"*In Vitro* Investigation of the Role of Human Cytomegalovirus Glycoprotein Polymorphisms in Disease Pathogenesis"

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LIST of ABBREVIATIONS

HCMV	Human Cytomegalovirus
SNHL	Sensorineural Hearing Loss
NK	Natural Killer
IgM	Immunoglobulin M
IgG	Immunoglobulin G
dsDNA	Double stranded DNA
KbP	Kilobase Pair
gB	Glycoprotein B
gM	Glycoprotein M
gN	Glycoprotein N
gH	Glycoprotein H
gL	Glycoprotein L
gO	Glycoprotein O
ER	Endoplasmic Reticulum
HIV	Human Immunodeficiency Virus
HCV	Hepatitis C Virus
SNHL	Sensorineural Hearing Loss
MCP	Major Capsid Protein
mCP	Minor capsid proteins
SCP	Smallest Capsid Proteins
VZV	Varicella-zoster Virus
EBV	Epstein-Barr virus
RNAs	Ribonucleic acids

IE	Immediate Early
LTP	Largest Tegument Protein
GC	Glycoprotein Complex
Wnt	Wingless-related integration
RFLP	Restriction Fragment Length Polymorphism
HSPGs	Heparin sulphate proteoglycan
EGFR	Epidermal Growth Factor Receptor
MCP	Major Capsid Proteins
mCP	Minor capsid proteins
SCP	Smallest Capsid Proteins
GalNAc	N-acetyl-D-galactosamine
GlcNAc	N-acetyl-D-glucosamine
Neu5Ac	N-acetylneuraminic acid
Glc	D-glucose
Gal	D-galactose
Man	D-mannose
Sia	Sialic acids
Asn	Asparagine residue
Thr	Threonine
Ser	Serine
GAGs	Glycosaminoglycans
HSGAGs	Heparan Sulphate Glycosaminoglycans

O.D.	Optical Density
Av/Po	Avidin/peroxidase
DMSO	Dimethyl Sulphoxide
MEM	Eagle's Minimum Essential Medium
Sab	Sabouraud
UPDW	Ultra-pure distilled water
SD	Standard deviation
P	Probability value
ECL	*Erythrina cristagalli* lectin
WGA	Wheat Germ agglutinin
EBL	*Sambucus nigra* lectin
GNL	*Galanthus nivalis* lectin
BPL	*Bauhinia purpurea* lectin
EEL	*Euonymus europaeus* lectin
PHA-E	*Phaseolus vulgaris* erythroagglutinin
PTL	*Psophocarpus tetragonolobus* lectin
AAL	*Aleuria aurantia* lectin
PSA	*Pisum sativum* agglutinin
HSV	Herpes simplex Virus
CID	Cytomegalic inclusion disease
ICTV	International Committee on Taxonomy of Viruses
HH-5	Human Herpesvirus 5

DB	Dense Bodies
NIEPs	Non-Infectious Enveloped Particles
Nm	Nanometer
UL	Unique Long
US	Unique Short
TRL	Terminal Repeat Long
TRS	Terminal Repeat Short
IRL	Internal Repeat Long
IRS	Internal repeat Short
G	Guanine
C	Cytosine
ORFs	Open Reading Frames
P	Protein
gp	Glycoprotein
STAT	Signal Transducer and Activators of Transcription
E	Early Gene Expression
L	Late Gene Expression
APs	Assembly Proteins
ACD	Amino Conserved Domain
PCR	Polymerase Chain Reaction
AIDS	Acquired Immunodeficiency Syndrome
INF	Interferon

CNS	Central Nervous System
ELISA	Enzyme Linked Immuno-Sorbent Assay
ATP	Adenosine triphosphate
HAART	Highly Active Antiretroviral Therapy
MHC	Major Histocompatibility complex
ACV	Acyclovir
GCV	Ganciclovir
FOS	Foscarnet
Fuc	L-fucose
PNGase F	Peptide -N-Glycosidase F
GBP	Glycan-binding Proteins
ELLA	Enzyme Linked Lectin-sorbent Assay
HEL	Human Embryonic Lung Fibroblasts
ATCC	American Type Culture Collection
CPE	Cytopathic Effect
µg	Microgram
µl	Microliter
mM	Micromolar
M	Molar
TBS	Tris-buffered saline
TMB	Tetra MethylBenzidine
FCS	Foetal Calf Serum

$TCID_{50}$	50% Tissue Culture Infectious Dose
CaCl	Calcium Chloride
M	Mean
Sig	Significance
SBA	Soybean agglutinin
LCA	*Lens culinaris* agglutinin
GSL II	Biotinylated Griffonia (Bandeiraea) Simplicifolia Lectin II
PHA-L	Phaseolus vulgaris Leucoagglutinin
LEL	*Lycopersicon esculentum* lectin
UEA	*Ulex europaeus* agglutinin
WFA	*Wisteria floribunda* agglutinin
ALL	*Amaranthus caudatus* lectin
MAA II	Maackia Amurensis Lectin II
GSL I	*Griffonia (Bandeiraea) Simplicifolia* Lectin I

ABSTRACT

HCMV is a common viral pathogen that infects most of the world's population by early adulthood. It is typically asymptomatic in immunologically healthy individuals but causes severe disease in immunocompromised patients and congenitally infected infants. HCMV glycoproteins are highly polymorphic, and various types of associations have been suggested between glycoprotein types and the pathogenicity of the virus. Several studies on viruses other than HCMV have related the glycosylation of the viral glycoproteins to virulence. This project aimed to determine whether there is a robust relationship between the individual glycoprotein sequence and its glycosylation, how this influences the growth characteristic of the virus and whether this is related to its pathogenicity. Glycosylation patterns of 89 clinical specimens of different infection categories and specimen types were correlated with genetic sequence alterations of the virus glycoproteins (gB, gH, gL, gM, gN, gO), followed by determining whether mutation results in specific changes in glycosylation. The aim was approached using a cell culture model and a quantitative lectin-based assay (ELLA). A significantly increased glycosylation level for the following genotypes: mixed gH, gN4a, gO4, mixed gL was detected. Whereas a decreased pattern was found to be associated with gH1, gH2, gN3a, gO1a and gL2 genotypes ($P<0.05$). Glycoproteins of strains isolated from respiratory specimens were significantly highly glycosylated compared to the blood and urine samples, and from blood specimens compared to the urine samples ($P<0.05$). Furthermore, strains from congenitally infected infants and urine samples had a significantly higher growth rate than others tested. No direct association between the virus growth and its virulence was found. These findings demonstrate that glycosylation of glycoproteins in HCMV is affected by the glycoprotein polymorphisms and signifies a potentially important mechanism for avoidance of antibody-mediated neutralization, which, in turn, facilitates HCMV pathogenicity. This phenomenon requires further study and may have application for the selection of novel targets for diagnosis, vaccine development and other preventive measures to combat diseases caused by this virus.

DECLARATION

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

COPYRIGHT STATEMENT

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DEDICATION

This work is dedicated to Mum, may Allah prolong her life and protect her; Dad, may Allah rest his soul in peace; my husband Ayman, my partner and the love of my life; my children (Manar and Mariam), my joy and happiness; and my brothers, who have always been there for me.

CHAPTER 1

Introduction

Human Cytomegalovirus (HCMV)— humanbetaherpesvirus 5 (also referred to as the human herpes virus 5) —is a ubiquitous, double-stranded DNA virus belonging to the Herpesviridae family, subfamily betaherpesvirinae (Puchhammer-Stöckl and Görzer, 2011). The viral genome is packaged as a single, linear, 235 kb dsDNA molecule packaged in a preformed capsid. The mature capsid is an icosahedron and is surrounded by an amorphous layer, the tegument, which is in turn surrounded by a lipid envelope studded with glycoprotein spikes. The virion envelope is essential for virus infectivity. HCMV, as with other betaherpesviridae subfamily, is species-specific and cell-type-specific in culture. The growth cycle is slow, and the virus tends to remain cell-associated. Infection results in a marked increase in cell volume (cytomegalia) and development of prominent and distinctive nuclear and cytoplasmic inclusions. Infection is often clinically non-apparent in immune-competent hosts. Latent infection is established in cells of the myeloid lineage including CD14+ monocytes and their CD34+ progenitor (Brooks *et al.*, 2010; Poole *et al*., 2014).

HCMV infections can be acquired *in utero* or during the postnatal period via infected maternal breast milk. Maternal-to-foetal viral transmission is facilitated by the altered cytokine profile of pregnancy, resulting in functional immune suppression. During primary maternal infection, reactivated infection or re-infection, the virus can cross the placenta and infect the developing foetus. This subsequently triggers a localised immune response in the form of cytokine release, foetal and transplacental IgM and IgG release and cytotoxic natural killer (NK) cell responses. However, once the virus infiltrates the foetal compartment, underdeveloped foetal CD4+ T-cells are unable to suitably proliferate in response to the viral invasion, impairing the foetal immune response (Schleiss, 2013).

The most common manifestations of infection at parturition include hepatosplenomegaly, thrombocytopenia, cholestatic hepatitis, purpura, retinitis, viremia, and pneumonia. These congenitally-infected infants are particularly susceptible to long-term neurodevelopmental sequelae, the prognosis of which is determined at least partially by the maternal immune status prior to conception, the timing of foetal infection, and whether or not the mother is re-infected with a new strain of HCMV during the pregnancy (Kenneson and Cannon, 2007; Schleiss, 2013).

What is poorly understood is why some infected infants are affected, either at birth or with late sequelae whilst others are completely unaffected. The virulence of the virus and/or the way it interacts with the maternal and/or foetal immune system may have a role. To date, no robust data have been produced to prove or refute this hypothesis.

It is known that there is a large inter-host genomic variability of HCMV amongst congenitally infected infants. HCMV has the largest genome of any human virus; and the virus exhibits considerable sequence variability and exists as a complex mixture of genotypes (Renzette *et al.*, 2011).

Sequencing and analysis of the HCMV genome have defined around 200 Open Reading Frames (ORFs) including those coding for six envelope glycoproteins (gB, gM, gN, gH, gL and gO). Functional studies subsequently identified ORFs coding for glycoproteins B, H, L, M and N, as being essential for viral replication (Dunn *et al*., 2003). There is good evidence that alterations in the gene sequence of an individual glycoprotein affect the way it is post-translationally glycosylated and that this, in turn, affects its interactions with the immune system. Little attention has been paid in the literature to the role of the carbohydrate component of the HCMV glycoproteins and the influence these exert on the function of the glycoprotein. HCMV glycoproteins are known to be highly polymorphic, and as mentioned earlier, various associations have been suggested between glycoprotein

type or sub-type and pathogenicity of the virus. A study has reported that the glycosylation of glycoprotein N (gN) of HCMV could contribute to the resistance of the virus to neutralizing antibodies (Kropff *et al*., 2012; Vulgaris, 2013).

The main aim of this project is to determine whether there is a relationship between the individual glycoprotein sequence and its glycosylation, whether this is true for all the glycoproteins of the virus, and whether this alters the pathogenicity of virus infection. The research question "Does the polymorphic nature of HCMV glycoproteins affect their glycosylation and is this a mechanism that explains the variable disease pathogenesis of the virus?", was answered by investigating the glycosylation pattern of HCMV glycoproteins, using a cell culture model and a quantitative lectin-based assay, Enzyme Linked Immunosorbent Assay (ELLA). RFLP analysis of genomic DNA was carried out to determine the glycoprotein genotype profile for all specimens. $TCID_{50}$ and PCR assay were used to investigate the growth of virus in culture.

The glycoprotein genotype was compared with the patient sample data (infection category and specimen type), which in turn was associated with the virus growth characteristics to determine whether the observations made *in vitro* correlate with the *in vivo* activity of the virus.

Finally, the glycan profile and glycoprotein genotype were compared to determine whether mutations or combinations of mutations result in changes in glycosylation.

CHAPTER 2

2 Literature review

2.1. Human cytomegalovirus

2.1.1 History of cytomegalovirus

In 1881, Ribbert first observed and recognised the presence of large inclusion-bearing cells in the kidneys of a stillborn infant with syphilis. This observation went unnoticed until twenty-three years later; the first images of these protozoan-like cells in kidneys, liver, and lungs of another stillborn infant were published by Jesionek and Kiolemenoglou (Figure 1) (Ho, 2008). Between 1909 and 1937, similar inclusions were observed and documented by several researchers (Harris and Riley, 1997).

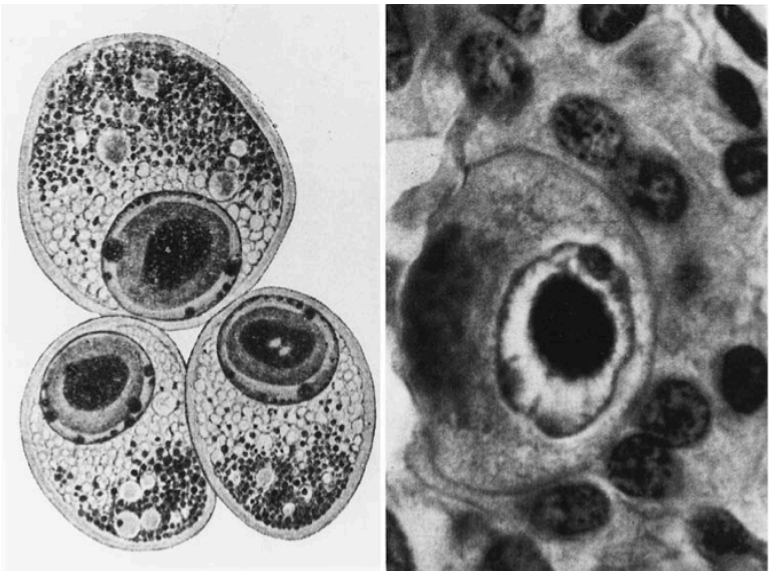

Figure 1: Cells with intranuclear inclusions with halo seen by Jesionek and Kiolemenoglou and described as a protozoan- like (Ho, 2008).

In 1921, Goodpasture and Talbot suggested that these cellular alterations were similar to the ones seen in skin lesions by Tyzzer in 1906. They hypothesised that these 'cytomegalia' might be a result of a viral agent rather than protozoa. In 1950, Wyatt *et al*., suggested a name "generalized cytomegalic inclusion disease

(CID)" for the condition, which was also associated with intranuclear inclusions found in 25 cases of uncommon lethal congenital infection described by Petechiae in 1932. Fetterman achieved the first *intravitum* diagnosis of CID when he used the urine sediment of a premature infant with congenital infection for cytological preparation (Dudgeon, 1971). Following that, Minder used electron microscopy to examine a case of CID and described the causative virus for the first time (Ho, 2008). Smith and Vellios reviewed 69 cases of inclusion disease previously reported, in addition to their series of 20 cases. They believed these inclusion-bearing cells were pathognomonic for the disease, with the majority of cases of generalised infection observed during the first two years of life; and, concluded the infection occurred in utero (Harris and Riley, 1997).

In the succeeding years, Smith in 1955, Rowe *et al*., in 1956, and Weller *et al.*, in 1957 independently isolated human cytomegalovirus from salivary gland, adenoid tissue and liver biopsy respectively (Baumann, 2011). By 1960, Weller and colleagues recommended the term 'cytomegalovirus' for the virus isolated since the cytomegalic inclusion disease-salivary gland virus disease categorisation, as used earlier, was cumbersome and confusing as the salivary gland is just one of many probable sites of infection (Ho, 2008). Although the importance of cytomegalovirus disease was known at that time, the fact that the disease may occur as a fatal complication either congenitally or after organ transplantation has made the scientific community more interested in studying it. During the 1970s and 1980s, cytomegalovirus was intensely studied and continues to be explored by numerous researchers (Baumann, 2011).

2.1.2 Classification and properties

According to the International Committee on Taxonomy of Viruses (ICTV), Human Cytomegalovirus (HCMV) is categorised into the Herpesvirales order, Herpesviridae family, Betaherpesvirinae subfamily, Cytomegalovirus genus, Human betaherpesvirus five species (ICTV Virus Taxonomy, 2017).

All members of Herpesviridae family were assigned according to the architecture of the virion (a linear double-stranded DNA genome enclosed within an icosahedral capsid). Additionally, they share essential biological properties such as; specification of a large range of enzymes involved in DNA synthesis and protein processing; a nuclear assembly of viral capsids and synthesis of viral DNAs, followed by final processing of the virion in the cytoplasm; establishment of a lifelong persistence after primary infection, and becoming latent within host cells (Pellet and Roizman, 2007).

Herpesviridae are classified into three subfamilies (α, β and γ herpesvirinae) based on genetic content (sequence-based phylogeny). Cytomegalovirus belongs to the β-herpesvirinae subfamily, and its genome is the largest among all Herpesvirales (Lane, 2006). HCMV is characterised by a limited host range, a long and slow replication cycle in cell culture (Rajcani and Durmanova, 2001). It infects various body tissues, but the main sites of latency are cells of the myeloid lineage including CD14+ monocytes and their CD34+ progenitors (Poole *et al.*, 2014).

2.1.3 HCMV structure

The human cytomegalovirus has a similar structure to other human herpesviruses. Although its genome is about 50% larger than herpes simplex virus type 1 (Gibson, 2008).

The HCMV virion is roughly 200–300 nm in diameter, with an icosahedral nucleocapsid measuring around 125 nm in diameter (Landolfo *et al*., 2003). The whole infectious virion estimated to have more than 50 viral proteins (Phillips and Bresnahan, 2011). The capsid contains the double-stranded linear DNA genome. Surrounding the capsid is a proteinaceous tegument layer, which is about 50 nm thick (Gibson, 2008), and composed of a minimum of 27 relatively abundant virus encoded proteins. This is then, enclosed by an approximately 10 nm thick outer lipid envelope (Mocarski *et al*., 2007; Gibson, 2008). Embedded within the lipid envelope are a number of virally encoded glycoproteins (Spaderna *et al*., 2002)

(Figure 2). Small RNAs, polyamines, phospholipids and some of the host cell proteins are also included in both tegument and envelope layers (Gibson, 2008).

