoglobin (HbA1c) $\geq 6.5\%$ /48 mmol/mol, or random plasma glucose ≥ 11.1 mmol/l (200 mg/dl) in the presence of classical diabetes symptoms. Asymptomatic individuals with a single abnormal test should have the test repeated to confirm the diagnosis unless the result is unequivocally elevated (WHO., 2003).

3. Materials and Methods

3.1. Ethical consideration

Permission to perform the study was taken from the College of Graduate Studies, Sudan University of Science and Technology. All subjects participate were informed before blood collected.

3.2. Study design

This study was a descriptive, prospective and cross-sectional study.

3.3. Study population

Type 2 diabetic already diagnosed patients of both genders males and females, with age ranged from (45-83 years).

3.4. Study area

The blood samples were collected from subjects with type 2 diabetes mellitus which were admitted to Zinam Specialist Hospital. The experimental work was carried out in Research Laboratory, Sudan University of Science and Technology, Khartoum, Sudan.

3.5. Study duration

The study was carried out during the period from February to April, 2015.

3.6. Sample size

Eighty seven venous blood samples (n=87) were collected from subjects with type 2 diabetes mellitus.

3.7. Sample technique

Randomized, non-probability convenience sampling technique during admission to these hospital was used in the study.

3.8. Data collection

Demographic data was collected by direct interviewed questionnaire (appendix 1).

3.9. Sample collection

Venous blood samples (5 ml) was collected from each subject after disinfection by 70% alcohol. Blood was collected in plain tubes, allowed to clot at room temperature and serum was separated by centrifugation at 3000 rpm/3 minutes. The serum was kept at -20⁰ C until tested.

3.10. Sample processing

All serum samples were tested for the presence of CMV IgG antibodies using commercially available ELISA kits (IgG).

3.11. Laboratory diagnosis of CMV

3.11.1. Detection of anti-CMV IgG antibodies using ELISA

Determination of IgG class antibodies to CMV in serum was performed using IgG ELISA kits (Foresight, ACON Laboratories, Inc.USA).

3.11.1.1. Principle

The microwell plate is coated with CMV antigens. During testing, the specimen diluent and the specimens are added to the antigen coated microwell plate and then incubated. If the specimens contain IgG antibodies to CMV, it will bind to the antigens coated in the microwell plate to form immobilized antigen-CMV IgG antibody complexes. If the specimens do not contain IgG antibodies to CMV, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. The enzyme-conjugated anti-human IgG antibodies are added to the microwell plate and then incubated. The enzyme-conjugated anti-human IgG antibodies will bind to the immobilized antigen-CMV IgG antibody complexes present. After the second incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of CMV IgG antibodies present in the specimens. Sulphuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of CMV IgG antibodies present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 nm.

3.11.1.2. Procedure

All materials were brought to room temperature before beginning the procedure. In brief, working wash buffer was prepared by diluting the concentrated wash buffer 1:25 in a graduated cylinder. A1 was leaved as blank well, 100µL of calibrator 1 the

negative control was added to B1 and C1, 100µL of calibrator 2 the cut-off calibrator was added to D1 and E1, 100µL of calibrator 3 the positive control was added F1 and G1, 100µL of calibrator 4 the positive control also was added to H1 and A2. 100µL of specimen diluent's was added to assigned wells starting at B2 (sample wells), 5µL of specimen starting at B2 (sample wells) was added, on a flat bench microwell plate was mixed gently by swirling for 30 seconds. The microwell plate was covered by the plate sealer and incubated at 37C° for 30 minutes; the wells was washed 5 times by working wash buffer to remove residual serum, 100µL of conjugate was added to each well except for the blank well. Microwell plate was covered by the plate sealer and incubated at 37C° for 30 minutes, then was washed by working wash buffer for 5 times. 50µL of substrate A (H₂O₂) and 50µL of substrate B (TMB) was added to each well, then was mixed gently, covered by the plate sealer and incubated at 37C° for 10 minutes. 50µL of stop solution (sulphuric acid) was added to each well. The optical density (O.D) in a microwell plate ELISA reader was read within 30 minutes at 450 nm.

3.11.1.3. Measurement

ELISA reader micro plate was adjusted at zero using the substrate blank in the first well, and the absorbance of all wells measured by UV light at 450 nm.

3.11.1.4. Calculation of control values and cut-off

Mean absorbance value (MNC) of Calibrator 1 the negative control, mean absorbance value (COC) of Calibrator 2 the cut-off calibrator, mean absorbance value (MPC) of Calibrator 3 the positive control, and mean absorbance value (MPC) of Calibrator 4 the positive control also were calculated.

The result run was validated according to the manufacture's criteria for validity as below:

- 1- Absorbance of substrate blank at 450 nm < 0.100
- 2-Mean absorbance (MNC) of calibrator 1 after subtraction of blank absorbance < 0.150
- 3-Mean absorbance (COC) of calibrator 2 after subtraction of blank absorbance > 0.150 and < 0.400
- 4- Mean absorbance (MPC) of calibrator 3 after subtraction of blank absorbance > calibrator 2 and < calibrator 4
- 5- Mean absorbance (MPC) of calibrator 4 after subtraction of blank absorbance > 1.200

The index value was calculated to obtain qualitative specimen results as following:

- 1-Cut-off value was obtained by this equation:

Cut-off value = mean absorbance (COC) of calibrator 2– blank absorbance.

- 2-The index value was calculated by dividing the specimen absorbance by the cut-off value.

3.11.1.5. Interpretation of the result

To determine the presence or absence of CMV IgG, the index value was obtained to each specimen, then read the result by referring to the interpretation of results below:

1-Negative samples < 0.9

2-Positive samples > 1.1

3-Equivocal ≥ 0.9 and ≤ 1.1

3.12. Statistical analysis

The data obtained were analyzed and presented using statistical package for social science (SPSS) version 16.0 computer software version. Significance of differences was determined using Chi-square test.

4. Results

Out of 87 subjects with type 2 diabetes mellitus tested, 84 subjects (96.6%) were CMV IgG positive, while 3 subjects (3.4%) were negative (Table 4.1) and (Figure C).