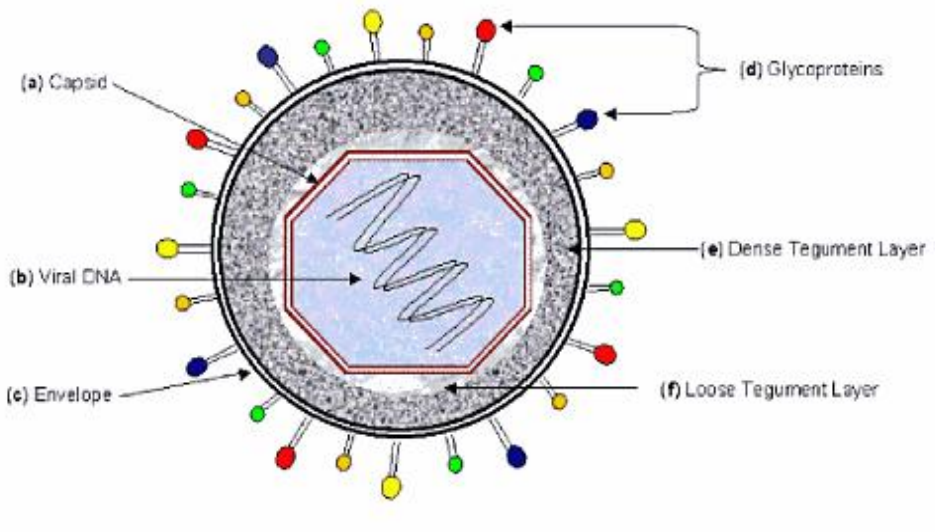

Figure 2: Diagrammatic illustration of a human cytomegalovirus virion (Gaddy, 2009).

Dense bodies (DB) and non-infectious enveloped particles (NIEPs), enveloped A and B-capsids (further explained in section 2.1.5.1), together with the infectious virions are produced in the cell cultures infected with HCMV, the relative percentage of the three forms is dependent on the viral strain and the number of passages in cell culture. Both dense bodies and the envelope particles are non-infectious because they lack some of the essential components that the virion possesses. Assembled nucleocapsid and viral DNA are absent in DB, which consist of tegument proteins, while the enveloped particles have immature capsids that lack DNA (Mocarski *et al*., 2007; Landolfo *et al*., 2003). Dense bodies are larger (250-600 nm) than the virion and the NIEPs, and bordered by an envelope that undistinguished from the one surrounding the virion (Gibson, 2008).

2.1.4 Genome organisation

HCMV genome is the largest of all herpesviruses with size around 235 kilobase pair (kbp), encoding more than 200 open reading frames (Phillips and Bresnahan, 2011), and about 50 glycoproteins (Boehme and Compton, 2006;

Isaacson *et al*., 2008). It comprises 58 % guanine+ cytosine content (G+C). It is a class E genome (Demmler, 2004), divided into two unique sequence regions, unique long (UL) and unique short (US) that are bounded by two sets of inverted repetition sequences: terminal repeat long (TRL) and internal repeat long (IRL), the latter is an inverted repeat of TRL, and terminal repeat short (TRS) and internal repeat short (IRS), the latter is an inverted repeat of TRS (Figure 3). The UL and US segments have the ability to separately invert with respect to one another during infection, resulting in four different genomic isomers (Mach *et al*., 1989; McVoy, 1994).

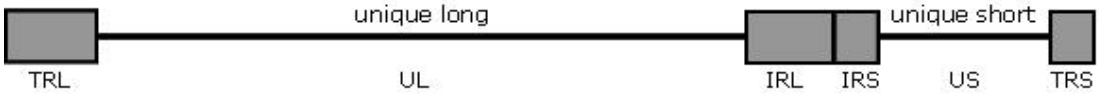

Figure 3: Schematic map of the genome, shows the unique sequences UL and US, bounded by the two sets of inverted repeats TRL/IRL and TRS /IRS (Schleiss, 2011).

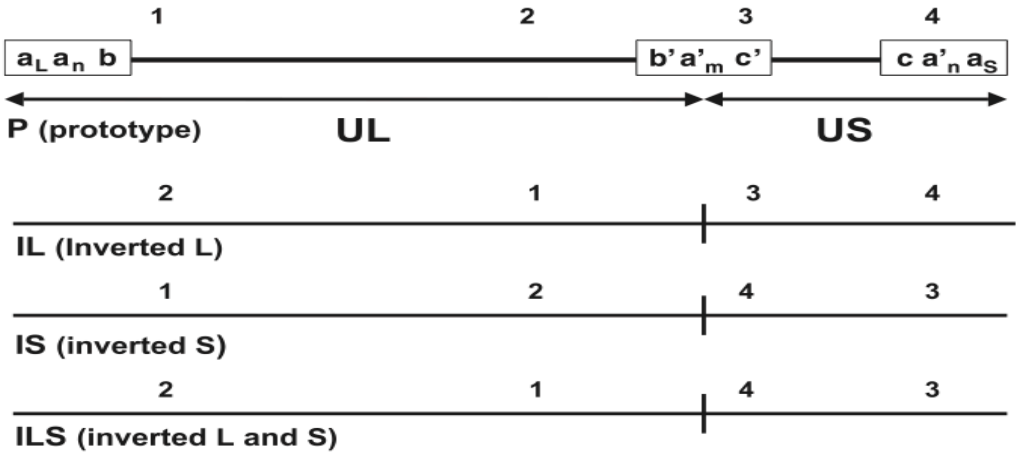

Figure 4: Structure of the four-human cytomegalovirus genomic isomers (Landolfo *et al*., 2003).

TRL segment surrounded with (a_n, b) repeats and IRL-IRS segment bounded with (b\, a_n, c\) repeats, whereas TRS surrounded with (c, a_n). Accordingly, the sequence of the repeats will be as follow: ($a_n$bb\$a_n$c\c$a_n$). These repeats mediate the inversion of the UL and the US regions. Thus, the HCMV genome isomerisation

occurs as a result of the recombination of terminal a_n and internal a'_n sequences (Figure 4) (Landolfo *et al*., 2003; Murphy and Shenk, 2008).

In each region (UL and US) there are a number of predicted open reading frames (ORFs), explained by abbreviations, p for protein, gp for glycoprotein and pp for phosphoprotein, tailed with the region acronym and the sequential number of the ORF, for example, gpUL55 (gB) is the 55th ORF recognized as glycoprotein B in the UL region (Dargan *et al*., 1997). Differing numbers of ORFs in the HCMV genome have been reported in different studies, from the initial estimation of 208 ORFs in 1990 to recent estimations ranging from a minimum of 165 potentially functional ORFs to a maximum of 252 ORFs (Murphy and Shenk, 2008).

2.1.5 Viral proteins

2.1.5.1 Capsid proteins

The HCMV T=16- icosahedral capsid is composed of 162 capsomeres, 12 pentons and 150 hexons. This consists of five different core proteins: 955 copies of major capsid protein (MCP or pUL86 gene product) forming 11 of the pentons (each has 5 copies of MCP to make up the triangular vertices) and hexons (each has 6 copies of MCP to make up the triangular faces); minor capsid proteins (mCP encoded by UL85) that is linked to the minor capsid binding protein (mC-BP, UL46) creating 320 copies of triplexes (TR1, TR2) located in between the pentons and hexons, thus connecting them; smallest capsid proteins (SCP, UL48/49), which are known to be crucial for the HCMV infection, bind to the MCP hexon subunit tips only, according to a study conducted by Xuekui *et al*., in 2005 using anti-SCP antibody labelling; and a portal protein (PORT, UL104), which forms a specific single penton, composed of 12 copies of PORT, essential for the viral DNA encapsidation that occurs within the host nucleus (Mocarski *et al*., 2007; Xuekui *et al*., 2005).

Various studies looked at the different capsid maturation stages, revealing three stages of capsid maturation in the infected cell nucleus: A-capsid, which is an

empty viral shell, B-capsid, which has viral proteins helps in the DNA to be contained inside the capsid, and C-capsid, which is a mature capsid with a dense DNA core. Both A and B capsids have been reported to be seen in the cell nucleus and cytoplasm, and also as cell-free viral particles known as non-infectious extracellular particles (NIEP) (Ryner *et al*., 2006; Sintorn *et al*., 2004; Mocarski *et al*., 2007; Chee *et al*., 1989) identified the MCP gene of human cytomegalovirus and compared its protein sequence with that of the herpes simplex virus (HSV), varicella-zoster virus (VZV), and Epstein-Barr virus (EBV). Their results showed a homology of 25% to HSV-1, 23% homology with the MCP of varicella-zoster virus, and 29% homology with the Epstein-Barr virus.

2.1.5.2 Tegument proteins

Most of the infectious virion proteins and some cellular and viral RNAs are contained in the tegument layer, which comprises about 40% of the virion's mass. It is assumed that the tegument layer is amorphous with no defined structure; this may be because it is beyond the power of the electron microscope to picture structure in this layer. Physically, it is known to be located between the capsid and the lipid envelope, plus some of its proteins are closely associated with the capsid (Chen *et al*., 1999; Mocarski *et al*., 2007).

The HCMV tegument comprises of the following: a lower matrix phosphoprotein (pp65, UL83), which is the most abundant protein, an upper matrix protein (pp71, UL82) that activates immediate-early (IE) gene expression (Tomtishen, 2012), a core virion maturation protein (pp150, UL32), a largest tegument protein (LTP, UL48) that, together with the binding protein UL47, plays a role in the un-coating of the nucleocapsid and releasing of the viral genome at the nuclear pores, and a minor tegument protein that is a viral protein kinase (UL97) (Mocarski *et al*., 2007; Varnum *et al*., 2004); plus UL99 (pp28), which is essential for the production of infectious virus when it interacts with another tegument protein (UL94) (Phillips *et al*., 2012).

According to the study by Kalejta (2008), the integration of proteins into the HCMV tegument is facilitated by the interaction with capsids or tails of envelope proteins, subcellular localisation to the assembly site, and phosphorylation. Although most of the tegument proteins are phosphorylated, the importance of this post-translational modification (phosphorylation) is not yet clear (Kalejta, 2008).

Several tegument proteins are of interest because of their involvement in a broad range of activities and the roles that they play in the replication cycle of human cytomegalovirus. These tegument proteins include pp28, pp65, pp71, and pp150 (Tomtishen, 2012). For example, they have a role in delivering the viral genome to the host nucleus during the entry process, the capsid-associated tegument proteins (UL47 and UL48), pp65 and pp71 are involved in this function, pp65 together with other tegument proteins also play an essential role in the virus immune evasion through inactivation of the cellular defence mechanisms (Kalejta, 2008). Moreover, pp65 has a vital role in HCMV lytic cycle development throughout its localisation to the nucleus in the early stages of the infection (Tomtishen, 2012).

2.1.5.3 Envelope glycoproteins

Aside from the complexity of the virion envelope structure, where most of its proteins have not yet been defined, there are three main disulfide-linked glycoprotein complexes found and characterized in an HCMV phospholipid envelope: Glycoprotein complex 1 (GC-I), glycoprotein complex 2 (GC-II) (Gretch *et al*., 1988) and glycoprotein complex 3 (GC-III) (Landolfo *et al*., 2003). According to the existence of these different glycoprotein complexes, the virus can infect most organs of the body and has a broad-spectrum cell tropism. Fibroblasts, dendritic cells, macrophages, smooth muscle cells, hepatocytes, neurons, glial cells, leukocytes, epithelial cells and endothelial cells are some examples of HCMV permissive cells (Sinzger *et al*., 1995; Theiler and Compton, 2001; Sinzger *et al*., 2008). In contrast, laboratory strains such as AD169 and Towne are unable to infect most cell types except fibroblasts likely due to a mutation in its ULb\ genes

such as UL128-131 (Sinzger *et al*., 2008). Gretch *et al*. in 1988 have reported that these complexes have been designated according to their monoclonal antibody reactivity and biochemical features.

These three envelope glycoprotein complexes are thought to have various functions in the entry of human cytomegalovirus into host cells; it's spread from cell to cell and in virion maturation. Human cytomegalovirus infection commences by the attachment of the virus to host cell heparan sulphate proteoglycans. Both GC-I and the GC-II complex have heparan-binding ability (Sinclair, 2000). The virus fusion requires at least two of the envelope glycoprotein complexes. Also, it is believed that these envelope glycoproteins have the ability to trigger the host immune response by provoking the production of neutralizing antibodies including strain- specific ones (Britt and Mach, 1996).

2.1.5.3.1 Glycoprotein complex I

The glycoprotein complex 1 (GC-I) contains disulfide-linked homodimeric molecules that form the glycoprotein B homologue (gB, gpUL55-116). Gp116 is the surface constituent, while gp55 is the transmembrane part of the complex (Britt and Vugler, 1992). It is the second most abundant envelope glycoprotein, and similar to the one described in HSV and EBV in regard to its structural and functional properties (Gretch *et al*., 1988; Kari and Gehrz, 1993; Sharma *et al.*, 2013). Glycosylation followed by proteinase cleavage at 461 and 460 codons of a 906 amino acids precursor molecule that forms gB occurs, resulting in the formation of a disulfide-link between gp116 and gp55 subunits. Both the cleavage site and the N-terminus region have been found to have major intragenic variability in the gB gene, while in the C-terminus region only minor variation has been detected (Chou, 1992; Haberland *et al*., 1999; Sarcinella *et al*., 2002; Meyer-König *et al*., 1998). The proteolytic cleavage is dispensable for viral replication and growth (Strive *et al*., 2002). Due to variability in the cleavage site, four major genotypes of gB have been identified (gB1, gB2, gB3 and gB4), using restriction

fragment length polymorphism (RFLP). This classification was made in order to study epidemiology and the disease outcome of a particular strain. The glycoprotein B is immunogenic (Billstrom and Britt, 1995) and has an essential role in binding of the virus to host cell receptors and in viral penetration and fusion (Landolfo *et al*., 2003; Isaacson *et al*., 2008). Heparin sulphate proteoglycan (HSPGs) involvement is crucial to the gB binding process as reported by Boyle and Compton (1998). Also, gB is known to bind to the epidermal growth factor receptor (EGFR) and αvβ3 co-receptor, and this is thought to be essential for HCMV entry (Wang *et al*., 2005).

2.1.5.3.2 Glycoprotein complex II

The second envelope glycoprotein complex 2 (GC-II) is composed of disulfide-linked glycoprotein M (gM, UL100) and glycoprotein N (gN, gpUL73 with 39- 200 KDa molecular masses. Sequence analysis of gN has shown four different genotypes (gN1, gN2, gN3 and gN4) with four subgroups (gN-4a, gN-4b, gN-4c and gN-4d) classification depends on the gene sequence variation at the N-terminus region (Pignatelli *et al*., 2010, Pignatelli *et al*., 2001; Rasmussen *et al*., 2002). Pignatelli *et al*., (2003) have reported that gN3 is sub-divided into two subgroups (gN3a and gN3b). These gM and gN glycoproteins are distinctive and have no similarity with other herpesvirus glycoproteins (Kari *et al*., 1992). A study conducted by Varnum *et al*. (2004) analysed the HCMV proteins and proposed that gM and gN are the most abundant glycoproteins in the virus envelope, relatively more than gB (Isaacson *et al*., 2008).

Mach *et al*., (2000) reported that gM:gN complex formation is required for protein transport from the endoplasmic reticulum to the Golgi and trans-Golgi compartments. Thus, the complex is crucial for HCMV replication (Shimamura *et al*., 2006; Mocarski *et al*., 2007). It was reported that gM and gN also interrelate with HSPGs during entry (Kari and Gehrz, 1992). Pati *et al*., (2012) suggested that gN could stimulate the production of specific neutralizing antibodies that may protect host cells from being re-infected by a different HCMV genotype. Shimamura

et al., (2006) showed that the anti-gM/gN antibodies could effectively neutralize the infectious HCMV. On the other hand, a study conducted by Kropff *et al*., (2012) revealed that the presence of the glycoprotein N could prevent HCMV neutralizing antibody activity.

2.1.5.3.3 Glycoprotein complex III

The third key envelope glycoprotein complex 3 (GC-III), consists of glycoprotein H (gH, gpUL75), which is known as a fusion promoter, glycoprotein L (gL, gpUL115), which acts in complex with gH (gH/gL) and plays an important role in virus fusion with the host cell (Gillet *et al*., 2007), and glycoprotein O (gO, gpUL74). Several previous studies reported that gO has a role in enhancing the entry process (Huber and Compton, 1998; Isaacson *et al*., 2008), while a relatively recent study conducted by Ryckman *et al*., (2010) have proposed that gO serves as a chaperone increasing the export of gH/gL from endoplasmic reticulum. The same study has confirmed that gH/gL together with UL128-131 mediates virus entry into epithelial and endothelial cells *in vivo*. Two different genotypes have been identified for gH (gH1, gH2), which can be distinguished by the N-terminus site variability, and for gL four genotypes (gL1, gL2, gL3, gL4), plus seven for gO (gO1, gO1a, gO1b, gO1c, gO2a, gO2b, gO3 and gO4) (Pingnatelli *et al*., 2004; Rasmussen *et al*., 2002).

The disulfide-linked heterooligomer complex (gH/gL/gO) plays an important role in the fusion process in the final stage of entry and has a similar characteristic to the other herpesviruses, except for gO, which is found only in the betaherpesvirinae (Kinzler and Compton, 2005; Gretch *et al*., 1988; Landolfo *et al*., 2003; Theiler and Compton, 2001). In contrast to gB, gH and gM the glycoprotein L is the least abundant glycoprotein in the virus envelope (Billstrom and Britt, 1995). In addition to their role in virus fusion, penetration of the host cell membrane, and cell-to-cell spread, the gH/ gL complex is also involved in the production of neutralizing antibodies (Rasmussen *et al*., 2002). Furthermore, gH alone has been

found to stimulate the antibody response in 100% of individuals included in one study (Urban *et al*., 1996). It has also been demonstrated that gH binds to EGFR and αvβ3 co-receptor (Wang *et al*., 2005).

2.1.5.3.4 Other envelope glycoproteins

In addition to the commonly known major envelope glycoproteins (gB, gM, gN, gH and gL and gO), which are conserved among herpesviruses, there are other HCMV specific envelope glycoproteins that have been identified. Recently, a study revealed that UL1 is a novel glycoprotein of HCMV envelope, found in association with gB and another viral protein pp28, expressed and detectable nearly 48 hours post HCMV infection. It was suggested that UL1 has a significant impact on viral growth and cell tropism (Shikhagaie *et al*., 2012). TRL10 is another immunogenic HCMV envelope glycoprotein found in complex with other viral proteins that have not yet been identified. It was argued that TRL10 requires a complex formation for proper folding and transport to the cell surface (Spaderna *et al*., 2002). Two years later, Spaderna *et al*. (2004) reported that gpTRL10 is dispensable for virus replication. Likewise, UL4 (gp48), TRL11, TRL12, RL13 (Cortese *et al*., 2012), US28, US27, UL132 and UL33 have not been found to have an essential role in the HCMV entry to the host cell (Boehme and Compton, 2006).

2.1.6 Replication

HCMV, as compared with other herpesviruses, has a slow replication cycle with early viral release occurring 48–72 hours after infection. It is sequentially regulated during the development of the infection. HCMV has the ability to bind to many different cell types (Compton and Feire, 2007). The replication cycle commences with virus entry, which is initiated by a minimum of five essential viral envelope glycoproteins (gB, gH:gL, gM:gN) attaching to cell-specific receptors. Since HCMV has a broad cellular tropism, this may suggest many different viral cellular receptors exist. For example, gB binding to heparan sulphate proteoglycan

(HSPGs) (Kari and Gehrz, 1993) which are widely present in the extracellular matrix. This is a significant initial step as this confers the virion stability on the cell surface at least until the engagement of other receptors. The entry can be boosted by mediator candidates, such as the co-receptor annexin II that enhances the virus binding and fusion, although cells lacking annexin II are still found to be infected with HCMV (Pietropaolo and Compton, 1999); and aminopeptidase N (CD13), which is located on peripheral blood mononuclear cells, its binding with HCMV is found to prevent the differentiation ability of monocytes into macrophages (Gredmark *et al*., 2004). The epidermal growth factor receptor (EGFR) is another receptor known as an entry receptor or a signalling receptor; it activates other signalling receptors such as protein kinase-B (Akt) and phosphatidylinositol-3-OH kinase and releases the cellular supply of calcium. A further 92.5 KD gH binding receptor exists, but little is known about it (Mocarski *et al*., 2007). The entry process continues either by direct fusion between cell plasma membrane and the viral envelope or by receptor-mediated endocytosis. Next, the release of the viral nucleocapsid into the host cell cytoplasm occurs where the cytoplasmic microtubules assist its translocation into the nucleus. After that, interaction with the nuclear pores occurs allowing the viral DNA to be released into the nucleus (Figure 5) (Crough and Khanna, 2009; Mocarski *et al*., 2007; Detrick *et al*., 1996).

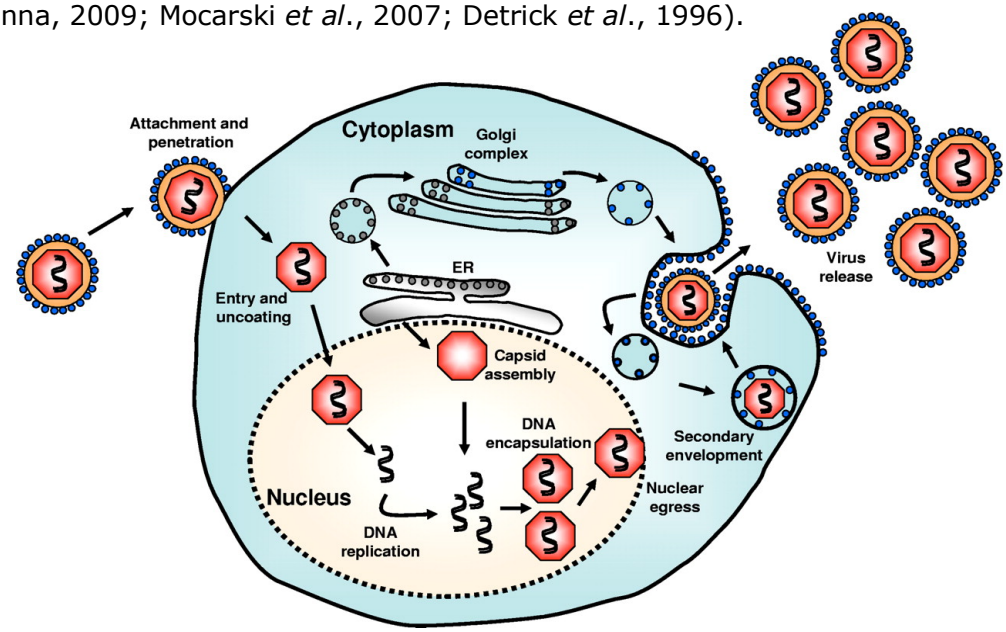

Figure 5: The life cycle of HCMV in human cell (Crough and Khanna, 2009). The figure shows the different replication steps of HCMV life cycle, the virus acquires its primary envelope from the nuclear membrane during the nuclear egress.

Subsequently, a temporally regulated cascade of the viral genome expression takes place in the cell nucleus. The immediate-early gene expression (IE) (α) that is detectable 0-2 hours after infection and viral protein production, has a vital role in regulating the HCMV gene expression (Pignoloni *et al*., 2016; Crough and Khanna, 2009; Thrower *et al*., 1996). During this phase, the expression of a few different regions of the HCMV genome have been detected (Major IE (IE1 (UL123), IE2 (UL122,)), (UL36, UL37), (US3) and (TRS1, IRS1) (Crough and Khanna, 2009; Mocarski *et al.*, 2007; Thrower *et al*., 1996). Alone or in synergism, the IE proteins autostimulate or transactivate viral genes for subsequent expression. This, has a dynamic impact on the host cell functioning, such as blocking the induction of the potentially antiviral host genes that depends on interaction with the signal transducer and activators of transcription (STAT) proteins by IE1 (Paulus and Nevels, 2009), inhibition of apoptosis by UL36, and down regulation of major histocompatibility complex class-I mediating antigen presentation by US3 (Figure 6), so enhancing infectious virus production (Landolfo *et al*., 2003; Oduro *et al*., 2012; Mocarski *et al.*, 2007).

The early gene expression (E) (β) starts after about 6 hours and persist up to 18-24 hours post infection. It requires the presence of IE gene products (IE2-86/IE1-72) in order to generate later gene products. About 23 genes are needed for viral DNA synthesis, alteration of the cell environment to make it appropriate for viral replication or capsid maturation. Some of these gene products are UL112, UL113, which initiate DNA replication, viral DNA polymerase catalytic subunit encoded by UL54 and UL44 performs as a polymerase processivity factor (Mocarski *et al*., 2007; Detrick *et al*., 1996; Castillo and Kowalik; Landolfo *et al*., 2003). In addition, Hobom *et al*., in 2000 reported a non- essential 48 kd virion envelope glycoprotein encoded by UL4.

Once early genes are expressed, the late gene expression (L) (γ) follows and takes from 24–36 hours post-infection. Most of the important functions such as DNA encapsidation, capsid maturation, virion maturation and egress from the cell is

carried out by late-early or late genes (Figure 6). It is known that transcribed IE genes are not enough for the L genes to be activated, thus other viral genes such as UL 79 (gH), UL87, UL 95, and UL99 (28)) are important for the expression of the L genes (Isomura *et al*., 2011; Mocarski *et al*., 2007). Both IE and L phases are further sub-classified into (β_1, β_2) and (γ_1, γ_2) respectively, based on timing (Mocarski *et al*., 2007; Detrick *et al*., 1996; Castillo and Kowalik, 2004; Crough and Khanna, 2009). By the end of L phase of HCMV infection, the host cell undergoes extensive changes and formation of cytoplasmic inclusions, where the nucleocapsid particles accumulate within the Golgi apparatus.

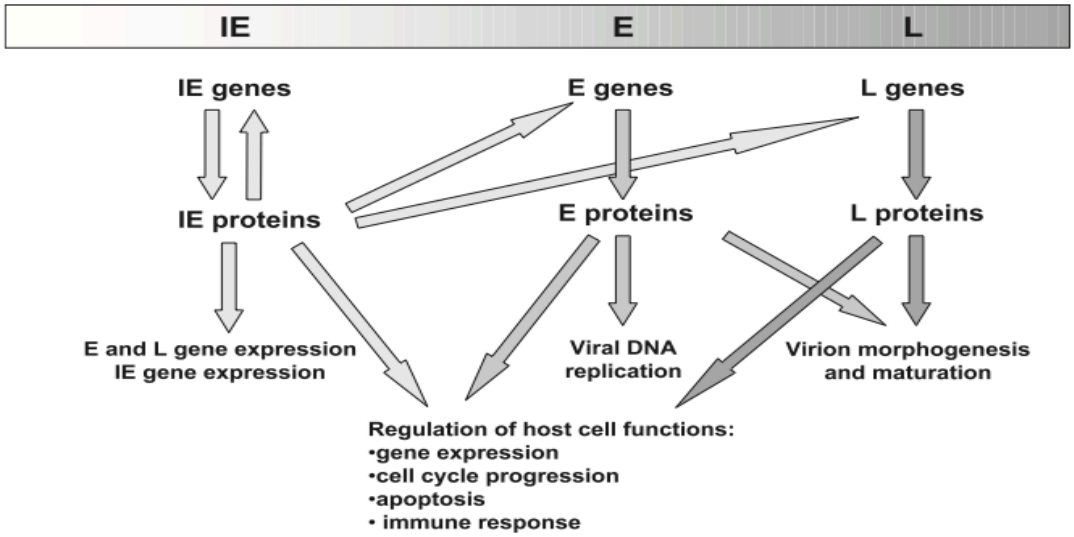

Figure 6: Gene expression and function of the viral production during HCMV infection (Landolfo *et al*., 2003).

Several enzymes are needed for DNA replication initiation in the nucleus. HCMV has a conserved lytic replication origin (oriLyt) from where the DNA synthesis begins (Mocarski, 2007). The capsid is assembled primarily with the development of an interior protein scaffold, maturation protein precursor, assemblin, (pUL80a) and assembly protein precursor (pUL80.5), The scaffolding proteins (assembly proteins (APs)) self-interact and interact with each other through the amino-terminus and then interact with the major capsid protein through the carboxyl-terminus to initiate capsid formation. Also, a disruption of the amino conserved domain (ACD) by a point mutation causes disruption in the scaffolding proteins and

their ability to interact with each other, consequently, affecting the assembly of the capsid and the production of the infectious viral particles (Loveland *et al*., 2007). This interaction of these proteins occurs in the cytoplasm and then translocate into the nucleus. This is followed by hexon and penton formation, which interact with other protein complexes (pIL85/pUL46) to form the capsid shell (B-Capsid) where the genome will be inserted, and APs will be removed, encapsidating the viral DNA (Mocarski, 2007).

Next, nucleocapsids will acquire a primary envelope from the nuclear membrane followed by their de-envelopment in the cytoplasm. The mature virion will acquire its tegument to be enveloped again through budding into Golgi apparatus vesicles (Golgi derived structure); this is followed by the acquisition of the virion envelope glycoproteins. Finally, the infectious virus will be transported to the cell surface for the release out of the host cell at about 72 hours post infection (Figure 5) (Landolfo *et al*., 2003; Mocarski, 2007; Mocarski *et al.*, 2007).

2.1.7 Epidemiology

2.1.7.1 Prevalence of human cytomegalovirus

HCMV is a common pathogen that infects most of the world's population by early adulthood, 50%-85% of adult individuals are infected by the age of 40 (Selinsky *et al*., 2005). The incidence of human cytomegalovirus infection in a number of population groups reaches 100% depending on race, age, gender, socioeconomic status, and ethnic background of the population being examined (Gaddy, 2009), with the highest prevalence being in developing countries (Mocarski *et al*., 2007). Various studies have shown that HCMV infection is common in infants and children day-care centres reflecting the transmission of the virus by close contacts (Ford-Jones *et al*., 1996).

In a serological survey conducted by Vyse *et al*., (2009) exploring the epidemiology of cytomegalovirus infections in England and Wales, the authors examined 5,237 sera collected in 1991 and 2002 and screened for HCMV-specific

IgG, representing a complete age range and reflecting the general population. Their results showed antibody prevalence increased with age from about 15% in those children aged one to four years to about 80% of individuals aged 65 years and older. Application to live birth approximations indicates that between 1991 and 2002, 159,996 HCMV infections took place in England and Wales, with an average of 2,133 infections per year affecting pregnant females (Vyse *et al*., 2009).

The frequency of congenital infection in different countries varies from 0.2% to 2.2% of all live births (Barbi *et al*., 1998). Of the 35,000 new-borns infected with HCMV in the United States, approximately 8,000 of these experience loss of vision, hearing loss, neurologic abnormalities, mental retardation, or death (Ross *et al*., 2006). A study by Paixão *et al*., (2009) suggested that the HCMV congenital infection prevalence in Portugal reaches 1.50% and might be the highest in Europe. Conversely, in 2005, Gaytant *et al*., reported the lowest prevalence of HCMV infection described to date is in the Netherlands. They stated that HCMV infection at birth was observed at a rate of 0.09% of all new-borns, with an overall seroprevalence of mothers at 41% (Gaytant *et al*., 2005). One lengthy Swedish study revealed 0.5% (76/ 16,474) of new-borns congenitally infected with HCMV, via the virus isolation testing done from 1977 to 1985 (Ahlfors *et al*., 1999). Also, a meta-analysis, of 55 published articles in MEDLINE database, conducted by Kenneson and Cannon (2007) among foetuses and infants for the period 1966-2006 accounted for a birth prevalence of 0.64% of congenital HCMV.

On the other hand, in Africa, a study claimed a congenital HCMV infection prevalence of 1.4% among 2,032 new-borns, for previously infected mothers, screened for HCMV infection (Schopfer *et al*., 1978). Whilst two separate studies conducted in the Gambia, West Africa among term infants utilising PCR detection methods revealed a prevalence of 3.9% and 5.4%, respectively (Kaye *et al*., 2008; Van der Sande *et al*., 2007). The rates of HCMV infection in these studies were higher when compared to birth prevalence in industrialised countries as described earlier. However, due to asymptomatic characteristics of the infection, the exact

burden of congenital infection remains unclear. Although HCMV congenital infection occurs rarely, this intrauterine infection is still probably the commonest in humans (Vyse *et al*., 2009).

2.1.7.2 Transmission of HCMV infection

HCMV transmission requires direct contact with body fluids of an infected person. This may occur horizontally or vertically. Body fluids include; urine, faeces, semen, cervical secretions, saliva, tears, breast milk and blood. Sexual contact and close contact with children are two forms of the horizontal transmission (Mocarski *et al*., 2007). Two specific modes of transmission are particularly important for cytomegalovirus: mother to unborn child transmission (vertical transmission) and organ donor-to-recipient transmission. Meier *et al*., (2005) stated that the primary reason for pre-natal transmission is the primary infection of the mother whilst the main reason for the post-natal mother-to-child transmission is breastfeeding. It was estimated that breastfeeding-associated transmission rates range from 58% to 76% (Van der Strate *et al*., 2001). Van der Strate *et al*., (2001) also explained that viral load in breast milk is highly correlated with transmission of the virus, although breast milk does contain lactoferrin, an iron-binding glycoprotein that exerts antiviral activities to protect the infants.

Although breast milk plays a role in the postnatal transmission of HCMV from mother to her new-born infant, it rarely results in serious diseases. In contrast, transmission via transplacental and intrapartum routes can cause serious congenital infection (Mocarski *et al*., 2007). Both primary infection during pregnancy or recurrent infection in the mother can lead to the congenital infection. Confirmation of maternal viremia resulting in uterine infection was provided by a study conducted by Loh *et al.,* (2006). Intrapartum HCMV infection is caused by local shedding of the virus in the mothers' vagina, genital tract or cervical secretions. Transmission via the genital tract is more common in younger women under the age of 30

(50%), while 10% have been found to shed the virus through the vagina or cervix near or at the time of delivery (Mocarski *et al*., 2007).

The pre-transplant serostatus of both donor and recipient has been identified by several studies to play a significant role in the development of HCMV infection after an organ transplant. A study conducted by Manuel *et al*., (2009) was one of the largest studies performed to analyse the transmission patterns of donor-to-recipient transmission of HCMV. Their data suggest that HCMV transmission has a complex and dynamic pattern, in that transmission of multiple HCMV strains is possible and can be detected simultaneously or sequentially. It was also found that seropositive recipients can either reactivate their own HCMV infection or be superinfected with a strain from the donor.

2.1.8 Pathogenesis

Regardless of its high infection rate, HCMV is an opportunistic pathogen that seldom results in clinical disease among healthy individuals but may promote severe illness in immunocompromised individuals including transplant patients, patients with AIDS. In this situation, most body organs could become infected.

In healthy individuals, the immune response against HCMV is robust and extensive, and even a long period after primary infection, a strong response can still be detected. However, the presence of this robust cellular immunity does not protect from the reinfection by a new HCMV strain, although it does control the infection and reduces the severity of the disease outcome in both immunocompetent individuals and immunocompromised patients (Yamamoto *et al*., 2010; Ross *et al*., 2010).