Table (4.1): Frequency of Cytomegalovirus IgG among subjects with type 2 diabetes mellitus:

Subjects with type 2 diabetes mellitus	Frequency	Percent %
Anti-CMV IgG positive	84	96.6%
Anti-CMV IgG negative	3	3.4%
Total	87	100%

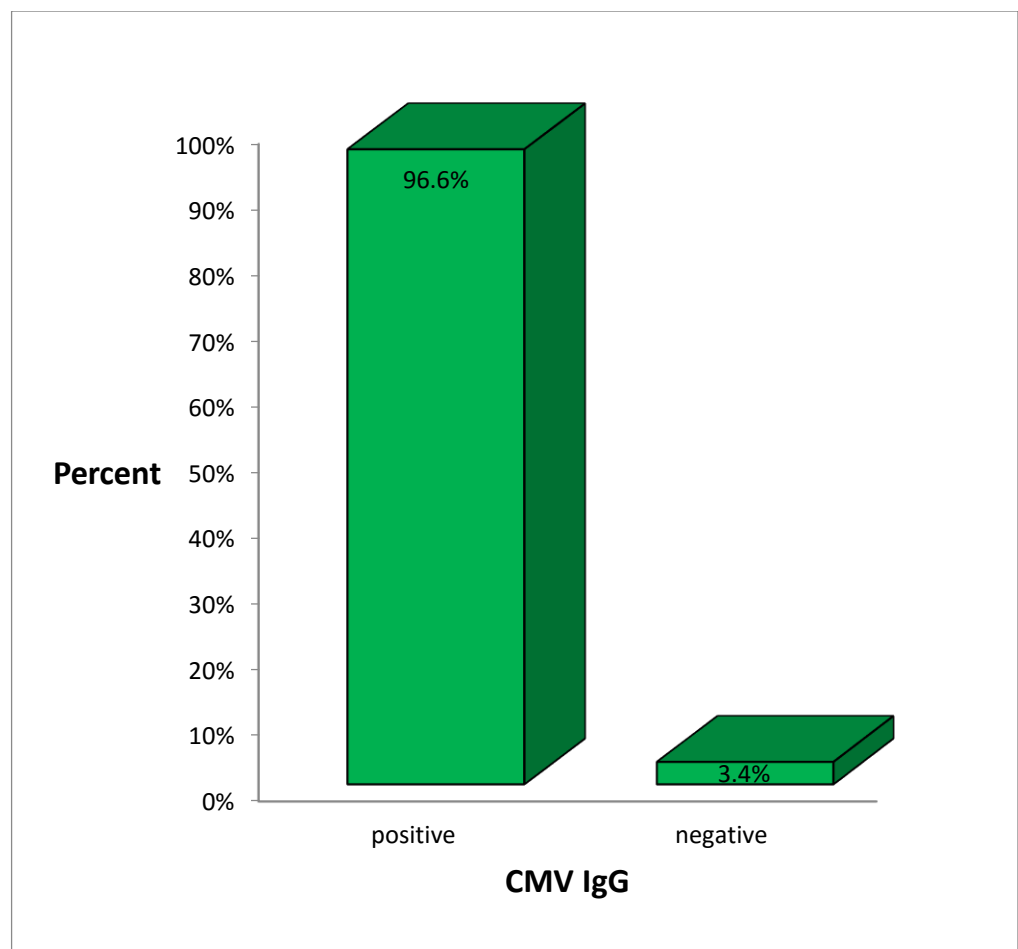


Fig (C): Frequency of Cytomegalovirus IgG among subjects with type 2 diabetes mellitus.

4.2. The relation of seropositivity of CMV IgG with age among subjects with type 2 diabetes mellitus:

The mean age of subjects was 59.18 years. Out of 87 subjects with type 2 diabetes mellitus, 42 subjects in age group 45-57 years with 39/42 (92.9%) positive case for CMV IgG, and 3/42 (7.1%) negative case found in the same group. There are 36 subjects in age group 58-70 years, all of them were positive for CMV IgG 36/36 (100%). 9 subjects in age group 71-83 years and all of them also were positive for CMV IgG 9/9 (100%) (Table 4.2) and (Figure D).

Table (4.2): The relation of seropositivity of CMV IgG with age among subjects with type 2 diabetes mellitus:

Age/year	Subjects with type 2 diabetes mellitus	+ve IgG to CMV	-ve IgG to CMV
45-57	42/87 (48.3%)	39/42 (92.9%)	3/42 (7.1%)
58-70	36/87 (41.4%)	36/36 (100%)	0
71-83	9/87 (10.3%)	9/9 (100%)	0
Total	87/87 (100%)	84/87 (96.6%)	3/87 (3.4%)

($P = 0.189$)