The virus has the ability to disseminate and become latent, even in the presence of active immunity. Reactivation and multiplication of the virus can occur resulting in severe damage (AIDS, allogeneic rejection) in the setting of weakened or suppressed immunity. Under host immune pressure, HCMV has the ability to

evolve and develop different immune modulatory strategies, which allow the virus to escape immune system mechanisms, resulting in a long viral survival time within the host cells. Therefore, these immune modulatory strategies may strongly contribute to the viral pathogenesis (Mocarski *et al.*, 2007; Noriega and Tortorella, 2009).

2.1.9 Immune response

The human immune response includes a combination of two forms of immunities that work in combination to eliminate infections caused by pathogens. The innate immunity offers an immediate non-specific response; it includes interferon (INF), natural killer (NK) cells and macrophages. For the innate immunity to control the infection, cellular transcription factors and interferon regulatory factor, together with the antiviral cellular genes such as the inflammatory cytokines and the interferon stimulated genes are activated in response to the HCMV envelope glycoproteins binding and entry. Natural Killer cells produce cytokines that regulate the development of subsequent adaptive immunity that is crucial for HCMV lifelong control (Isaacson *et al*., 2008). The adaptive immune response is an antigen-specific immune response, designed to attack a specific antigen and produce memory cells during the response to prevent re-infection and re-activation. It includes humoral immunity mediated by antibody produced by B cells, and cellular immunity mediated by T cells, such as cytotoxic T lymphocytes ($CD8^+$) and T helper lymphocytes ($CD4^+$) (Mocarski *et al.*, 2007). The role of antibodies in reducing destructive infection is essential. For example, the presence of maternal IgG antibodies following primary infection has a vital role in protecting the foetus from congenital infection (Fowler *et al*., 1992). Controlling viral production and protection from HCMV disease is mediated by activated HCMV specific $CD8^+$ T cells, which recognize infected cells and destroy them. It was found that patients who had undergone stem cell transplants who lack $CD8^+$ cells experienced more reactivation of HCMV compared to those who had a $CD8^+$ response (Avetisyan *et al*., 2007). HCMV specific $CD4^+$ T cells also have an important role in limiting viral

replication through cytokine production that boosts $CD8^+$ T lymphocytes and B-lymphocytes response to the infection (Jackson *et al*., 2011; Miller *et al*., 2001). Jonjic *et al*., 1989 showed that HCMV persistent replication was associated with depletion of $CD4^+$ cells compartment. In HCMV seropositive individuals an enormous specific T cells response (about 10% of $CD8^+$ and $CD4^+$ memory compartments) was observed and documented by Sylwester *et al*., (2005).

2.1.10 Immune modulation

The ability of HCMV to escape innate and adaptive immune reactions facilitates virus multiplication to levels that allow it to cause disease. A large proportion of the HCMV genome is used to encode specific glycoproteins that function as immune modulators of the host immune response and make the host cell environment permissive for virus replication and persistence. For instance, upon entry into the uterine wall and/or placenta, HCMV hijacks cellular replication machinery, interfering with cell cycle progression and exploiting host immune responses. In addition, HCMV dysregulates a number of signalling pathways, in particular, the canonical Wnt/β-catenin pathway (Wnt: Wingless-related integration site), which is implicated in cell cycle control, cellular differentiation, embryonic development, and placentation (Angelova *et al*., 2012).

HCMV encodes a number of glycoproteins, such as US2, US11, U6, U3, US2 and US3 (Noriega and Tortorella, 2009), that modulate antigenic presentation on the infected cell surface through down regulating the expression of the major histocompatibility class (MHC) molecules that normally play a major role in the destruction of virally infected cells (Noriega and Tortorella, 2009). MHC-I has a significant role in presenting the intracellular antigenic peptides formed by the virus on the infected cell surface for $CD8^+$ cells recognition. These antigenic peptide products are generated in the infected cell cytoplasm (by proteasome) and then transported by a transporter-associated antigen processing (TAP) to the endoplasmic reticulum (ER), where the assembly of MHC-I molecules occurs. After

assembly, MHC-I leaves the ER via the Golgi apparatus to be presented on the cell surface (Abele and Tampé, 1999). Down regulation of the MHC-1 molecule by HCMV glycoproteins prevents the viral antigen from being presented on the cell surface and triggering an attack by $CD8^+$ cytotoxic cells. However, MHC-I down regulation makes the infected cell vulnerable to NK attack. To counteract this, HCMV proteins with sequence homologue to MHC-I are produced (Ul18 and UL40) and expressed on the infected cell preventing the NK activity. Other HCMV encoded proteins (UL16, UL83, UL141 and UL142) are also suppressors of NK activity (Prod'homme *et al*., 2012). There are other immune mechanisms inhibited by viral processed proteins. For example, down regulation of MHC-II molecules by inhibiting their antigen presentation to $CD4^+$ T cells recognition through collaboration of the HCMV gpUS3 and gpUS2 (Miller *et al*., 2001; Hegde *et al*., 2002); prevention of apoptosis by the major IE proteins (UL123, UL122) (Yu and Alwine, 2002); and controlling the mitochondrial ATP production needed for the virus production and replication by the HCMV β2.7 RNA (a viral non-coding RNA) that targets the mitochondrion to maintain high levels of ATP production during infection, are other schemes for the HCMV cellular modulation of the immune response (Reeves *et al*., 2007).

2.1.11 Latency and reactivation

As with the other viruses in the herpes family, HCMV is capable of instituting a lifelong persistent infection. HCMV establishes latency within cells of the infected individual; periodic reactivation leads to sporadic shedding of infectious virions (Sinzger *et al*., 1995; Drew, 1992). Although the mechanism of the HCMV latency and reactivation is not clearly understood, it is known that latency is established in bone marrow haematopoietic stem cells, mainly within undifferentiated cells of myeloid lineage and monocytes. During latency, it has been found that only IE genes are expressed, while IE and E gene expression was observed in macrophages that differentiated from monocytes. Accordingly, it was concluded that reactivation of the virus and the induction of IE gene expression arises upon differentiation of

$CD34^+$ hematopoietic progenitors or $CD14^+$ monocytes into dendritic cells or macrophages. However, not all differentiated cells carry the viral genome, T lymphocytes, B-lymphocytes and $CD33^+$ cells were found to be negative (Sinclair, 2008; Soderberg-Naucler *et al*., 1997; Reeves *et al*., 2005). In addition, HCMV reactivation due to specific monocyte-macrophage differentiation was observed, induced by the allogeneic stimulation of T-cells (Soderberg-Naucler *et al*., 1997). A study by Cicin-Sain *et al*., 2012 suggested T-cell function may be impaired by the virus latency.

2.1.12 Diseases associated with human cytomegalovirus

2.1.12.1 Infections in immunocompetent individuals

Human cytomegalovirus infection in immunocompetent individuals is asymptomatic in almost 90% of cases (Drew, 1992). The remaining 10% of immunocompetent patients with primary HCMV infection experience a mononucleosis-like syndrome characterised by malaise, fever, lymphocytosis with atypical lymphocytes and abnormal liver function (Friel *et al*., 2012). However, there are also studies reporting severe cytomegalovirus infection amongst healthy individuals. The quantitative descriptive study conducted by Wreghitt *et al*., (2003) in the UK reported 124 cases of cytomegalovirus infection out of 7,630 immunocompetent patients with symptoms of HCMV infection. These patients had a higher incidence of abnormal liver function tests, respiratory symptoms, fever, sweats and malaise. Prösch *et al*., (1998), in their study, revealed that HCMV also causes encephalitis amongst immunocompetent individuals. Cerebrospinal fluid and blood specimens of 35 patients with neurological disorders were tested for HCMV, 11 of these were found to be positive for HCMV, in addition, HCMV encephalitis in a 23-year-old female was proven in the same study. Interestingly, the virus was cleared after three weeks of hospitalisation and remained undetectable for up to 5 months after the infection onset. Maiorana *et al*., (2003) described gastrointestinal tract infections caused by HCMV in 11 non-immunocompromised patients, infection included the large intestine, stomach and lower oesophagus, in 5 patients atypical

inclusions were found. Also, several cases of hepatitis caused by HCMV were reported by different studies, most of them were cured without the use of antivirals (Al-Mahtab *et al*., 2009; Azad *et al*., 2008; Ma *et al*., 2011). Arthralgia, ulcerative colitis, pneumonitis, aseptic meningitis, myocarditis, splenomegaly and arthritis are also some of the complications that might be seen in immunocompetent patients (Gandhi and Khanna, 2004).

2.1.12.2 Congenital infections

HCMV congenital infection occurs as a result of primary or recurrent maternal infection and is the commonest congenital infection among humans. It is thought that the primary infection has a more serious impact with regard to foetal damage (Fowler *et al*., 1992), but several more recent studies found that *in utero* CMV infection occurred in over 60% of infants as consequences of a secondary infection. About 1-4% of pregnant women have a high risk of developing a primary infection, 30-40% of these transmit the virus to their foetus especially during the first six months of gestation (Boppana and Britt, 2006; Benshushan *et al*., 1998; Leung *et al*., 2003). The virus transmission may occur in three ways: placental infection (intrauterine); ascending infection from the mothers' genital organ secretions to the amniotic fluid (intrapartum); infection of the foetal oropharynx (Ornoy and Diav-Citrin, 2006).

Infants born with the infection are either asymptomatic at birth (about 85-90%) or have symptoms that can be mild or severe such as jaundice (67%), hepatosplenomegaly (60%), petechiae (76%), microcephaly (53%), elevated alanine aminotransferase (83%), conjugated hyperbilirubinemia (81%), thrombocytopenia (77%), chorioretinitis (20%) and seizures (7%). It was reported that 86% of 106 HCMV congenitally infected infants had at least two of the symptoms mentioned (Boppana *et al*., 1992). However, variable percentages were reported by different studies in regard of symptomatic infants ranging from 10 to 15% (Munro *et al*., 2005; Kenneson and Cannon, 2007; Boppana *et al*., 2013;

Crough and Khanna, 2009). Moreover, symptomatic infection in the case of primary infection (5-15%) is higher than in the case of the secondary infection (1-< 2%). Within the first year of life, this may progress to more severe symptoms, such as vision impairment, sensorineural hearing loss, mental retardation and seizure. Furthermore, asymptomatic individuals could progress to have severe symptoms within the first 4 years in life in 5-17% of the cases (Fujikawa *et al*., 2003; Leung *et al*., 2003; Ornoy and Diav-Citrin, 2006). Also, about half of the symptomatic infected infants and 10% of the asymptomatic develop sensorineural hearing Loss (SNHL) (Boppana *et al*., 2013), which is the single most common manifestation of this infection (Leung *et al*., 2003). However, percentages of mortality were found to be <5% among infants born with HCMV infection (Boppana *et al*., 2013)).

A seven-year prospective study conducted by Griffiths and Baboonian in 1984 investigated the association between the early HCMV infection during pregnancy and foetal death. They found that the incidence of foetal loss occurred because of early primary infection (15.4%) was higher than that caused as a result of secondary HCMV infection (2.2%). In the same study, at age 2 and 4 children developed moderate and severe global retardation respectively; also, microcephaly at age 4 was identified. The central nervous system damage linked with HCMV infection may be explained by a productive replication of the virus that leads to damage in individual cells (lytic infection) or may be due to immunopathology, indirect damage through the action of the immune system (Scheld *et al*., 2004).

2.1.12.3 Infections in immunocompromised patients

Although primary HCMV infection is mostly subclinical, the virus remains latent within the host thereafter. However, amongst immunocompromised individuals such as acquired immunodeficiency syndrome (AIDS) patients and transplant recipients on immunosuppressant medications, the latent virus may reactivate and produce a wide variety of diseases.

In most industrialised countries, before the use of highly active antiretroviral therapy (HAART), HCMV disease was observed in about 40% of AIDS patients with CD4+ counts less than 50 cells/ml (Erice *et al*., 2003). In a study by Verbraak *et al*., (1999) the impact of the introduction of HAART on HIV patients in relation to HCMV was examined. Increasing CD4+ counts from 34 cells/ml to 194 cells/ml were seen after HAART introduction with a significant decrease in the risk of developing CMV disease. However, several studies reported that HCMV infection in HIV patients continued to be a major problem even after HAART was introduced; HCMV retinitis and other CMV end-organ diseases have been described in HAART recipients (Lilleri *et al*., 2003; Springer and Weinberg, 2004; Erice *et al*., 2003; Sugar *et al*., 2012). Drew (1992) conducted a literature review reporting non-pulmonary diseases in immunocompromised patients caused by HCMV such as retinitis, adrenalitis, colitis, esophagitis, hepatitis, and subacute encephalitis. A study by Spector *et al*., (1992) also detected HCMV in plasma of AIDS patients during acute visceral disease. Furthermore, HCMV can also cause pneumonitis in patients taking corticosteroids and immunosuppressant drugs which can be expressed by multiple masses in the patient lung, while in AIDS patients complete lung damage may occur (Ayyappan *et al*., 2006). HCMV could be coupled with Epstein-Barr virus and causes a wide range of neurological disease after reactivation in immunosuppressed patients, such as a cancer chemotherapy population in addition to AIDS and transplant patients (Tselis, 2013). Moreover, HCMV is considered a common source of oral disease in AIDS patients, causing ulceration, canker sores, necrotising gingivitis and periodontal abscess as some examples of these oral infections (Hai *et al*., 2006).

HCMV infection is also one of the most important viral pathogens affecting solid organ transplant recipients, causing considerable morbidity and mortality. The infection is more severe when a seropositive donor donates his/her organ to a seronegative patient because a host-derived cytomegalovirus-specific immune response toward the primary infection is not present. However, it is less severe in

other cases of a seropositive recipient and a seronegative donor or a seropositive recipient and donor assortments, due to the lower viral load in the transplanted organ. It is ideal for a seronegative recipient to receive an organ from a matching seronegative donor, although this is not always possible (Gandhi and Khanna, 2004). According to Brennan *et al*., (1997), latent HCMV infection is detectable in 60%-90% of all renal transplant candidates, and 20%-60% of all recipients become symptomatic three months post-transplantation. A study by Courivaud *et al*., (2013), also performed amongst kidney transplant patients, revealed that human cytomegalovirus exposure contributes to increased risk of developing a cardiovascular disease. The authors attributed this to the ability of the CMV to invade the cardiovascular tissues, stimulate immune responses causing inflammation. Likewise, among immunosuppressed patients, the presence of the HCMV infection activates the lytic replication of Kaposi's sarcoma-associated herpesvirus (Vieira *et al.*, 2001). In general, rejection of an organ, graft dysfunction and secondary fungal and bacterial infections are complaints triggered by HCMV recurrence (Allice *et al*., 2008). Graft rejection associated with CMV has been investigated in different studies, among renal transplants due to renal artery stenosis, heart transplants due to coronary artery stenosis, among lung transplants due to bronchiolitis obliterans and in liver transplants due to vanishing bile duct syndrome (Gandhi and Khanna, 2004).

Aside from solid organ transplant patients, where HCMV infection is usually organ-specific, allogeneic stem cell transplant patients typically have a systemic infection (Tselis, 2013). The probability of HCMV reactivation in a previously seropositive recipient reaches 80%, while in the case of a seronegative recipient and a seropositive donor it is about 30% due to a primary infection. The best ways to decrease the risk of HCMV infection in hematopoietic stem-cell transplants is by using seronegative blood products with a depleted leucocyte count (Gandhi and Khanna, 2004; Crough and Khanna, 2009). Pneumonitis and enterocolitis associated with significant myelosuppression and consequent fungal and bacterial

infections are most common manifestations of HCMV disease seen in the early period post stem cell transplant (<100 days), while in the late period (>100 days) involvement of lung and gastrointestinal tract, retinitis and encephalitis due to HCMV has been observed. However, the introduction of antivirals and the long-term management of patients after the transplant have decreased the risk of HCMV infections in the early period, but not in the late period (Boeckh *et al*., 2003).

2.1.12.4 Potential role of human cytomegalovirus envelope glycoproteins in disease outcome

The availability of a simple test, such as simple restriction-length polymorphism (RFLP) that makes the study of gene strain variation possible, and the availability of the complete sequence of HCMV laboratory strain (AD169) raised interest in studying the variations of HCMV strains and their effect on disease outcome. Even more curiosity was raised in studying the envelope glycoprotein genetic variations and their relationship with disease outcome. As gB is the most common glycoprotein presented on the virus envelope, has a significant role in establishing infection, and is a main target for HCMV neutralizing antibodies, most early studies focused on variation in the gene sequence of gB (Meyer-König *et al*., 1998; Haberland *et al*., 1999; Rasmussen *et al*., 2003).

Rasmussen and colleagues (2003) attempted to determine the variation in glycoprotein genes as a factor in the outcome of HCMV infection. They proposed that intragenic variability is one of the most important factors that could complicate epidemiological studies. Also, the intragenic variability of HCMV strains isolated from clinical specimens indicated that use of gene sequencing unaided is not sufficient in predicting the disease outcome. Thus, identifying strain phenotype might be more significant than the sequence variability as they concluded (Pignatelli *et al*., 2004). Variation within gB gene frequently occurs due to homologue recombination. This recombination may have generated a higher number of non-prototypic gB strains in addition to the non-prototypic strains gB5,

gB6 and gB7 that have been previously published (Meyer-König *et al*., 1998; Haberland *et al*., 1999; Pignatelli *et al*., 2004; Qian and Jin, 2009).