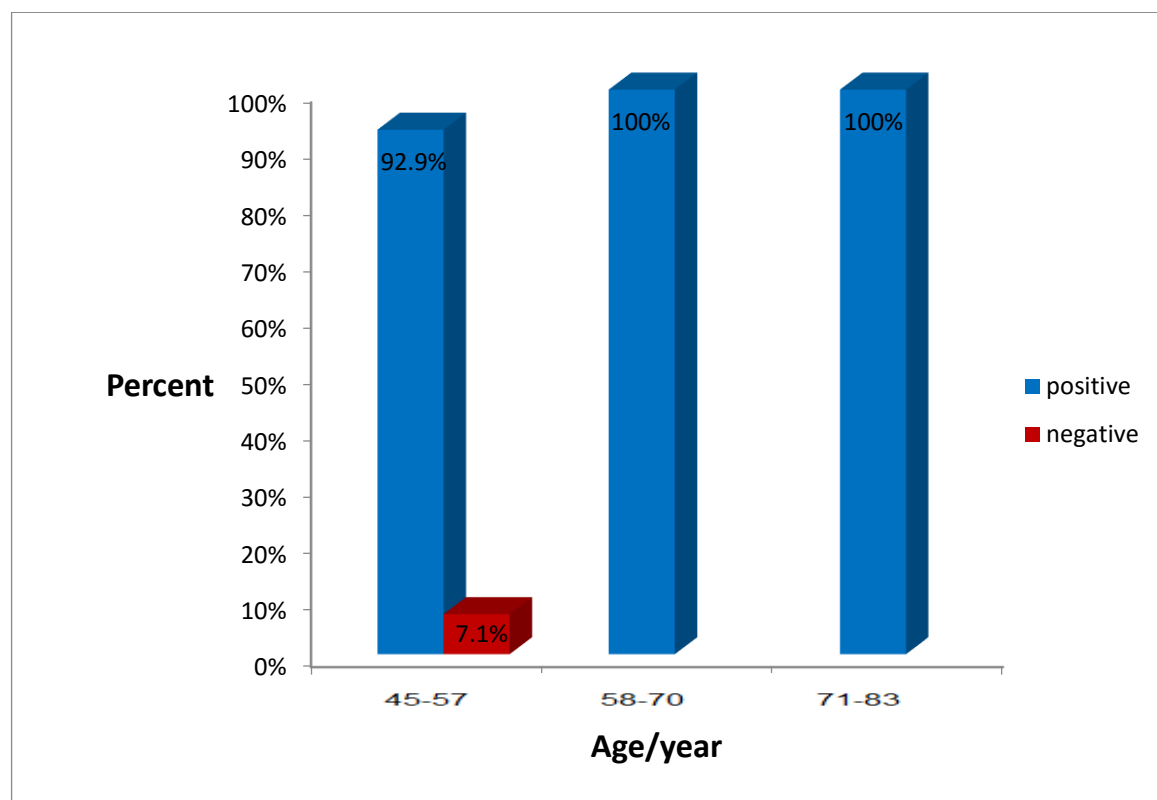


Fig (D): The relation of seropositivity of CMV IgG with age among subjects with type 2 diabetes mellitus.

4.3. The relation of seropositivity of CMV IgG with gender among subjects with type 2 diabetes mellitus:

Out of 87 subjects with type 2 diabetes mellitus, 52 of them were males with 51/52 (98.1%) positive case for CMV IgG, and 35 were females with 33/35 (94.3%) positive case for CMV IgG. The highest seropositivity was found in males group (Table 4.3) and (Figure E).

Table (4.3): The relation of seropositivity of CMV IgG with gender among subjects with type 2 diabetes mellitus:

Gender	Subjects with type 2 diabetes mellitus	+ve IgG to CMV	-ve IgG to CMV
Male	52/87 (59.8%)	51/52 (98.1%)	1/52 (1.9%)
Female	35/87 (40.2%)	33/35 (94.3%)	2/35 (5.7%)
Total	87/87 (100%)	84/87 (96.6%)	3/87 (3.4%)

($P = 0.342$)

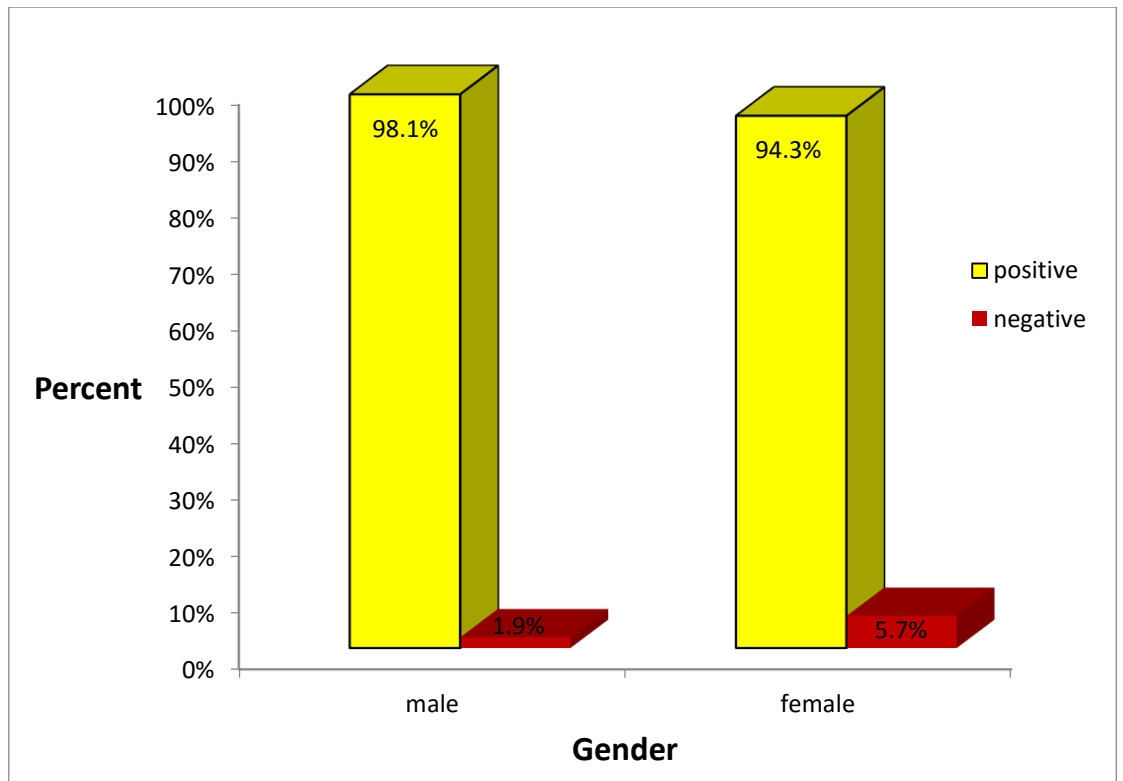


Fig (E): The relation of seropositivity of CMV IgG with gender among subjects with type 2 diabetes mellitus.

4.4. The role of family history of diabetes mellitus and history of blood transfusion on CMV IgG seropositivity among subjects with type 2 diabetes mellitus:

There were 6 subjects with family history of DM (first relative degree), and also 6 subjects with history of blood transfusion, 5/6 (83.3%) of them were positive for CMV IgG and only one case was negative for CMV IgG (Table 4.4) and (Figure F). No significant association between family history of DM, history of blood transfusion and CMV IgG seropositivity ($P > 0.05$).

Table 4.4. The role of family history of diabetes mellitus and history of blood transfusion on CMV IgG seropositivity among subjects with type 2 diabetes mellitus:

Medical history	Subjects with type 2 diabetes mellitus	+ve IgG to CMV	-ve IgG to CMV
Family history of diabetes mellitus	6/87 (6.9%)	5/6 (83.3%)	1/6 (16.7%)
History of blood transfusion	6/87 (6.9%)	5/6 (83.3%)	1/6 (16.7%)
Both	12/87 (13.8%)	10/87 (11.5%)	2/87 (2.3%)

($P = 0.066$)

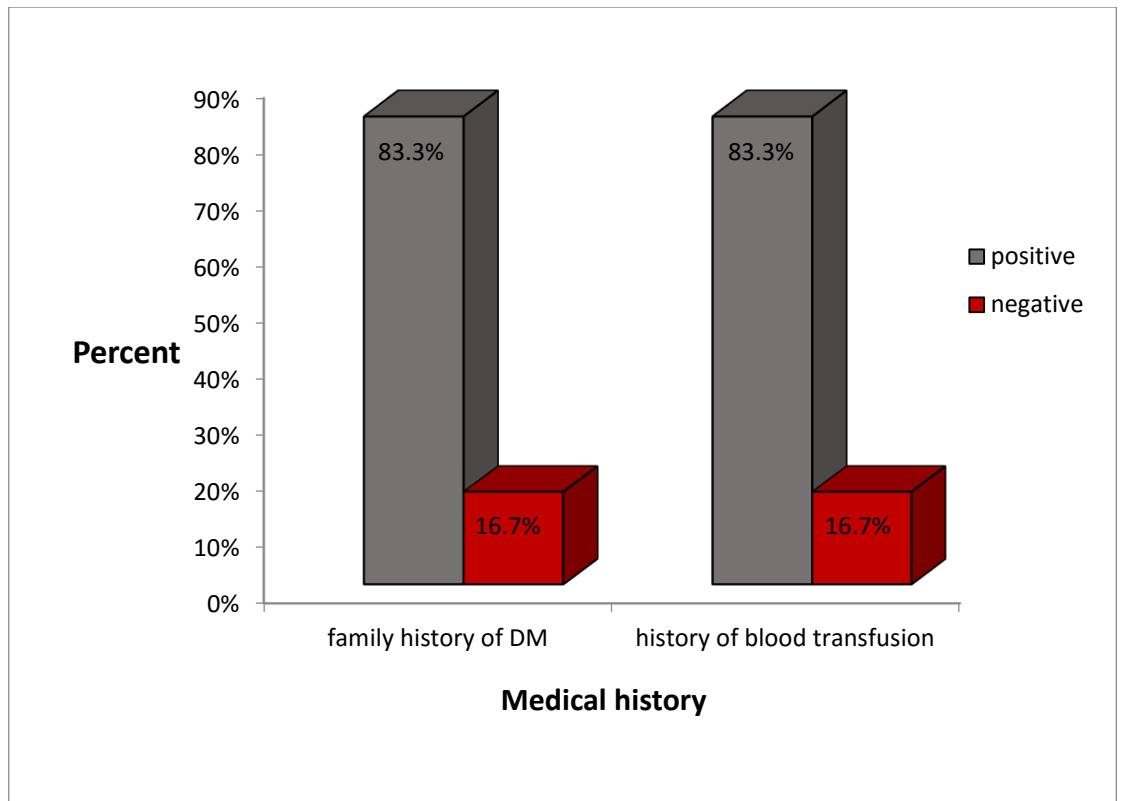


Fig (F): The role of family history of diabetes mellitus and history of blood transfusion on CMV IgG seropositivity among subjects with type 2 diabetes mellitus.

4.5. The relation of seropositivity of CMV IgG with level of education among subjects with type 2 diabetes mellitus:

Out of 87 subjects with type 2 diabetes mellitus, 14 subjects had high education level (attending a university), 13/14 (92.9%) of them were positive for CMV IgG. About 23 subjects with intermediate education level (attending high school), 21/23 (91.3%) of them were positive for CMV IgG.

The highest percentage of positivity 50/50 (100%), were found in subjects with low education level (attending primary school or never go to school), which were 50 subjects and all of them positive for CMV IgG (Table 4.5) and (Figure G).

Table (4.5): The relation of seropositivity of CMV IgG with level of education among subjects with type 2 diabetes mellitus:

Education level	Subjects with type 2 diabetes mellitus	+ve IgG to CMV	-ve IgG to CMV
High	14/87 (16.1%)	13/14 (92.9%)	1/14 (7.1%)
Intermediate	23/87 (26.4%)	21/23 (91.3%)	2/23 (8.7%)
Low	50/87 (57.5%)	50/50 (100%)	0
Total	87/87 (100%)	84/87 (96.6%)	3/87 (3.4%)

($P = 0.119$)