A large and growing body of literature has investigated the relation of the gB HCMV glycoprotein with disease outcome and so virulence. Shepp *et al*., (1996) reported an association between gB2 presence in the blood of AIDS patients and the development of HCMV retinitis. Another recent study by Vogel *et al*., (2013) has supported this finding. Moreover, Woo *et al*., (1997) claimed that more than half of bone marrow transplant recipients with HCMV disease exhibited gB2 genotype, while gB1 genotype was found in viraemic recipients who did not develop HCMV disease. Also, Fries *et al*., (1994) found that the majority of bone marrow recipients with gB1 (67%) survived, whereas only 38% of the patients with the same genotype died suffering from pneumonia. Similarly, another study showed that patients with gB1 and gB2 (2.2%) survived, while others with gB3 and gB4 (21.3%) died due to myelosuppression of the patients (Torok-Storb *et al*., 1997). This may indicate that after bone marrow transplantation gB3 and gB4 genotypes are more virulent than gB1 and gB2. Likewise, a study of HCMV seropositive children showed that either gB1 or gB3 was associated with aminotransferase elevation, plus a significantly longer duration of this elevation was detected with gB1 (Terabe *et al*., 2004). Another study showed that, among Chinese haematopoietic stem cell transplant recipients, gB1 genotype presence was higher than gB3, but gB3 genotype had a greater risk of reactivation than gB1 (Xia *et al*., 2012). A study conducted by Coaquette *et al*., (2004) showed a relation between mixed gB genotypes infection occurred in a high number of transplant patients and the progression of their CMV diseases, such as increasing graft rejection rate and co-infection with other herpesviruses. Moreover, it was proposed that in the case of the ectopic pregnancy, gB1, gB2 and gB3 genotypes were more likely (Qian and Jin, 2009). In addition, gB3 was found to be the most prevalent genotype among congenitally infected symptomatic infants (Gandhoke *et al*., 2013), while other

study reported that no predominant genotype was presented in the samples of the same patients' category (Ross *et al*., 2011).

In contrast, other studies suggested that there is no association between a certain genotype and the disease outcome. A study conducted by Yamamoto *et al*., (2007) reported that intrauterine transmission was found to be not influenced by the HCMV gB genetic variability. Similarly, Paradowska *et al*., (2011) reported no correlation between HCMV genotypes and the disease outcome among congenitally infected newborns. Also, Vilas-Boas *et al*., (2003) found no relation between CNS diseases caused by HCMV and the HCMV gB genotypes among AIDS patients. Furthermore, among liver transplant patients, there was no correlation between a gB genotype and the development of HCMV disease, and the graft rejection as reported by Sarcinella *et al*., (2002). Likewise, Barbi *et al*., (2001) demonstrated that all HCMV gB genotypes could cause congenital infection, yet none appear to be linked with progression and severity of illness. Nonetheless, it was found that gB, gB2 and gB3 are relatively common among liver transplant patients and congenitally infected infants, while gB4 and the mixed genotypes infection were rarely found within the HCMV infected patients (Sarcinella *et al*., 2002; Barbi *et al*., 2001; Gandhoke *et al*., 2013).

Thus, it is still hard to determine whether an association of a specific genotype and HCMV disease severity and progression is present. This might be due to the wide genetic variability of HCMV genotypes and the occurrence of mixed genotype infections, especially among immunocompromised patients. Also, the geographic and demographic status influences the genotype variability and the pathogenicity of the virus. Zipeto *et al*., (1998) in their study claimed that the gB strain distribution might be affected by the patients geographic and demographic differences, as they found gB2 occurrence in Italian homosexual AIDS patients was higher than AIDS heterosexual drug users in Italy and Zimbabwe, plus the gB4 rate in Italian patients was higher when compared to patients in California. Furthermore, alteration of gB may occur due to the host immune pressure, which may affect the

viral binding ability to the host cell receptors and so the cells tropism and virulence, or it may be simply the differences in the genotype gene sequences that affect the cell tropism. This may explain the discrepant results obtained in regard to the gB genotype variability in HCMV patients and their relation to the disease development and progression.

Other studies have focused on studying the relation between the other glycoproteins such as gH and gN genotypes. In a study conducted by Mujtaba *et al*., (2016), both genotypes (gH and gN), in addition to the gB genotype, were found to be prevalent among congenitally infected children. Another study by Paradowska *et al*., (2014) reported that gH may be related to the hearing loss development in congenitally infected children. Also, Madi *et al*. (2011) demonstrated that both gH and gB genotypes and the severity of the HCMV disease are associated.

Furthermore, gN genotypes relation to the severity of the congenital infections at birth and afterwards was investigated in a large study conducted by Pignatelli *et al*., in 2010, they argued that gN1 and gN3a are significantly correlated with reducing the risk of developing HCMV congenital infections, while gN4 with increasing that risk. Similar findings published by Rossini *et al*., (2005) among solid transplant patients supporting that gN4 is more virulent as it was associated with the earlier initiation and increasing levels of HCMV antigenemia. Furthermore, Paradowska *et al*., (2013) have reported that gN genotypes could be associated with the neurological illnesses in HCMV infection new-borns.

2.1.13 Diagnosis and screening

Early detection helps reduce morbidity and mortality amongst patients with HCMV infection. Following a primary or recurrent infection, the virus can be isolated from different body fluids, such as urine, blood, saliva, amniotic and vaginal secretions, or can be recognised in biopsy specimens taken from different infected

organs such as liver or lung. There are different methods available to detect, characterise, and monitor the virus (Bieniek *et al*., 2011).

There is no universal screening protocol for CMV infection in pregnancy. Pregnant women who become infected with CMV are often asymptomatic, but symptoms such as fatigue, lymphadenopathy, and hepatosplenomegaly may be present. Detection of CMV in clinical samples, such as blood, urine and saliva, obtained from the mother and/or infant in the first 2-3 weeks of life, forms the basis of diagnosis (Albanna *et al.*, 2013). When a pregnant woman presents with these symptoms, serological testing such as ELISA for CMV could be performed. Detection of specific IgM antibodies may indicate a primary infection, as they are detectable up to 3-4 months post infection (though in some cases up to 2 years after transplantation) (Kangro *et al*., 1982). However, the presence of rheumatoid factors may influence test results giving false positive results that reduce test reliability (Revello and Gerna, 2002). Test specificity can be improved through the use of tests that measure HCMV- specific IgG avidity; low HCMV IgG avidity indicates a primary infection and high HCMV IgG avidity implies a recurrent infection (Grangeot-Keros *et al*., 1997; Lazzarotto *et al*., 1999).

Currently, most sensitive and rapid clinical results can be provided by the use of molecular methods, such as quantitative polymerase chain reaction (PCR). These techniques allow early detection and monitoring of infection and nucleic acid sequencing for drug resistance testing (Schindele *et al.*, 2010; Härter and Michel, 2012; Ross *et al.*, 2011b). Either PCR for detection of the virus DNA (Brantsaeter *et al*., 2007) or reverse transcriptase PCR or NASBA (nucleic acid sequence based amplification) for RNA (Revello *et al*., 2001) have been used.

Diagnosis of the infection in the foetus usually depends on the detection of the virus in the amniotic fluid using PCR; after the 21st week of pregnancy. A sensitivity (80%) and specificity (100%) of detection can be achieved provided that the test is performed at least 6 weeks after the onset of maternal infection. This is

because detectable HCMV DNA only appears in amniotic fluid 5-7 weeks after the virus starts replicating in the foetal kidney (Liesnard *et al*., 2000).

Cranial ultrasound can also be used for diagnostic purposes in newborns with suspected HCMV and can be a useful prognostic indicator in symptomatic newborns with cerebral abnormalities (Kadambari *et al*., 2011). The long-term follow-up and monitoring of children with HCMV include neurodevelopmental, ophthalmological, and audiological assessments on an ongoing basis throughout the child's development (Kadambari *et al.*, 2011).

2.1.14 Management

2.1.14.1 Antiviral treatment

Ganciclovir (GCV), Cidofovir (CDV), Foscarnet (FOS), Letermovir, Maribavir, and Brincidofovir are antivirals used against HCMV; they function by targeting viral DNA polymerase and prevent virus replication (Razonable, 2011; Härter and Michel, 2012). The current mainstay of treatment CMV infection in most patient settings is Ganciclovir, which is highly active against CMV. It is indicated for the initial treatment of CMV infection and treatment of symptomatic congenital CMV infection, administered in the form of an intravenous infusion. Ganciclovir is a potentially toxic drug and should only be prescribed when the potential benefits of treatment outweigh the risks (Biron, 2006). Some of the GCV side effects involve diarrhoea, rash, increase of the liver enzymes, creatinine levels, and bilirubin, and haematologic effects, such as thrombocytopenia, anaemia and neutropenia (Upadhyayula and Michaels, 2013).

Ljungman *et al*., (2001) investigated CDV efficacy and toxicity in HCMV disease in allogeneic stem cell transplant recipients. More than half of the patients responded well to the therapy, and more than 60% showed no toxicity, while the others developed renal and other toxicities. The study concluded that CDV could be considered as a second line of treatment for HCMV amongst patients who fail to

respond with previous antiviral GCV treatment, especially HIV patients who developed CMV retinitis. CDV side effects include renal dysfunction and nephrotoxicity, in addition to neutropenia, ocular hypotony, and metabolic acidosis (Upadhyayula and Michaels, 2013). FOS as HCMV antiviral drug should be used with caution, especially with patients who suffer even mild renal impairment. It also causes nephrotoxicity, increases the serum creatinine level, and seizures.

There are several drugs that were under clinical development, such as Letermovir, which was approved by the FDA to be used in hematopoietic stem cell transplant patients in 2017, and no resistance was reported among patients in the phase-II clinical trial (Biron, 2006; Härter and Michel, 2012). It has a new mechanism of action interfering with the viral genes that are involved in DNA processing and packaging; Maribavir, which interferes with viral DNA synthesis and nucleocapsid egress from the nucleus to inhibit viral replication, but after considered as promising drug, it has exhibited some limitations in phase III clinical trials maybe because of the too low dosage given to the patients (Snydman, 2011); Brincidofovir, which is a lipid-linked CDV that might be an alternative to the CDV as it has a lower nephrotoxicity.

2.1.14.2 Vaccination

A major goal in HCMV prevention programme is to develop vaccines that can protect seronegative individuals, especially pregnant women (Revello and Gerna, 2002). Considerable attempts have been made to develop efficient HCMV vaccines. CMV live attenuated vaccines were one of the first vaccine types developed using AD196 and Towne strains, the latter showed a significant decrease in disease severity among renal transplant recipients but did not completely prevent the disease (Plotkin *et al*., 1990; Brennan, 2001). Due to the variation observed in the virus genome, development of recombinant virus vaccines has emerged, where four CMV strains are recombined to induce a virus-specific cellular response (Revello and Gerna, 2002). Other attempts to use subunit vaccines have been made delivering

recombinant proteins representing the major antigenic viral components pp65, pp150, gB and gH (Revello and Gerna, 2002).

Considerable effort has been made to develop vaccines against HCMV envelope glycoproteins, due to the role they play in developing the infection. So far, none have proved to be completely effective in preventing infection (Revello and Gerna, 2002). However, a vaccine based on recombinant HCMV glycoprotein B was shown to be about 50% effective in reducing the incidence of maternal and congenital HCMV infection (Pass *et al*., 2009) and similarly encouraging results were found for the vaccine in organ transplant recipients (Snydman *et al*., 1993; Griffiths *et al*., 2013). In humans infected with HCMV, specific neutralizing antibodies to the virus, which react with the HCMV envelope glycoproteins M and N are produced (Shimamura *et al*., 2006). Immunization of mice with the mouse CMV homologs of glycoproteins M and N as a complex gave complete protection against challenge with mouse CMV (Wang *et al*., 2013) suggesting that HCMV glycoproteins M and N could potentially also be used as an HCMV vaccine.

At present, no vaccines exist for the prophylaxis of CMV infection in pregnant women. Previous CMV vaccines provoked inadequate immune responses in subjects, possibly due to the lack of the antigenic glycoprotein H (gH) complex, essential for mediating viral entry into the host cell. Current efforts are focused on this pentameric gH construct (gH, gL, UL128, UL130 and UL131 proteins) due to its ability to induce a range of neutralizing antibodies in the host to combat the infection. The gH complex is an ideal vaccine target as it is conserved among clinical isolates, and preliminary *in vivo* studies are generating promising results (Freed *et al*., 2013; Fu *et al*., 2012; Genini *et al*., 2011).

However, the use of HCMV specific hyperimmune globulin prophylaxis, which has neutralizing antibodies activity in pregnant women with primary HCMV infection has proven to reduce the intrauterine CMV transmission and the symptomatic foetal infection as reported by Nigro *et al*., in 2005 and Buxmann *et al*., in 2012 and

2017. In contrast, a study conducted by Revello *et al*., (2014) reported that the hyperimmune globulin has no significant effect on the primary infection during pregnancy.

2.1.14.3 Prevention

It is difficult to prevent the spread of HCMV due to its ubiquitous nature in the population; most individuals will come in contact with the virus at some point in their lives. The most common primary source of HCMV infection is young children. In some cases, a woman may acquire the infection from her own children, who may have been exposed to the infection in-group day care. Through adopting simple hygiene measures such as hand washing and avoiding the close contacts with an unknown HCMV serostatus children, as recommended by the Centre for Disease Control and Prevention (CDC), can cut the infection rates in pregnant women dramatically (Cheeran *et al*., 2009), especially in the case of the seronegative women (Ross *et al*., 2008). Additionally, awareness and knowledge about HCMV prevention measures should be available to all women by their healthcare providers regardless of their serostatus.

2.2 Glycosylation

2.2.1 Glycans

It is well known that glycans play an important role in the metabolic, functional and the structural properties of biological systems. They are large biological molecules consisting of carbon, hydrogen and oxygen with a general chemical formula of $C_n(H_2O)_n$. They can be classified into several groups according to the number of sugar units: Monosaccharides (the simplest form of sugars), disaccharides (two monosaccharides linked together via a glycosidic bond), oligosaccharides (up to ten monosaccharides linked via glycosidic bonds), and polysaccharides (more than ten monosaccharides). Monosaccharides are classified

into aldoses (have an aldehyde group- carbonyl group at the end of the carbon chain) or ketoses (have a ketone group- carbonyl group in the middle of the carbon chain). Monosaccharides are referred to as trioses when they consist of 3 carbon atoms; Tetroses consist of 4 carbon atoms; Pentoses consist of 5 carbon atoms, or hexoses if they consist of 6 carbon atoms. The glycosidic bonds link an anomeric carbon of one monosaccharide to a hydroxyl group of another monosaccharide; it is either an alpha (α) linkage (CH_2OH group is on an opposite side of OH group within the molecule structure- CH2OH on the top and OH on the bottom- of the ring) or a beta (β) linkage (CH2OH group and OH group are on the same side of the molecule structure- top of the ring) (Varki *et al*., 2009).

There are several common types of glycans present in nature. However, most of them are found in plants, and only limited numbers can be found in humans. Some examples of human glycans are: hexoses such as D-glucose (Glc), D-galactose (Gal), D-mannose (Man); hexosamine such as N-acetyl-D-glucosamine (GlcNAc) and N-acetyl-D-galactosamine (GalNAc); Deoxyhexoses, such as L-fucose (Fuc); Sialic acids (Sia), such as N-acetylneuraminic acid (Neu5Ac). The overall configuration of a monosaccharide is either D, when the OH group is on the right side of the Fischer projection (two-dimensional representation of 3-Dimensional organic molecule), or L when the OH is on the left side (Varki *et al*., 2009).

2.2.2 Protein glycosylation

After translation and releasing from ribosomes in ER, most proteins undergo post-translational modification (protein glycosylation) to be completely functional. Glycosylation of proteins is an important process in various stages of the virus replication cycle and has a vital role in stability, proper folding (Newrzella and Stoffel, 1996), trafficking, pharmacodynamics, pharmacokinetics and immunogenicity of glycoproteins (Varki *et al*., 2009; Aebi *et al*., 2013). The two common types of glycosylation can be accomplished by the action of different enzymes activity called glycosyltransferases, which are specific for targeting

oligosaccharides and adding of sugar residues from a donor to a recipient to form either linear or branched glycan chains (Varki *et al*., 2009;). N-linked glycosylation takes place in both ER and Golgi apparatus, and is considered as a base for further glycosylation and can be used as an indicator of protein-folding errors. O-linked glycosylation begins after transporting of proteins, via vesicle transport, into the Golgi apparatus using Golgi-specific enzymes such as GalNac transferase that links an N-acetylgalactosamine to a polypeptide hydroxyl group. Around 50% of glycoprotein mass is acquired through this type of glycosylation (Lodish *et al*., 2000; Lepenies, 2015).

To what extent the protein structure is modified by glycosylation, what class of glycans are attached (via N or O linkages), and which glycosylation-associated cellular enzymes, are used differs considerably. However, most proteins are glycosylated through N- or O-linked glycans (N-linked or O-linked oligosaccharides), with N-linked oligosaccharides being more complex, commonly having several branches that contain mannose and N-acetylglucosamine and terminate with a sialic acid residue. N-linked oligosaccharides are linked to an asparagine residue (Asn) of a polypeptide via the amide nitrogen, in a conserved sequence (Asn-an amino acid-Ser/Thr). In contrast, O-linked oligosaccharides are shorter, frequently containing between one and four sugar residues, with oligosaccharides being linked via N-acetylgalactosamine to the hydroxyl group of threonine (Thr) or serine residues (Ser), or to a hydroxyl group of hydroxylysine (Hyl) residues through galactose as in collagen (Figure 7) (Lodish *et al*., 2000).

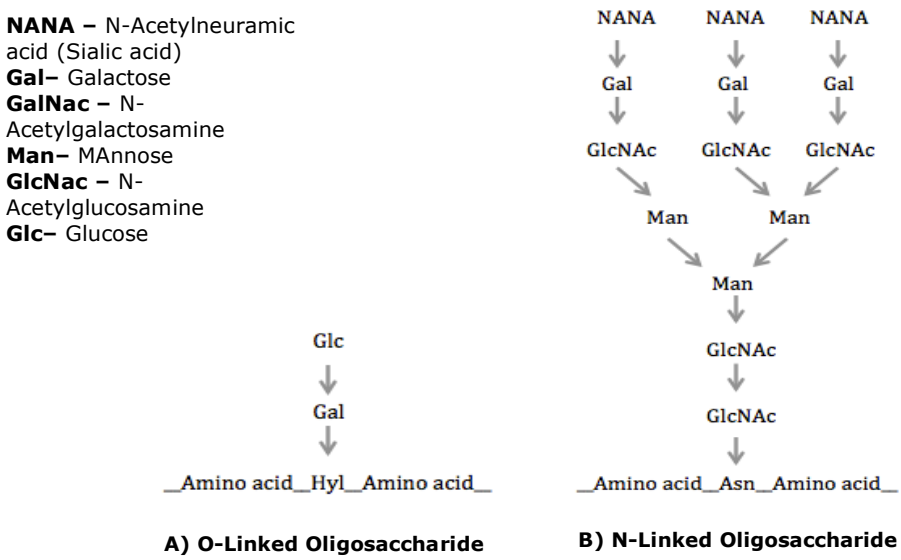

Figure 7: Structures of O-Linked oligosaccharide and N-Linked oligosaccharide (Lodish *et al.*, 2000).