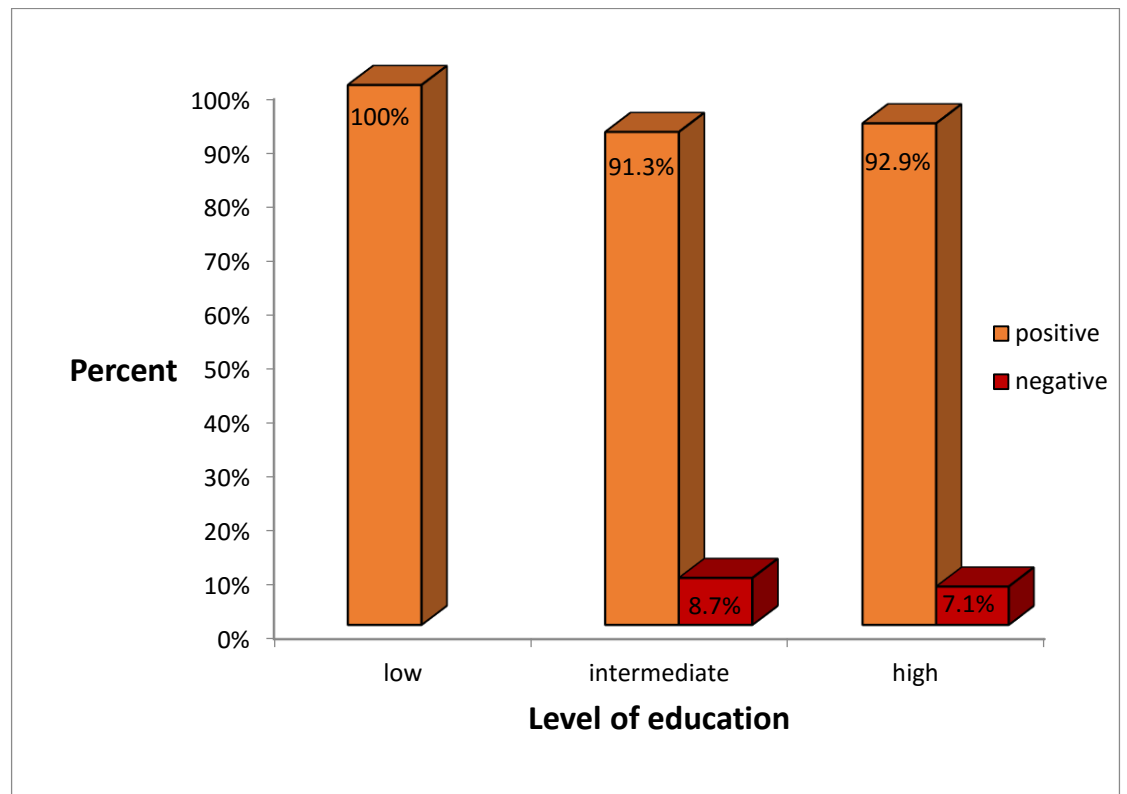


Fig (G): The relation of seropositivity of CMV IgG with level of education among subjects with type 2 diabetes mellitus.

5. Discussion

5.1. Discussion

The positive cases for CMV IgG were detected among type 2 diabetic patients using ELISA, and the results are nearly in accordance with that conducted by Roberts and Cech (2005), and Rachael (2012) but disagree with Norra (2012).

The detection of CMV indicated that the type 2 diabetic patients 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 type 2 diabetic patients have not been infected with CMV.

However, according to Lohr and Oldstone (1990), HCMV was demonstrated in the pancreas of about half the patients with type 2 diabetes mellitus, this indicated that HCMV infection in the pancreas is associated with type 2 diabetes.

This study showed no significant association between age, sex, family history of diabetes, history of blood transfusion or level of education and CMV IgG antibodies ($P > 0.05$).

The differences in results may be due to smaller sample size, shorter period of study, or presence of other etiologic causes of type 2 diabetes mellitus like lack of physical activity and obesity, which need to be confirmed by other studies with larger sample size, longer period of study and use of other modern, specific and sensitive methods of diagnosis.

Locally, there is no study on CMV with type 2 diabetes mellitus. There is only a case control study conducted on the pathogenesis of CMV on type 1 diabetes mellitus (Eltayib *et al.*, 2014), who used sera of 81 children, 27 (33.3%) with diabetes which represent the study group and 54 (66.7%) without diabetes which represent the control group. The samples were tested for IgG anti-cytomegalovirus using (ELISA) technique. 18 (22.2%) of the total population of study were positive, 55.6% were diabetic patients, the results indicated significant association of CMV IgG antibodies with type 1 diabetes mellitus in children.

5.2. Conclusion

This study concluded that CMV IgG antibodies was highly detected in subjects with type 2 diabetes mellitus (96.6%), and the study revealed that there was no significant association between age, sex, family history of diabetes, history of blood transfusion or level of education and CMV IgG antibodies ($P > 0.05$).

5.3. Recommendations

1. Minimize the exposing of immunosuppressed patients to outside sources of CMV like saliva, blood products or transplant organ that containing the virus.
2. All people including diabetic patients should be educated concerning CMV, its transmission, and hygienic practices, such as hand washing, which minimize the risk of infection.

3. Routine laboratory testing for CMV antibody in people under risk of CMV infection is not recommended, but can be performed to determine their immune status.

4. More investigation is needed to explain the results obtained by various authors in different geographical locations using larger sample size, and further to study the role of CMV in diabetes mellitus.

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Appendix (1)

Sudan University of Science and Technology

College of Graduate Studies

Medical Laboratory Science

Department of Microbiology

Questionnaire

Seroprevalence of Cytomegalovirus IgG Abs among type 2 diabetic patients.

1. Sample No

2. Age groups.....

() 45-57

() 58-70

() 71-83 years

3. Gender.....

() male

() female

4. Family history of diabetes mellitus.....

() Yes

() No

5. History of blood transfusion.....

() Yes

() No

6. Education level.....

() Low

() Intermediate

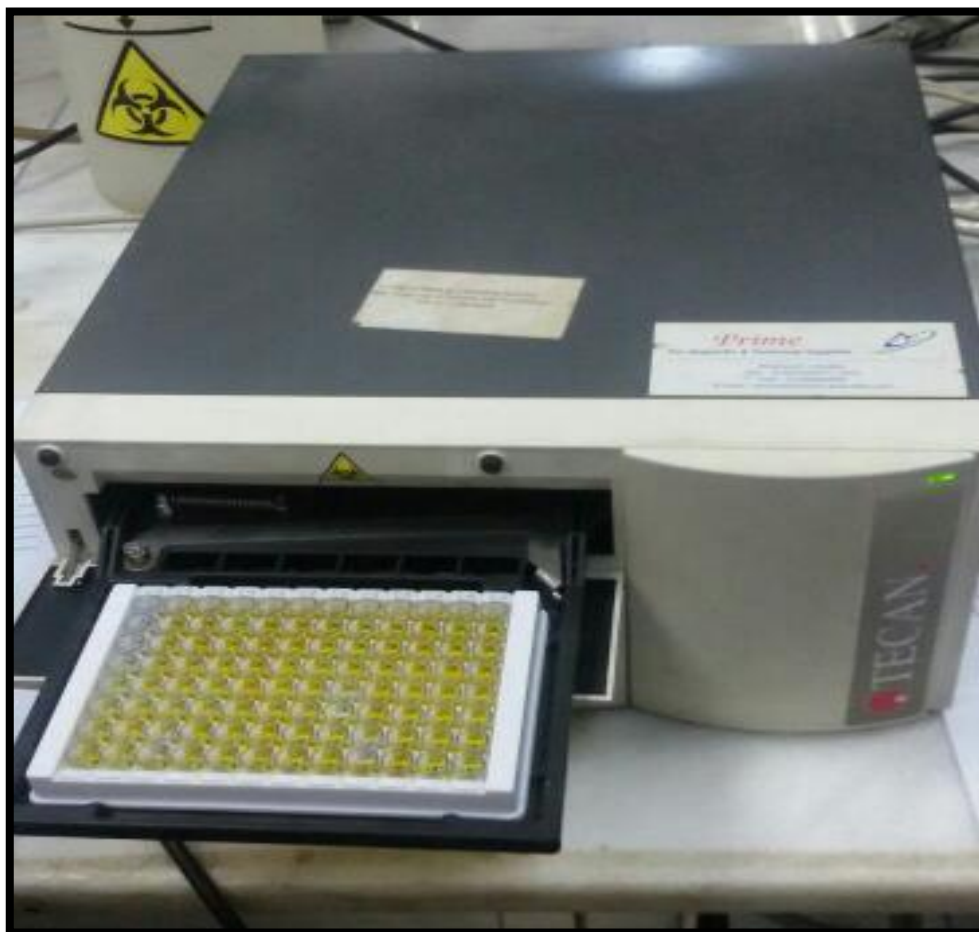
() High

Appendix (2)



ELISA microplate

Appendix (3)



ELISA reader

Appendix (4)

Foresight®

CMV IgG EIA Test Kit Package Insert

REF I231-1131**English**

*An enzyme immunoassay (EIA) for the qualitative and quantitative detection of IgG antibodies to Cytomegalovirus (CMV) in human serum or plasma.
For professional in vitro diagnostic use only.*

INTENDED USE

The CMV IgG EIA Test Kit is an enzyme immunoassay for the qualitative and quantitative detection of IgG antibodies to CMV in human serum or plasma. It is intended as an aid in the diagnosis of possible CMV infection.

SUMMARY

Cytomegalovirus (CMV) is a member of the Herpes virus family which includes Herpes Simplex virus (HSV) type 1 and 2, Varicella Zoster virus (VZV) and Epstein-Barr virus (EBV). It is a ubiquitous human pathogen transmitted through saliva, sexual contact, perinatally, organ transplantation or blood transfusion.

In majority of the cases, the infection remains asymptomatic. However, CMV infection can cause serious illness in newborns and immunosuppressed individuals such as patients with AIDS, HIV, cancer or patients that received organ transplants.¹ During immunosuppressive therapy, a reactivation of the latent virus or primary infection occurs frequently. For most newborns, CMV infections can be acquired before birth, during birth and later in life. The infection may cause severe congenital abnormalities such as microcephaly, motor disability, and mental retardation.^{2, 3, 4} Therefore, determining primary maternal infections and distinguishing primary from latent infection is of great importance. The presence of IgM antibodies indicates the presence of primary infection, while presence of IgG antibodies indicates immune status of patients.

The CMV IgG EIA Test Kit is an immunoassay for the qualitative and quantitative detection of the presence of IgG antibodies to CMV in serum or plasma specimen. The test utilizes purified CMV antigens to selectively detect antibodies to CMV in serum or plasma.

PRINCIPLE

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

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not mix reagents from other kits with different lot numbers.
- Avoid cross contamination between reagents to ensure valid test results.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipet tip for each specimen assayed.
- Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Do not allow wells to dry out during the assay procedure.
- Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with fingertips.
- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.

HEALTH AND SAFETY INFORMATION

- Some components of this kit contain human blood derivatives which were found to be non-reactive for the HIV-1/HIV-2/HIV-O, Syphilis and HCV antibodies, as well as HBsAg. But No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection

- while handling kit reagents and specimens. Wash hands thoroughly when finished.
- ProClin™ 300 is included as a preservative in the Conjugate, Concentrated Wash Buffer, Specimen Diluent, Substrate and Calibrators. Avoid any contact with skin or eyes.
 - Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not mouth pipette.
 - Avoid any contact of the Substrate A, Substrate B, and Stop Solution with skin or mucosa. The Stop Solution contains 0.5M sulfuric acid which is a strong acid. If spills occur, wipe immediately with large amounts of water. If the acid contacts the skin or eyes, flush with large amounts of water and seek medical attention.
 - Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
 - Handle and dispose all specimens and materials used to perform the test as if they contained infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.
 - Observe Good Laboratory Practices when handling chemicals and potentially infectious material. Discard all contaminated material, specimens and reagents of human origin after proper decontamination and by following local, state and federal regulations.
 - Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

STORAGE AND STABILITY

- Unopened test kits should be stored at 2-8°C upon receipt. All unopened reagents are stable through the expiration date printed on the box if stored between 2-8°C. Once opened, all reagents are stable for up to 3 months after the first opening date if stored between 2-8°C. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch and remove the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch with desiccant supplied at 2-8°C and can be used within 3 months of the opening date. Return the remaining unused strips and supplied desiccant to the original resealable pouch, firmly press the seal closure to seal the pouch completely and immediately store at 2-8°C.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
- Do not store Stop Solution in a shallow dish or return it to the original bottle after use.

SPECIMEN COLLECTION AND PREPARATION

- The CMV IgG EIA Test Kit can be performed using only human serum or plasma collected from venipuncture whole blood.
- EDTA, sodium heparin, and ACD collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxidase and may lead to erroneous results.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not use specimens with fibrin particles or contaminated with microbial growth.
- Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

REAGENTS AND COMPONENTS

A human serum sample demonstrating high levels of anti-CMV IgG activity was defined as containing 150 units of CMV IgG antibody per mL (U/mL). The calibrators for the CMV IgG EIA assay are manufactured by dilution and are referenced to this standard.

Materials Provided

No.	Reagent	Component Description	Quantity		
			96 wells/kit	480 wells/kit	48 wells/kit
	CMV IgG Microwell Plate	Microwell plate coated with purified CMV antigens	1 plate (96 wells/plate)	5 plates (96 wells/plate)	1 plate (48 wells/plate)
1	CMV IgG Conjugate	Anti-human IgG antibody bound to peroxidase; Preservative: 0.1% ProClin™ 300	1 x 12 mL	5 x 12 mL	1 x 6 mL
2	Concentrated Wash Buffer (25x)	Tris-HCl buffer containing 0.1% Tween 20; Preservative: 0.1% ProClin™ 300	1 x 50 mL	5 x 50 mL	1 x 25 mL

2A	Specimen Diluent	Tris buffer; Preservative: 0.1% ProClin™ 300	1 x 12 mL	5 x 12 mL	1 x 6 mL
3	Substrate A	Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL	1 x 4 mL
4	Substrate B	Buffer containing tetramethylbenzidine (TMB); Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL	1 x 4 mL
5	Stop Solution	0.5M Sulfuric acid	1 x 8 mL	5 x 8 mL	1 x 4 mL
6	CMV IgG Calibrator 1	Diluted human serum non- reactive for CMV IgG antibodies; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL	1 x 0.5 mL
7	CMV IgG Calibrator 2	Diluted human serum containing 15 U/mL CMV IgG antibodies; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL	1 x 0.5 mL
8	CMV IgG Calibrator 3	Diluted human serum containing 60 U/mL CMV IgG antibodies; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL	1 x 0.5 mL
9	CMV IgG Calibrator 4	Diluted human serum containing 150 U/mL CMV IgG antibodies; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL	1 x 0.5 mL
	Plate Sealer		3	15	3
	Package Insert		1	1	1