In Herpesviruses, both N and O linked oligosaccharides are found. For example, O-linked oligosaccharides are predominantly found in HSV-2 envelope glycoprotein G (Dall'Olio *et al*., 1987), whereas N-linked oligosaccharides were demonstrated in the HCMV glycoprotein UL18 (MHC-I homologue) (Griffin *et al*., 2010). Infrequently, in some cases, viral glycoproteins are glycosylated by attaching to a glycolipid molecule, called glycosylphosphatidylinositol; anchored via their C-terminus site (Protein-Glycan Chain-lipid anchor). One example of such a virus is dengue virus (Jacobs *et al*., 2000).

The combination of the glycans with other macromolecules to form glycoconjugates, such as glycoproteins, lead to the introduction of significant heterogeneity in the protein composition. As glycans undergo a large number of structural modifications, they consequently have a great impact on protein functions (Aebi *et al*., 2013). In nature, all cells contain carbohydrates (glycans) that have essential biological roles depending on their physical properties, such as shape, mass and charge. Many of these glycans present on the cells surface, which enable them to play a vital role in the interactions between the cells and the surrounding of matrix, which is essential for the function and development of

complex multicellular organisms, and in the interaction between the two different organisms (Varki *et al*., 2009).

Glycosaminoglycans (GAGs) are a linear, heterogeneous, complex and high-density type of glycans (Sugrue, 2007), ubiquitous in nature surrounding all types of cell surfaces and in the intracellular matrix area. They play a critical role in facilitating the interaction between the host cell and the infectious virus during early periods of infection. Thus, understanding of this interaction, how it occurs and whether it influences the viral glycoprotein function is essential. This understanding could open a gate to discover novel carbohydrate-based drug therapies (Kamhi *et al*., 2013). For instance, heparan sulphate glycosaminoglycans (HSGAGs), the most studied subtype of GAGs, act as receptors of many acceptors, such as HCMV envelope glycoproteins (Kari and Gehrz, 1993). The enzymatic process in which the viral envelope glycoproteins become attached to HSGAGs receptors is a type of glycosylation (Sasisekharan and Myette, 2003). Also, herpes simplex virus type 1 (HSV-1), another herpes family member, binds and enters the cell surface through envelope glycoprotein interaction with the cell surface glycans. Both HSV-1 glycoprotein B and C interact with HSGAGs on the cell surface while glycoprotein D binds to specific HSGAGs sequence to facilitate the virus entry and fusion. This binding needs a specific enzyme for an addition of a specific sulphate molecule and chain of four disaccharides to the glycan chain, cells lacking this enzyme are found not infected (Sasisekharan and Myette, 2003). This confirms the vital role of glycosylation in the virus pathogenicity.

Glycosylation can be inhibited selectively through suppression of specific enzyme activities that mediate the process, for example (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVdU), which was found to inhibit both N- and O-linked oligosaccharide glycosylation reaction in HSV-1 and HSV2 through phosphorylation to its 5‵monophosphate (BVdUMP). The BVdUMP, in turn, prohibited the transport of pyrimidine nucleotides through Golgi membrane and thus the sugars incorporation into the virus glycoproteins (Olofsson *et al*., 1988). Also, benzhydrazone, is a

herpesviruses specific glycosylation inhibitor as described by Serafini-Cessi and Campadelli-Fiume in their report (1981).

Modification of glycosylation could arise through changes on sites where the glycan link to a protein, glycan assembly (branched or unbranched chains and their length), carbohydrate type to which protein will attach, as in the case of the N-linked glycosylation, and/or the glycan molecular mass (increase or decrease) in the O-linked glycosylation (Sugrue, 2007; Patton, 2002).

2.2.3 Methods to analyse and detect glycoprotein glycosylation

There are different generic techniques to detect viral glycoproteins: Radioactive detection, where the infected cells are incubated in a glucose-free medium followed by addition of a radio-labelled monosaccharide. This is a sensitive yet slow, potentially hazardous and expensive technique (Sugrue, 2007; Patton, 2002). Non-radioactive detection includes using conventional stains (Coomassie blue, silver stain) or with different fluorescence stains. This is as sensitive and slow as a radioactive detection method but less expensive. Another divergent technique termed fluorophore-assisted carbohydrate electrophoresis (FACE) is used. In FACE, glycans are labelled with a fluorescence tag after being removed from the proteins and then resolved on a polyacrylamide gel. This procedure is affected by heating and pore size. Amongst these, the radioactive detection has perhaps been the most commonly used method to detect glycoproteins. This method was the strategy implemented by Gretch *et al*., (1988b) to detect and characterise HCMV GC-I.

Viral glycoproteins are analysed by the use of different enzymes. The most commonly used are glycosidases, which removes the entire glycan chain from the glycoprotein or cleaves it at a specific site. Most of these enzymes process the N-linked glycans, such as PNGase F (N-Glycosidase F), which removes the complete glycan chain; Endoglycosidase H, which targets N-glycan structure can be used to determine glycan chain complexity, as complex glycans are resistant to it. Further specificity in cleaving the glycan structure is achieved by Endo F1, F2 and F3 or

sigma enzymes and fucosidase. Moreover, N-acetyl-hexosaminidase, N-acetyl-galactosaminidase, and neuraminidase eliminate terminal N-acetyl-galactosamine, N-acetyl glucosamine, and sialic acid from a glycan chain, respectively. Enzymes targeting O-linked glycan have also been identified, such as endo-a-N-acetylgalactosaminidase that targets the N-acetylgalactosamine linkage (Sugrue, 2007).

O-linked glycosylation can be analysed by mass spectrometry, but due to the considerable diversity of oligosaccharide and proteins, it is a complicated and a time-consuming technique (Peter-Katalinić, 2005), although it is very useful for elucidating the details of carbohydrate structure (Pilobello *et al*., 2005). Capillary electrophoresis has been combined with mass spectrometry for monitoring of the recombinant glycoproteins. Although some enhancement in the sensitivity of this technique has been attained, it has several drawbacks, such as requiring high sample concentrations and the poor sensitivity of the technique interfaces (Volpi *et al*., 2009). Also, liquid chromatography and nuclear magnetic resonance are other techniques used for glycosylation detection and analysis; they need expensive special equipment and highly skilled personnel (Thompson *et al*., 2011; Pilobello and Mahal, 2007).

Techniques based on glycan-binding proteins (GBP), such as lectins, which are known to recognise specific glycans structures, are also available (Pilobello *et al*., 2005; Varki *et al*., 2009). Lectin histochemistry has been used to detect and visualise glycan expression and the changes in glycosylation (Leathem and Brooks, 1998; Malkinson *et al*., 1986). Also, detection and quantification of specific glycans can be achieved using lectin microarray (Fais *et al*., 2009; Pilobello *et al*., 2005). Enzyme linked lectin-sorbent assay (ELLA) assay has been recently developed from a previously used technique described in the literature by Leathem and Brooks (1998). It adopts the same principle (protein- glycan binding) but gives quantitative results instead of qualitative data (Phung, 2011; Bala, 2010).

2.2.4 Lectins

Lectins are carbohydrate-binding proteins or glycoproteins that are not induced as a result of an antigenic stimulus within the immune system. The term lectin, which means to select or to pick, acquired from the lectins' capacity to select and bind to specific glycan structures (Cummings and Etzler, 2009; Varki *et al*., 2009). They are naturally abundant and can be derived from several sources, plant, animals or microbes. Lectins have mainly been classified according to their amino acid sequence homology and biochemical properties, some of these types are: C-type lectins, which require calcium for recognition,; Galectins, which bind to β-galactose- containing glycoconjugates; P-type, which bind to Man-6-P; I-type, which are immunoglobulin members; Siglecs, which recognize sialic acid and consider a subgroup of the immunoglobulin family members (Varki *et al*., 2009). Moreover, based on their affinity to specific glycans, lectins have been classified into 5 groups, and these are: glucose/mannose, galactose, N-acetyl-D-galactosamine (GalNAc), N-acetylglucosamine (GlcNAc), L-fucose, and sialic acids. Generally, most of the lectins bind to complex carbohydrates, as they possess two or more glycan-binding sites (Varki *et al*., 2009; Slifkin and Doyle, 1990). Lectins have different biological roles as adhesions and agglutinations. Microbial lectins play an important role in the host-pathogen interaction, and food lectins, such as the tomato lectin, resist the denaturation caused by some enzymes by binding to mucosal cells. Based on their biological properties, investigators have used lectins in different aspects of research. For example, blood grouping, a study of cell surface structures and functions, acquiring information about the presence of a specific glycan structure and/or the position of specific carbohydrate residues within a polysaccharide molecule (Slifkin and Doyle, 1990).

CHAPTER 3

3 Materials and methods

3.1 Materials

3.1.1 Cells culture and viral Stocks

Minimal Essential Medium (MEM) Glutamine, Penicillin/Streptomycin (10,000 I.U/ml Penicillin/ 10.000 µg/ml Streptomycin), Antibiotic Antimycotic, Phosphate buffered Saline (PBS), Trypsin, Phenol Red, Trypan Blue, Dimethylsulphoxide (DMSO) - All purchased from Sigma-Aldrich, Poole, Dorset, UK.

Fast-Read 102 disposable counting chamber purchased from Immune Systems, UK.

Foetal Calf Serum (FCS) purchased from Biosera, East Sussex, UK.

70% Industrial Methylated Spirit (IMS), Methanol purchased from Fisher Scientific, Loughbrough, UK.

Liquid Nitrogen Freezer, Liquid Nitrogen Ampolues, Nalgene Cryo Freezing Container (special freezing block).

MRC-5 cell line stock from 8/9/2009 (Culture Collections, Public Health England).

75cm^2 plastic tissue culture flasks piurchased from Greiner Bio -One, UK.

16 X 110 mm screw caped Cell Culture Tubes Nunc™ purchased from ThermoFisher Scientific, Paisley, UK.

3.1.2 Viral DNA extraction

QAamp MinElute virus spin kit (QIAGEN) contains (QIAamp MinElute columns, collection tubes, Buffer AL, buffer AW1, Buffer AW2, Buffer AVE, Carrier RNA, and QIAGEN protease) purchased from QIAGEN Ltd., Manchester, UK.

Methanol, Ethanol purchased from Fisher Scientific, Loughbrough, UK.

Ultra-Pure Distilled water (UPDW) purchased from Invitrogen by Life Technologies, Paisley, UK.

3.1.3 Polymerase chain reaction (PCR)

Amplitaq Gold 360 Master Mix with Gene Amp 10X PCR buffer, TaqMan™ Multiplex Master Mix, all purchased from Applied Biosystems by Life Technologies, Warrington, UK.

2X MyTaq HS Mix purchased from Bioline, London, UK.

Oligonucleotide primers and HCMV Probe purchased from Eurofins, UK, (Table 1).

Deoxyribonucleotide triphosphates (dNTPs), E-Gel^{PowerBase} Version 4, E-Gel Agarose Gels, UPDW, Blue juice-loading buffer, 1kb plus DNA ladder- All purchased from Invitrogen, Paisley, UK.

PCR standards for HCMV virus ordered from Eurofins Genomics, Ebersberg, Germany.

Table 1: Oligonucleotide primers sequences used in HCMV PCR reaction.

Primer	Primer Sequence (5'>3')	Gene	Amplicon Size	Reference
P2	5'-TCGCTGTCTTCGACCGGTGA-3'	PP	194 bp	McElhinney *et al*., 1995
724C	5'-AAGAATCCTCACCTGGCTTA-3'			
gB1319	5'-TGGAACTGGAACGTTTGGC-3'	gB	305 bp	Chou and Dennison,

gB1604	5′-GAAACGCGCGGCAATCGG-3′			1991
gM509JE	5′-GCTCAAACCGCGTCGTGA-3′	gM	1298 bp	Ellis, 2006
gM1801JE	5′-ACGGTCTGCGTGTCTCTT-3′			
gNup	5′-TGGTGTGATGGGTGGAAC-3′	gN	420 bp	Pignatelli *et al*., 2003
gNlow	5′-TAGCCTTTGGTGGTGGTTGC-3′			
gH203	5'-CCACCTGGATCACGCCGCTG-3′	gH	215 bp	Chou, 1992
gH172	5′-TGGTGTTTTCACGCAGGAA-3′			
115upout	5′-TTGATGTGCCGCCGCCCGGAT-3′	gL	555 bp	Rasmussen *et al*., 2002
115loout	5′-GCACCAGCTCGAAGCCTAAC-3′			
UL74ZB5	5′-CGTTGGAACACCAAATTGTA-3′	gO	840 bp	Buhamad, 2018
UL74ZB3	5′-ACCAAAGGCTATTGAGGGTG-3′			

3.1.4 E-Gel Electrophoresis and Restriction Fragment Length Polymorphisms (RFLP)

Restriction enzymes (RsaI, HinfI, HpaII, StuI, TaqI, SacI, ScalI, and SalI) and their appropriate cut buffers, Ultra-Pure Distilled water (UPDW), purchased from Invitrogen (Table 3).

Restriction enzymes (RsaI, EarI, BfaI, ApoI, BanII and HhaI) and their appropriate cut buffers, Ultra-pure TBE buffer (10X), 1 kb plus DNA ladder, E-Gel™ Agarose Gels with SYBR™ Safe DNA Gel Stain, 1.2%, Novex® TBE gels, purchased from ThermoFisher Scientific (Table 2).

25bp Hyper ladder, 50bp Hyper ladder purchased from Bioline.

Gel Red nucleic acid gel stain purchased from Biotium, Cambridge, UK

Table 2: Restriction enzymes used in RFLP assay.

Glycoprotein gene	Enzyme	Reference
gB	RsaI HinfI	Chou and Dennison, 1991
gH	HpaII StuI	Sowmya and Madhavan, 2009
gL	RsaI TaqII	Sowmya and Madhavan, 2009
gM	EarI BfaI	Ellis, 2006; Buhamad, 2018
gN	SacI ScaI SalI	Pignatelli *et al*., 2003
gO	ApoI BanII HhaI	Buhamad, 2018

3.1.5 Enzyme linked lectin-sorbent assay (ELLA)

Biotinylated lectins (Table 3), Tween 20%, Tetra MethylBenzidine (TMB) substrate, Hydrogen peroxide, avidin/peroxidase conjugate– All purchased from Sigma-Aldrich, Poole, Dorset, UK.

96-well plate (flat bottomed) purchased from Greiner Bio-One, UK.

ELISA plates washer and reader purchased from BioTek, Swindon, UK.

Tris-buffered saline, HCL purchased from Fisher Scientific, Loughbrough, UK).

Sulphuric acid (H_2So_4) purchased from Sigma-Aldrich.

19 Biotinylated lectins (Table 4), NovaRed (Vector Labs), Methyl Green – All purchased from Vector Labs, UK.

Table 3: Biotinylated lectins used (20) and their binding specificity.

Source of Lectin	**Source**	**Acronym**	**Sugar Specificity**
Soybean agglutinin	Glycine max (soybean) seeds	SBA	α> βGalNAc
Erythrina cristagalli	*Erythrina cristagalli* (Coral Tree) seeds	ECL	Galβ4GlcNAc
Lens culinaris	*Lens culinaris* (lentil) seeds	LCA	αMan, αGlc
Wheat Germ	*Triticum vulgaris* (wheat germ)	WGA	GlcNAc
Griffonia (Bandeiraea) simplicifolia II	*Griffonia (Bandeiraea) simplicifolia* seeds	GSL-II	α or βGlcNAc
Sambucus nigra	*Sambucus nigra* (Elderberry) bark	EBL	Neu5Acα6Gal/GalNAc

Phaseolus vulgaris Leucoagglutinin	*Phaseolus vulgaris* (Red Kidney Bean) seeds	PHA-L	Galβ4GlcNAcβ6(GlcNAcβ 2Manα3) Manα3
Galanthus nivalis	*Galanthus nivalis* (Snowdrop) bulbs	GNL	αMan
Lycopersicon esculentum	*Lycopersicon esculentum* (Tomato) fruit	LEL	$(GlcNAc)_{2-4}$
Bauhinia purpurea	*Bauhinia purpurea alba* (Camel's Foot Tree) seeds	BPL	Galβ3GalNAc
Ulex europaeus	*Ulex europaeus* (Furze Gorse) seeds	UEA	αFuc
Euonymus europaeus	*Eunonymus europaeus* (Spindle Tree) seeds	EEL	Galα3Gal
Wistera floribunda	*Wisteria floribunda* (Japanese Wisteria) seeds	WFA	GalNAc
Phaseolus vulgaris Erythroagglutinin	*Phaseolus vulgaris* (Red Kidney Bean) seeds	PHA-E (PVL)	Galβ4GlcNAcβ2Manα6 (GlcNAcβ4) (GlcNAcβ4Manα3) Manβ4
Amaranthus caudatus	*Amaranthus caudatus* seeds	ALL (ACL)	Galβ3GalNAc
Psophocarpus tetragonolobus I	*Psophocarpus tetragonolobus* (Winged Bean) seeds	PTL-I	GalNAc, Gal
Maackia Amurensis II	*Maackia amurensis* seeds	MAA-II	Neu5Acα3Galβ4GalNAc
Aleuria aurantia	*Aleuria aurantia* mushrooms	AAL	Fucα6GlcNAc
Griffonia (Bandeiraea)		GSL-I	αGal, αGalNAc

simplicifolia I	*Griffonia (Bandeiraea) simplicifolia* seeds		
Pisum sativum	*Pisum sativum* (Pea) seeds	PSA	αMan, αGlc

Sugar Abbreviations: Fuc: L-Fucose, **Gal:** D-Galactose, **GalNAc:** N-Acetylgalactosamine, **Glc:** D-Glucose, **GlcNAc:** N-Acetylglucosamine, **Man:** Mannose, **Neu5Ac:** N-Acetylneuraminic acid (sialic acid), **SA:** Sialic Acid (Table of Lectin Properties. Vector Laboratories. (2012)).