Materials Required But Not Provided

- Freshly distilled or deionized water
- Sodium hypochlorite solution for decontamination
- Absorbent paper or paper towel
- Water bath or incubator capable of maintaining 37°C ± 2°C
- Calibrated automatic or manual microwell plate washer capable of aspirating and dispensing 350 µL/well
- Disposable gloves
- Calibrated micropipettes with disposable tips capable of dispensing 5, 50 and 100 µL
- Graduated cylinders for wash buffer dilution
- Vortex mixer for specimen mixing (optional)
- Timer
- Disposable reagent reservoirs
- Calibrated microplate reader capable of reading at 450 nm with a 630-700 nm reference filter, or reading at 450 nm without a reference filter
- Automated processor (optional)

DIRECTIONS FOR USE

Allow reagents and specimens to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange the calibrators so that well A1 is the Blank well. From well A1, arrange the calibrators in a horizontal or vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.

Step	Detailed Procedure	Simplified Procedure
	<ul style="list-style-type: none"> Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the contents of the bottle containing the concentrated wash buffer in a graduated cylinder and fill it with freshly distilled or deionized water to 1250 mL for 96 wells/plate testing, or 625 mL for 48 wells/plate testing. The Working Wash Buffer is stable for 2 weeks at 15-30°C. NOTE: If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C until all crystals dissolve. 	<ul style="list-style-type: none"> Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25
0	<ul style="list-style-type: none"> Leave A1 as Blank well. 	<ul style="list-style-type: none"> Leave A1 as Blank well
1	<ul style="list-style-type: none"> Add 100 µL of Calibrator 1 in wells B1 and C1. (Yellow Reagent) Add 100 µL of Calibrator 2 in wells D1 and E1. (Blue Reagent) Add 100 µL of Calibrator 3 in wells F1 and G1. (Blue Reagent) Add 100 µL of Calibrator 4 in wells H1 and A2. (Blue Reagent) 	<ul style="list-style-type: none"> B1 and C1: Add 100 µL Calibrator 1 D1 and E1: Add 100 µL Calibrator 2 F1 and G1: Add 100 µL Calibrator 3 H1 and A2: Add 100 µL Calibrator 4
2	<ul style="list-style-type: none"> Add 100 µL of Specimen Diluent to assigned wells starting at B2. (Green Reagent) Add 5 µL of specimen to assigned wells starting at B2. Then a color change from green to blue will occur to verify that the specimen has been added. Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C. 	<ul style="list-style-type: none"> Starting B2: Add 100 µL Specimen Diluent Starting B2: Add 5 µL specimen Remove and store unused strips at 2-8°C

3	<ul style="list-style-type: none"> Mix gently by swirling the microwell plate on a flat bench for 30 seconds. Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes. 	<ul style="list-style-type: none"> Mix gently Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 min
4	<ul style="list-style-type: none"> Remove the Plate Sealer. Wash each well 5 times with 350 µL of Working Wash Buffer per well, then remove the liquid. Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried. <p>NOTE: Improper washing may cause false positive results.</p>	<ul style="list-style-type: none"> Remove the Plate Sealer Wash each well 5 times with 350 µL of Working Wash Buffer Turn the microwell plate upside down on absorbent tissue
5	<ul style="list-style-type: none"> Add 100 µL of Conjugate to each well except for the Blank well. (Red Reagent) 	<ul style="list-style-type: none"> Add 100 µL of Conjugate to each well except for the Blank well
6	<ul style="list-style-type: none"> Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes. 	<ul style="list-style-type: none"> Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 min
7	<ul style="list-style-type: none"> Repeat Step 4. 	<ul style="list-style-type: none"> Repeat Step 4
8	<ul style="list-style-type: none"> Add 50 µL of Substrate A to each well. (Clear Reagent) Add 50 µL of Substrate B to each well. (Clear Reagent) <p>Then a blue color should develop in wells containing Positive specimens.</p>	<ul style="list-style-type: none"> Add 50 µL of Substrate A to each well Add 50 µL of Substrate B to each well
9	<ul style="list-style-type: none"> Mix gently then cover microwell plate with Plate Sealer and incubate in a water bath or incubator at 37°C ± 2°C for 10 minutes ± 1 minute. 	<ul style="list-style-type: none"> Mix then cover microwell plate with Plate Sealer and incubate at 37°C for 10 min
10	<ul style="list-style-type: none"> Remove the Plate Sealer. Add 50 µL of Stop Solution to each well. (Clear Reagent) <p>Then a yellow color should develop in wells containing Positive specimens.</p>	<ul style="list-style-type: none"> Remove the Plate Sealer Add 50 µL of Stop Solution to each well
11	<ul style="list-style-type: none"> Read at 450/630-700 nm in 30 minutes. <p>NOTE: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450/630-700 nm for better results.</p>	<ul style="list-style-type: none"> Read at 450/630-700 nm in 30 min

AUTOMATED PROCESSING

Automatic EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens. Incubation times may vary depending on the processors used but do not program less incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

VALIDATION REQUIREMENTS AND QUALITY CONTROL

1. Calculate the Mean Absorbance of Calibrators 1-4 by referring to the table below.

Example of Calibrator 2 Calculation

Item	Absorbance
Calibrator 2: Well D1	0.268
Calibrator 2: Well E1	0.254
Total Absorbance of Calibrator 2	0.268 + 0.254 = 0.522
Mean Absorbance of Calibrator 2	0.522/2 = 0.261

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

Item	Validation Requirements
Blank Well	Blank Absorbance should be < 0.050 if read at 450/630-700 nm NOTE: It should be < 0.100 if read at 450 nm
Calibrator 1	Mean Absorbance after subtraction of Blank Absorbance should be < 0.150
Calibrator 2	Mean Absorbance after subtraction of Blank Absorbance should be > 0.150 and < 0.400
Calibrator 3	Mean Absorbance after subtraction of Blank Absorbance should be > Calibrator 2 and < Calibrator 4
Calibrator 4	Mean Absorbance after subtraction of Blank Absorbance should be > 1.200

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

INTERPRETATION OF RESULTS

Qualitative

Calculate the Index Value to obtain qualitative specimen results.