3.2 Methods

3.2.1 Cell culture

3.2.1.1 Cell culture media preparation

For growth medium preparation, 500 ml of Eagle's Minimum Essential Medium (MEM) was used, this was supplemented with 10% Foetal Calf Serum (FCS) as a growth factor, 5ml Glutamine, 5 ml of Antibiotic antimycotic solution (100X) stabilized with 10,000 units/ml Penicillin, 10 mg/ml Streptomycin and 25 µg/ml amphotericin B. For Maintenance Medium preparation the same components were used but FCS was reduced to 2%. Both were stored at +4°C.

3.2.1.2 Sterility test

To test the medium for any indication of microbial growth, a sterility test was done by adding 1 ml of the medium to each of 4 bottles, 2 Brain Heart Infusion (BHI) and 2 Sabouraud (Sab). One bottle of each was incubated at 37°C with CO_2 for 3-4 days and the other two bottles kept at room temperature for several days. All bottles were examined daily.

3.2.1.3 Cultivation of cells

Once a confluent monolayer cell sheet (95%-100% confluence) was formed in a 75cm^2 plastic tissue culture flask and observed using an inverted microscope, cells were washed and trypsinised twice with 5 ml trypsin in 80 ml of phosphate buffered saline (PBS), and then incubated at 37°C for 3-4 minutes. Once seen under the microscope to be rounded and detached, the cells were suspended in 10 ml of growth medium supplemented with FCS, antibiotics and glutamine. The suspended cells were counted and resuspended with the growth medium in: a 75cm^2 plastic tissue culture flask (total volume of 25 ml), a 96 wells plate (total volume of 10 ml (0.1 ml for each well), and screw caped cell culture tubes (total volume of 3 ml)). The sub-cultured cells were incubated again at 37°C and left for 3-4 days until a confluent monolayer of the cell was formed again.

3.2.1.4 Counting of cells

Following washing and removing the cells from the flask using trypsin in PBS, cells were resuspended in 10 ml of the growth medium. After that, a dilution of the suspension (0.2 ml) and Trypan blue stain (0.1 ml) was prepared. Then, 0.1 ml of the dilution was filled into one of the chambers of the Fast Read 102 Disposable Counting Chamber using the pipette tip. Using microscope, the cells were counted in the four corner squares (dead cells stained with Trypan blue and were not counted). The total number of cells in all four squares was divided by 4 and multiplied by 10^4 and multiplied further by 3/2 to give the number of cells/ml in the original cell suspension. The cells were seeded at 1×10^5 (flasks) or 2×10^5 cells/ml concentration (Plates).

3.2.1.5 Freezing of HEL (MRC-5) cells

For freezing medium preparation, 10% of dimethyl sulphoxide (DMSO) with FCS was added. Cells were removed from the flask by trypsinisation (as previously described in section 2.1.3). Cells were resuspended in 5ml of growth medium in a 50

ml centrifuge tube. The suspension was centrifuged at 1500 rpm for 5 minutes. The pellet was taken and resuspended in 1 ml of freezing medium and transferred to a special freezing ampoule to be placed inside a special freezing block at 4°C. The freezing block was transferred to -80°C for about 4 hours. Then the ampoule was transferred to the liquid nitrogen freezer at - 196°C for long-term storage.

3.2.1.6 Thawing of HEL (MRC-5) cells

The ampoule was taken from the liquid nitrogen freezer and placed in a beaker of warm water at 37°C for the cells to be thawed. Once thawed, the ampoule was removed from the water and dried using a paper towel. The outside of the ampoule was washed using 70% ethanol before opening. The content was transferred using a plastic Pasteur pipette to a $75cm^2$ cell culture flask. Approximately 25 ml of growth medium was added to the flask, which was then incubated at 37°C with 5% CO_2 to be observed daily until a confluent monolayer sheet was formed.

3.2.1.7 Infection MRC-5 cells

Growth medium was discarded from the flask into a plastic waste beaker once the cells were 90% confluent. About 0.5 ml of human cytomegalovirus strain (unknown concentration), was added. The flask ($75cm^2$) was incubated at 37°C with 5% CO_2 for an hour for the virus to be adsorbed and rocked every 15 minutes. 10 ml of the maintenance medium was added. The flask was re-incubated at 37°C with 5% CO_2, with replacing of the maintenance medium every 2-3 days until cytopathic effect (CPE) was observed. Each strain with the known concentrations was used for infecting cells grown in 96 well plates using the same principle. The same process was repeated for infecting the cells with all laboratory strains (AD169, Towne, Toledo, Merlin, and clinical samples. For the clinical samples, the cell culture tubes were used, 3 ml ($2X10^5$) of the cells suspension was added until the cells are confluent, 3 ml of the maintenance medium was used, then infected with 10,000 TCID 50 of the viral strain).

3.2.1.8 Harvesting the virus and preparing a stock of HCMV

The frozen flask was thawed at room temperature, shaken vigorously, re-frozen for 2 hours at -80°C. After being subjected to another 2 cycles of thawing-freezing, the contents of the flask were transferred to a centrifuge tube and centrifuged at 1500 rpm for 5 minutes; the supernatant was transferred to a universal tube and aliquoted into small tubes at 1ml volumes. These were kept frozen at -80°C and then at -196°C until needed for the assays.

3.2.1.9 Virus infectivity titration (Determination of 50% tissue culture infectious dose ($TCID_{50}$))

Cells were trypsinized and counted and the concentration adjusted to 2×10^5 cells/ml using 10 ml of the growth medium. About 0.1 ml of the cell suspension was added to each well of a 96 well microtiter plate and incubated at 37°C with 5% CO_2. After 24 hours, a confluent monolayer was formed. Using 0.9 ml of maintenance media and 0.1 ml of the HCMV strain, a serial ten-fold dilution series was prepared from 10^{-1} to 10^{-11} in Bijou bottles. The growth medium was discarded from each well using a multichannel pipette. To each well of the 96 well microtitre plate, 0.1 ml of each virus dilution was added (8 wells from A to H per dilution). In the last column (Number 12) of the plate, 0.1 ml of the maintenance media with no virus was added as a control. The plate was incubated at 37°C with 5% CO_2 for several days (3-5) until CPE was observed. For each strain, the number of wells with apparent CPE was recorded and $TCID_{50}$ was calculated using Spearman–Karber formula (Log_{10} Median Dose= X_0- (d/2) + d (Σ (r_i/n_i)).

X_0 is the highest dilution where all the wells are positive

d is the difference between the log dilution intervals (1)

n_i is the number of wells used for each dilution (8)

r_i is the number of the positive wells showing CPE in each dilution

r_i/n_i is the proportion of the positive wells (P).

The same process was repeated for all viral strains used.

3.2.1.10 Fixation of MRC-5 cells and MRC-5 infected cells

Cells were trypsinized and counted with a concentration adjustment of 2×10^5 cells/ml using 10 ml of the growth medium. About 0.1 ml of the cell suspension was added to each well of a 96 well microtitre plate and incubated at 37°C with 5% CO_2. After 24 hours, a confluent monolayer was formed. The growth medium was discarded from each well using a multichannel pipette and replaced by 0.1 ml of the virus inoculum of 10,000 $TCID_{50}$ and incubated at 37°C with 5% CO_2. The plate was incubated at 37°C with 5% CO_2 for several days (3-5) until CPE was observed. The medium was discarded and replaced by 0.1 ml methanol in each well, incubated at room temperature for 1 hour, rinsed with distilled water, and left to dry at room temperature. The same process was repeated for all the strains of HCMV.

3.2.2 Real time polymerase chain reaction (PCR)

3.2.2.1 Viral DNA extraction

The nucleic acid extraction kit (Qiagen) was used as instructed by the manufacturer. Reagents were prepared as follows: 200 µl of the virus suspension (Stock) was prepared as previously described (section 3.2.1.8). The protease was dissolved by heating at 65°C in 1.4 ml of AVE buffer, 310 µl of AVE buffer was added to the tube contains 310 µg lyophilized carrier RNA to have a solution of 1 µg/µl, both were mixed gently to avoid foaming. Then, 6.16 µl per sample of carrier RNA solution was added to 220µl per sample of the AL buffer. The AW1 and AW2 buffers were prepared by adding 25 ml and 30 ml of cold absolute ethanol, respectively, to 19 ml of both buffers and then mixing by shaking. Then, 25 µl of the protease was added into a 1.5 micro-centrifuge tube, 100 µl of each virus suspension was added to each tube containing 100 µl of 0.9% Sodium chloride. Next, 200 µl of buffer AL (28 µg/ml of carrier RNA + lysis buffer) was added and mixed well for 15 seconds using a vortex mixer. The mixture was then incubated in a heating block at 65°C for 15

minutes and centrifuged for 1 or 2 minutes to remove any drops present inside the lid. 250 µl of cold Ethanol was added to each sample column, followed by vortexing for 15 seconds, and then incubated at room temperature for 5 minutes. The mixture was transferred to QIAamp MinElute columns (provided with the kit) and centrifuged at 8000 rpm for 1 minute. Then, the columns were placed in clean 2 ml collection tubes, and the ones containing the filtrate were discarded. After that, 500 µl of buffer AW2 was added to each column, closed and centrifuged at 8000 rpm for 1 minute. Again, the columns were placed in clean 2 ml collection tubes, and the ones containing the filtrate were discarded. Next, 500 µl of cold ethanol was added to each tube, before centrifuging at 14000 rpm for 3 minutes to dry the membrane completely, the columns were placed in clean 2 ml collection tubes, and the ones containing the filtrate were discarded. The columns were transferred to new collection tubes and incubated for 3 minutes in the heating block at 65°C. Then the columns were placed in 1.5 ml sterile microcentrifuge tubes (Eppendorf tubes), 150 µl of buffer AVE was added to each tube and centrifuged at 14000 rpm for 5 minutes. The mixture then was transferred to clean 1.5 ml microcentrifuge tubes and the ones containing the filtrate were discarded. Lastly, the tubes containing the nucleic acid were stored at – 20 °C until needed. The same process was applied to extract the DNA of all HCMV strains.

3.2.2.2 Conventional PCR

PCR mixture was prepared for each genotype of all laboratory strains and clinical specimens by pipetting the following reagents together in a 1.5 ml sterile micro-centrifuge tubes (Eppendorf tube): 25 µl of AmpliTaq Gold 360 master mix or 2X my taq HS mix in case of gO genotype, 1µl of each forward and reverse primers (each genotype with its assigned primers) (Table 5), 18 µl of sterile distilled water (SDW). 45 µl of the mixture was aliquoted in each PCR tube needed (depends on the number of samples and genotypes including negative and positive controls). After vortexing, the tubes 5 µl of the PCR products was added to each tube (each laboratory strain and the clinical specimen has 8 tubes). Then, the PCR reaction was

carried out in the PCR thermocycler machine (Gene Amp® PCR system9700) and exposed to specific thermal cycling parameters (Table 4).

Table 4: PCR Cycling Parameters.

Primer	Primer Sequence (5'>3')	Cycling Parameters	Reference
P2	5'-TCGCTGTCTTCGACCGGTGA-3'	95°C-12 min, 55°C-1 min, 72°C-1 min (40 cycles): 95°C-30 sec, 55°C-30 sec, 72°C-30 sec	McElhinney 1995
724C	5'-AAGAATCCTCACCTGGCTTA-3'		
gB1319	5'-TGGAACTGGAACGTTTGGC-3'	95°C-12 min, 55°C-1 min, 72°C-1 min (40 cycles): 95°C-30 sec, 55°C-30 sec, 72°C-30 sec	Chou and Dennison 1991
gB1604	5'-GAAACGCGCGGCAATCGG-3'		
gM509JE	5'-GCTCAAACCGCGTCGTGA-3'	95°C-12 min (40 cycles): 95°C-45 sec, 55°C-45 sec, 72°C-1 min Final extension: 72°C-10 min	Ellis 2006
gM1801JE	5'-ACGGTCTGCGTGTCTCTT-3'		
gNup	5'-TGGTGTGATGGGTGGAAC-3'	95°C-12 min, 55°C-1 min, 72°C-1 min (35 cycles): 95°C-1 min, 55°C-1 min, 72°C-1 min Final extension: 72°C-10 min	Pignatelli *et al.*, 2003
gNlow	5'-TAGCCTTTGGTGGTGGTTGC-3'		

gH203	5'-CCACCTGGATCACGCCGCTG-3′	95°C-12 min, 55°C-1 min, 72°C-1 min (40 cycles): 95°C-30 sec, 55°C-30 sec, 72°C-30 sec	Chou 1992
gH172	5′-TGGTGTTTTCACGCAGGAA-3′		
115upout	5′-TTGATGTGCCGCCGCCCGGAT-3′	95°C-12 min (40 cycles): 95°C-15 sec, 55°C-20 sec, 72°C-2 min Final extension: 72°C-10 min	Rasmussen *et al*., 2002
115loout	5′-GCACCAGCTCGAAGCCTAAC-3′		
UL74ZB5	5′-CGTTGGAACACCAAATTGTA-3′	(40 cycles): 95°C-15 sec, 55°C-15 sec, 72°C-10 min	Buhamad 2018
UL74ZB3	5′-ACCAAAGGCTATTGAGGGTG-3′		

3.2.2.3 Real time PCR

The PCR master mix was prepared by pipetting the following reagents together in a 1.5 ml sterile micro-centrifuge tubes (Eppendorf tube): 10 µl of TaqMan Fast Universal PCR master mix (2X), 1 ml of each HCMV forward primer (CTGCGTGATATGAACGTGAAGG) (6µM), HCMV reverse primer (ACTGCACGTACGAGCTGTTGG) (6µM), and probe (CGCCAGGACGCTGCTACTCACGA) (4µM), 5 ml of a DNA- free sterile distilled water (per sample), these volumes were multiplied by the number of samples needed. To each well of a fast-thermal cycling reaction strip, 18 µl of the prepared master mix and 2 µl of each extracted DNA sample including the positive and the negative controls were aliquoted (the total volume is 20 µl). The PCR reaction was carried out using the following thermal

cycling parameters: 95°C for 20 sec., 40 cycles of 95°C for 1 sec and 60°C for 20 sec.

3.2.2.4 PCR conditions

Separation of the PCR DNA free room and the DNA preparation room was essential to avoid contamination. The PCR mixture (master mix) was prepared in the DNA free room, where no PCR products or any DNA samples were allowed. The virus DNA was extracted and added to the PCR master mix in the extraction room. No equipment was transferred between rooms, except the prepared master mix. The working cabinets in both rooms were cleaned regularly and kept DNA free by frequent short-wave UV sterilization. In both rooms, gloves and coat were worn at all times and changed if moving between rooms.

3.2.3 HCMV growth characteristics

To assess the viral replication kinetics and characteristic in cell culture, the exact process of the $TCID_{50}$ assay was used. Stocks of both laboratory strains and clinical samples were prepared. MRC-5 cells ($2X10^5$ cells/ml) were infected at 10,000 $TCID_{50}$ for each virus (standard) (Multiplicity of infection (MOI)= 0.05). The CPE was observed at specified different time points (7, 14, 21, 28 days' post infection) and the infectious titer was calculated as described previously in section (3.2.1.9). Duplicate wells were used for each virus titer to increase accuracy. Finally, $TCID_{50}$ values were calculated to plot the growth curves.

3.2.4 E-Gel® electrophoresis

To detect the PCR products E-gel electrophoresis was used. The E-Gel® agarose gel used in this study was a 12-well, single comb gel with SYBR® Safe DNA Gel Stain. Samples were prepared using a 0.5 ml Eppendorf tube as follow: 12 µl Ultra-Pure Distilled Water (UPDW), 8 µl of PCR products for each sample. Then the mixture was mixed using a pipette. The 1 kb plus ladder was prepared by adding 2 µl

of the latter with 18 µl of UPDW. The E-gel was inserted into the base until the red-light illuminated on the base, and then any button was pressed until the red light turned into green light (pre-running of the E-gel). Then the E-gel was removed and 20 µl of the ladder and each sample was loaded into the E- gel lanes, and the empty lanes filled with 20 µl of UPDW. After running the electrophoresis for 30 minutes, the E-Gel cassette was transferred to the UV transilluminator system to visualize the DNA bands.

3.2.5 Restriction fragment length polymorphisms (RFLP)

1 or 2 µl of each restriction enzyme was mixed with 1 or 2 µl of the restriction enzymes specific buffer, 5µl of the DNA being analysed, and 10 or 12 µl of Sterile Distilled Water, in a 0.5ml Eppendorf tubes (separate tubes for each enzyme, strain and genotype). Tubes were incubated for 1 hour at 37°C or 65°C, allowing the restriction enzyme to cut its recognition site (restriction enzymes and their buffers volumes and the incubation time and its appropriate temperature used according to the manufacturer instructions). Lastly, 2 µl of the loading buffer was added to each tube to stop the restriction digestion reaction (Table 5).

Table 5: Restriction enzymes used for each genotype and their specific size.