1. If the test is valid, obtain Cut-Off Value by subtracting the Blank Absorbance from the Mean

Absorbance of Calibrator 2. See an example of Cut-Off Value calculation below.

Item	Absorbance
Blank Absorbance: Well A1	0.009
Cut-Off Value: Mean Absorbance of Calibrator 2 – Blank Absorbance	$0.261 - 0.009 = 0.252$

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

Item	Absorbance
Specimen: Well B2	0.836
Cut-Off Value	0.252
Index Value: Specimen/Cut-Off Value	$0.836/0.252 = 3.317$

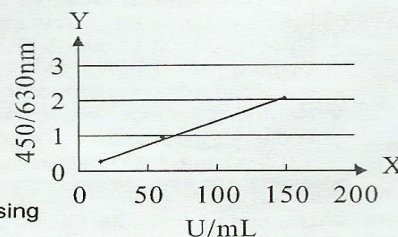
Quantitative

Draw the calibration curve and obtain quantitative specimen results.

1. Subtract the Blank Absorbance from the Mean Absorbance of each Calibrator, then plot them on the Y-axis against their concentration in U/mL on the X-axis on a linear graph paper and draw the calibration curve. Draw the best fitted line through data points to obtain a standard curve. Refer to an example of the calibration curve at right.

NOTE: Do not use the calibration curve at right to make any calculation. A calibration curve must be performed for each run.

2. Obtain quantitative specimen results from their absorbance by using the calibration curve.



***NOTE:** For Equivocal results, the specimens should be re-tested in duplicate and calculate the average value to make judgment. Specimens that are repeatedly Equivocal after re-test should be tested using an alternate method. If the results remain Equivocal, collect a new specimen in two weeks. If the new specimen is Positive, the specimen is presumed to be Positive.

Interpretation of Results – Qualitative and Quantitative

Results	Qualitative	Quantitative
	Index Value	Concentration
Negative	< 0.9	< 13.5 U/mL
Positive	> 1.1	≥ 16.5 U/mL
Equivocal*	≥ 0.9 and ≤ 1.1	$13.5 - 16.5$ U/mL

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

LIMITATIONS

1. The CMV IgG EIA Test Kit is used for the detection of IgG antibodies to CMV in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test result. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A negative test result does not exclude the possibility of exposure. Specimens containing precipitate may give inconsistent test results.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information by the physician.
3. As with other sensitive immunoassays, there is the possibility that the positive result cannot be repeated due to inadequate washing from the initial test. The results may be affected due to procedural or instrument error.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The CMV IgG EIA Test Kit has correctly identified specimens of a mixed titer performance panel (PTC202, Boston Biomedica Inc) when compared to a leading commercial CMV IgG EIA test. It has also been compared to a leading commercial CMV EIA test using clinical specimens. The results show that the clinical sensitivity of the CMV IgG EIA Test Kit is 98.0%, and the clinical specificity is 98.3%.

CMV IgG EIA vs. Other EIA

Method	Results	Other EIA		Total Results
		Positive	Negative	
CMV IgG EIA	Positive	100	1	101
	Negative	2	58	60
Total Results		102	59	161

Clinical Sensitivity: 98.0% (93.1-99.8%)*

Clinical Specificity: 98.3% (90.9-100.0%)*

Overall Agreement: 98.1% (94.7-99.6%)*

*95% Confidence Interval

Reproducibility

Intra-Assay: Within-run precision has been determined by using 15 replicates of three specimens: a low positive, a medium positive, and a high positive.

Inter-Assay: Between-run precision has been determined by 3 independent assays on the same

three specimens: a low positive, a medium positive, and a high positive. Three different lots of the CMV IgG EIA Test Kit have been tested using these specimens over a 5-day period.

Specimen	Intra-Assay			Inter-Assay		
	Mean Absorbance/ Cut-Off	Standard Deviation	Coefficient of Variation (%)	Mean Absorbance/ Cut-Off	Standard Deviation	Coefficient of Variation (%)
1	1.752	0.128	7.306	1.838	0.120	6.529
2	4.431	0.349	7.876	4.439	0.290	6.533
3	9.041	0.723	7.997	9.017	0.774	8.584

Interferences

Interferences are not observed up to a concentration of 1 mg/mL Acetaminophen, 0.2 mg/mL, Gentistic Acid, 0.1 mg/mL Ascorbic Acid, 0.1 mg/mL Acetosalisilic Acid, 0.1 mg/mL Caffeine, 0.6 mg/mL Oxalic Acid, 2 mg/mL Bilirubin, 2 mg/mL Hemoglobin, 1% Methanol and 1% Ethanol. Rheumatoid factors do not interfere with the test.










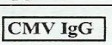
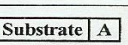
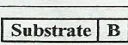
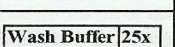
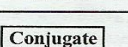
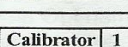
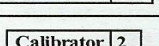
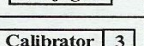
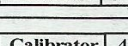
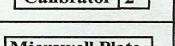
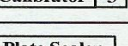
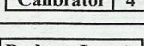
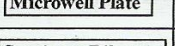
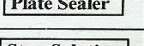
Cross-Reactivity


Syphilis, HBsAg, HIV, HCV, HSV IgG, Toxo IgG, and Rubella IgG positive specimens tested do not interfere with the Acon EIA Test Kit to generate correct positive results.

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4. Stern, H. Cytomegalovirus Vaccine: Justification and Problems. In: Waterson AP (ed.) Recent Advances in Clinical Virology (1977) 117-134.

Index of Symbols

	Consult instructions for use		Tests per kit		Manufacturer
	For <i>in vitro</i> diagnostic use only		Use by		Authorized Representative
	Store between 2-8°C		Lot Number		Catalog #
	CMV IgG		Substrate A		Substrate B
	Wash Buffer (25x)		Conjugate		Calibrator 1
	Calibrator 2		Calibrator 3		Calibrator 4
	Microwell Plate		Plate Sealer		Package Insert
	Specimen Diluent		Stop Solution		

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Number: 1150739002
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