Glycoproteins	**Restriction Enzymes**	**Genotypes**			
gB		gB1	gB2	gB3	gB4
	Rsa I	239, 66	239, 63	195, 63, 41	196, 65, 44
	Hnif I	202, 67, 33	202, 100	202, 97	203, 67, 35
gM		gM1	gM2	gM3	
	Ear I	679, 511, 105	1300 (uncut)	679, 511, 105	
	Bfa I	1089, 211	1300 (uncut)	1300 (uncut)	

gN		gN1	gN2	gN3a	gN3b	gN4a	gN4b	gN4c	gN4d
	Sac I	297, 123	299,123,65	420 (uncut)	420 (uncut)	291, 123	420 (uncut)	420 (uncut)	420 (uncut)
	Sca I	420 (uncut)	420 (uncut)	420 (uncut)	221, 172, 27	221, 166, 27	420 (uncut) or 287, 27	238, 172	239, 145, 27
	Sal I	420 (uncut)	296, 121	420 (uncut)	420 (uncut)	341, 73	341, 73	337, 73	338, 73
gH		gH1				gH2			
	Hpa II	162, 51				210 (uncut)			
	Stu I	210 (uncut)				158, 52			
gL		gL1		gL2		gL3		gL4	
	Rsa I	287, 117, 96, 50		337,117, 96		287, 117, 96, 50		337,117, 96	
	Taq I	386, 156, 8		386, 156, 8		542, 8		542, 8	
gO		gO1a	gO1b	gO1c	gO2a	gO2b	gO3	gO4	gO5
	Apo I	517, 320	370, 311, 147	517, 321	687, 147	517, 320	828 (uncut)	505, 322	370, 321, 147
	Ban II	592, 245	592, 236	592, 246	828 (uncut)	414, 245, 178	583, 245	580, 247	592, 246
	Hha I	613, 225	729, 99	486, 225, 127	370, 239, 126, 99	370, 239, 126, 99	729, 99	375, 224, 126, 102	613, 126, 99

3.2.6 Electrophoresis of TBE gels using the XCell SureLock® Mini-Cell

The XCell SureLock® Mini-Cell was assembled according to the manufacturer's instructions. After insertion of the TBE gel cassette into the tank the Gel Tension Wedge was pulled forward and the inner chamber filled with 200 ml of 1X TBE buffer and the outer chamber with 600 ml of 1X TBE buffer. Then about 5 µl

of the 25 bp or 50 bp hyper ladder (dependent on the genotype being analysed), and the samples were loaded into the gel wells. The electrodes were connected to the electrophoresis device and run under TBE gels default settings (200 Volt, 12 mA and 2.0 w for 45 minutes). In small Scott bottle about 30 µl of gel red stain was mixed with 50 µl of UPDW. Then, the gel was removed from the cassette, immersed in the stain using a square Petri dish and placed in the shaker for about 15 minutes. Lastly, the gels were visualized on the UV transilluminator.

3.2.7 Enzyme linked lectin-sorbent assay (ELLA)

In this assay, HCMV strain-infected cells with density of 2×10^5 cells/ml at virus infectious titer of 10^4 TCID50/ml in the 96 well plates were used. Cells with CPE were fixed using methanol as previously described (section 3.2.1.10). Blocking the endogenous biotin before starting the assay was completed in two steps: First, all endogenous biotin moieties were reacted with streptavidin by covering the wells in use with 0.1 mg/ml streptavidin diluted in wash buffer; incubation for 15 minutes at room temperature, then washing three times for 10 minutes each with wash buffer. To ensure blocking of remaining streptavidin biotin-binding sites 0.5 mg/ml of a free biotin dissolved in wash buffer was added, incubated for 30 minutes at room temperature, then washed three times for 10 minutes each with wash buffer. To each well, 150 µl of the prepared biotinylated lectin dilution (2.5 µg/ml in Tris-buffered saline (TBS) +1mM CaCl) was added including the control wells (non-infected cells). Some wells with infected cells were used as controls (lectins were not added). The plate was incubated at room temperature for 30 minutes. Then washed 5 times in TBS + 0.05% Tween 20, using the ELISA washer. Then, 150 µl of 01% hydrogen peroxide, to block the endogenous peroxidase activity, in TBS was added to all wells including the control wells. The plate was incubated at room temperature for 30 minutes. Then washed 5 times in TBS + 0.05% Tween 20, using the ELISA washer. Next, 150 µl of streptavidin/peroxidase conjugate was added to all wells in use including the control wells (No lectins added only conjugate). The

plate was incubated at room temperature for 30 minutes then washed 5 times in TBS + 0.05% Tween 20, using the ELISA washer. Next, 100 µl of the TMB substrate (3,3',5,5'Tetramethylbenzidine), which is a chromogenic substrate that interacts with the Hydrogen Peroxide (H_2O_2), was added to each well in use, incubated at 37°C for 20 minutes (until colour blue developed). Then the reaction was stopped by adding 10 µl of 0.5M Sulphuric acid, which is the stop reagent that inhibits the development of the TMB colour by inhibiting the enzymatic activity of the peroxidase, to each well in use (yellow colour developed). Finally, the optical density (O.D.) for each well was determined by reading at 450 nm on ELISA reader. The same method was repeated for the laboratory strains and clinical samples (Figure 8).

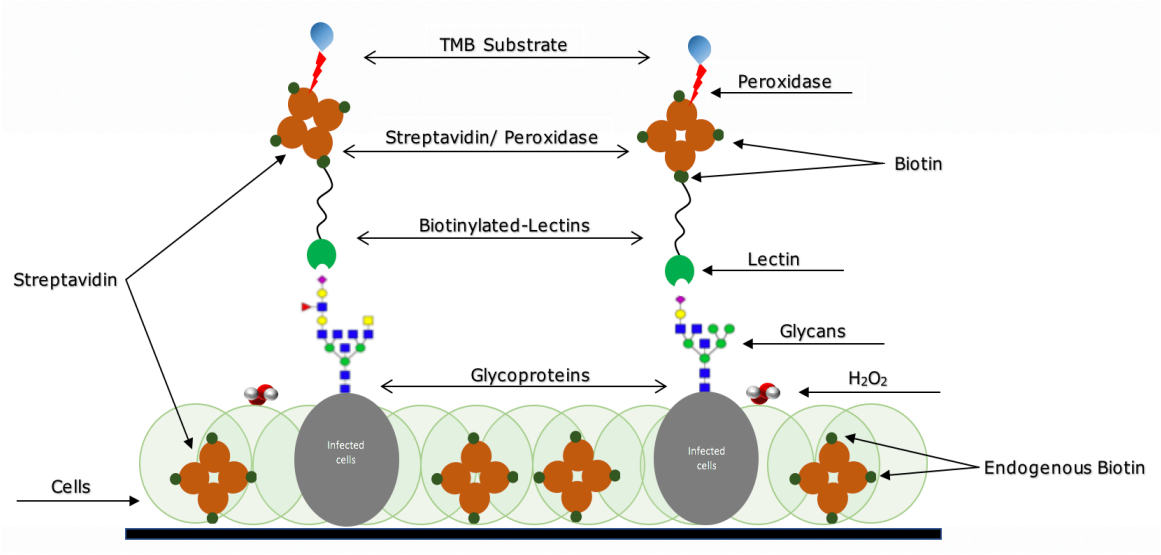

Figure 8: Schematic diagram presenting the method of Enzyme Linked Lectin-Sorbent Assay used in this project.

3.2.8 Samples information

3.2.8.1 Laboratory strains

HCMV Laboratory strains used in this project were AD169, Towne, Davis, Toledo and Merlin:

Viral stocks stored in liquid nitrogen in the University of Manchester Virology Laboratory were AD169 (Passage No. 3, lab stock from 12/02/03), Towne (Passage

No. 2, laboratory stock from 18/05/00), Davis (Passage No. 2, laboratory stock from 22/05/00). All were purchased from the American Type Culture Collection (ATCC), Middlesex, UK.

Viral laboratory strain Toledo was purchased from the National Collection of Pathogenic Viruses (NCPV), Public Health England, Salisbury, UK.

Viral laboratory strain Merlin was purchased from ATCC.

3.2.8.2 Clinical samples

Clinical samples (N=114) were obtained from the Public Health England, North West Regional Virus Laboratory, Manchester Royal Infirmary, Central Manchester University Hospitals, NHS Foundation Trust, UK., and Nova Medical School, Faculty of Medical Sciences, University of Lisbon, Lisbon, Portugal. All specimens were surplus residual, unlinked and anonymized clinical samples.

An HCMV specific Phosphoprotein PCR was used to confirm positive CMV results for all 114 clinical samples. Prior to handling in different assays, all samples were cultured in MRC-5 cells.

The specimens were classified according to two major groups (Infection category) and (Specimen type) and then each sample was assigned into different sub-groups as follows:

Group 1: Infection category

A: Congenitally or early post-natally infected infants (N=13)

A_1: Confirmed congenital (N=12)

A_2: Unconfirmed congenital or early post-natal (N=1)

B: Immunocompetent patients with primary infection (N=8)

B_1: Confirmed Primary (N=7)

B_2: Primary/recurrent not defined (N=1)

C: Immunocompromised patients (N=63)

C_1: Primary infection (N=26)

C_2: Recurrent infection (N= 35)

C_3: Primary/recurrent not defined (N=2)

D: Not known infection (N=5)

Group 2: Specimen type

A: Blood specimens (N=77)

B: Urine specimens (N=8)

C: Respiratory specimens (N=4)

3.2.9 Ethical considerations

This study was reviewed and approved (15/NW/0368) by The National Research Ethics Services (NREC) committee in North West- Lancaster.

Specimens used are residual, surplus, diagnostic human body fluids, such as blood, saliva and urine. These were unlinked anonymized specimens, and no contact with the patients was required. The anonymized data was handled and stored on a password secured encrypted computers held within locked, controlled access, secure University premises. Data storage computer systems are managed in full compliance with the requirements of the Data Protection Act. Data transported between computers held on a different site (e.g. NHS Hospital premises) was accomplished using password protected and encrypted secure portable data storage devices. All human tissues were discarded according to human tissue act regulations, and the remaining samples after study completion were stored in the human tissue bank in the laboratory. The results of this study were not reported back to the requesting clinician or patient.

3.2.10 Statistical analysis

All the data were analysed using IBM SPSS statistics, version 23.0. Statistical tests used for data analysis included: Cross-tabulation test to determine the distribution of all HCMV glycoprotein genotypes among different infection categories /and specimen types; Chi-square test was used to show whether there is a significant association of any of HCMV genotypes and the infection categories/ specimen types or not. Also, a paired Sample t-test was used to measure the glycosylation differences between non-infected and infected cells. To measure the difference between the same groups (non-infected and infected cells) with regard to a particular variable/measurement (specimen type, category), Paired samples t-test was used. Finally, Analysis of Variance (ANOVA) test to measures the differences between three groups or more, and the independent samples t-test, which is similar to ANOVA but measures the differences between two groups, was used. Finally, the significance of the relationship between variables was determined using Post hoc statistical test ($P<0.05$ indicates statistically significant results).

CHAPTER 4

4. Results and data analysis

4.1 Cultivation of MRC-5 cells

Laboratory strains and clinical isolates of HCMV were grown in human foetal cells, which were permissive to infection and characterized by their stability and integrity. MRC-5 cells (Human Embryonic Lung Fibroblast cells) were used, which are derived from a 14-week male foetus and known to tolerate environmental changes and attain confluency within 3 days when seeded at $1x10^5$ cells /ml (Jacobs *et al*., 1970). Cells were successfully cultivated in flasks, 96 well plates and cell culture tubes and a stock of cells was grown and stored frozen to ensure passage number consistency between all assays. Figure 9 shows MRC-5 cells grown in a $75cm^2$ cell culture plastic flask at a seeding concentration of $1x10^5$ cells/ml. After 72-96 hours of incubation, the cells were examined using an inverted microscope and found to be 100% confluent (Figure 9). Cells were also seeded at $2x10^5$ cells/ml in 96 well plates and in cell culture tubes and became 90% confluent after 24- 36 hours of incubation.

Figure 9: 100 % confluent MRC- cells observed after 72-96 hours of culturing.

4.2 Infection of MRC-5 cells

HCMV can induce two distinctive cytopathic effects (CPE): an early CPE, when the infected fibroblasts appear to be rounded and swollen, and a late CPE, when granular intracytoplasmic and intranuclear inclusion bodies appear within these swollen cells.

All 5 lab strains and 114 samples were cultured in MRC5 fibroblast cells as described previously, section (3.2.1.7). All lab strains and 89 of the 114 clinical samples started to show a clear CPE within 3 to 10 days after infection (Figure 10), while the complete CPE was observed 5-28 days after infection. Samples where no CPE was seen (n=25) were excluded from this study.

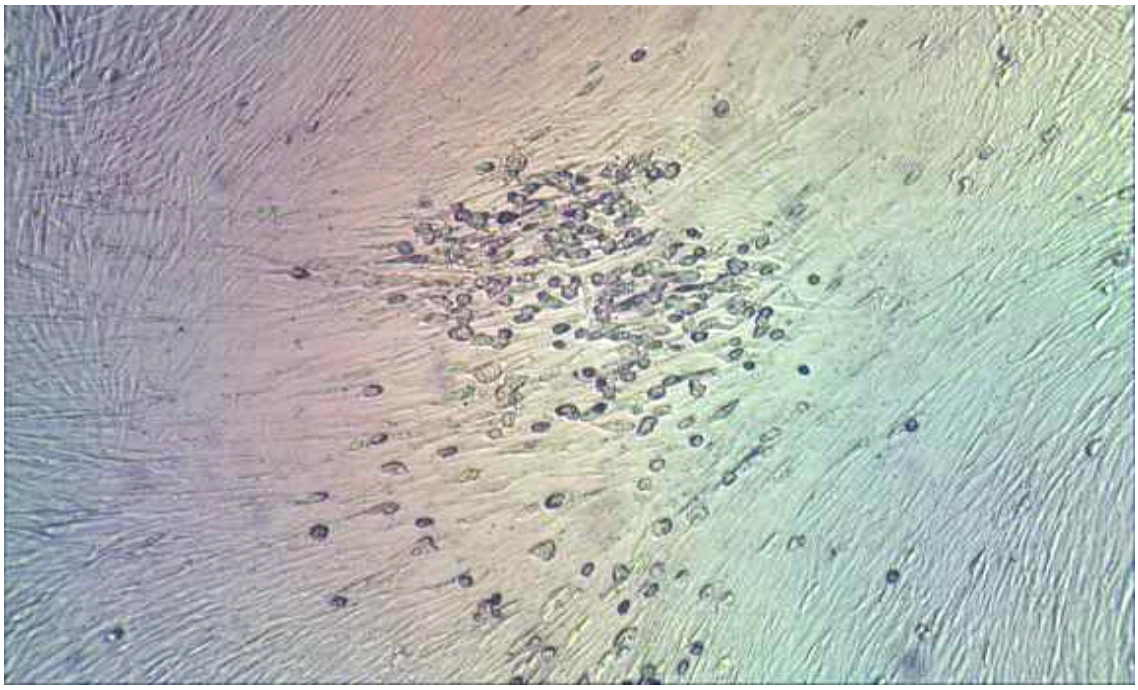

Figure 10: HCMV specific Cytopathic Effect (CPE) on fibroblasts cells, as the cells appear flat, rounded and swollen. Granular intracytoplasmic and intranuclear inclusion bodies appear within these cells in the centre of the picture.

4.3 Fixation of MRC-5 cells

Efforts to fix the infected cells in the 96 well plates without making them permeable was performed using: 1% formaldehyde in PBS or a formaldehyde-free-methanol, for 20, 30, or 60 minutes. After a first wash, using the ELISA washer, 20%

of the fixed cells were washed away and the remaining cells were removed by a second wash. Manual washing of the cells was tried, and the result was similar, but the percentage of cells lost was less (15%). There was no apparent difference in the percentage of cells lost whether the cell sheet was allowed to dry or remained wet after fixation. Fixation of cells without permeabilizing them was thus not achievable. As a compromise, cells were fixed using methanol for 60 minutes (as described in section (3.2.1.10)). This fixation will likely affect cell surface permeability. However, as both internal and external protein glycosylation is important, this was not considered a major setback.

4.4 Determination of 50% tissue culture infectious dose

Several assays are available to quantitate infectious virus including plaque assay and 50 % tissue culture infectious dose ($TCID_{50}$) assays. In this project, $TCID_{50,}$ which quantitates the number of infectious units of virus per unit volume by looking for the presence of virus-induced cytopathic effect, was used to determine the infectious titre of the different HCMV laboratory strains and clinical isolates. The 50% infectious dose was calculated using the Spearman and Karber method (Flint *et al.,* 2009) (Section 3.2.1.9). The infectious titre determined for each stock is presented in the table below (Table 6).

Table 6: The infectious titre of the stocks of different HCMV strains.

HCMV strain	The infectious titer
AD169	$10^{5.8}$ $TCID_{50}$ / ml
Towne	10^{5} $TCID_{50}$ / ml
Davis	$10^{3.5}$ $TCID_{50}$ / ml
Toledo	$10^{4.6}$ $TCID_{50}$ / ml
Merlin	$10^{3.6}$ $TCID_{50}$ / ml
Clinical samples	Ranging from $10^{2.2}$ to $10^{6.1}$ $TCID_{50}$ / ml

4.5 Identification of HCMV glycoprotein genotypes and their effect on the virus distribution

Glycoprotein genotype profiles were identified for all HCMV positive samples. This was done using PCR/RFLP assays to amplify and identify each glycoprotein gene in each sample. A total of 5 lab strains and 87 out of 89 clinical samples were genotyped successfully (although not all glycoprotein genotypes were determined for every sample). Of these 53 specimens were typed as part of this project and the remaining 35 specimens were typed as part of a related project (Buhamad, 2018) and pooled with this data to allow all 89 specimens to be included in the growth characteristics and glycosylation studies. The complete genotyping profile, infection category and specimen type for all HCMV lab strains and clinical samples can be found in Appendix 1.

The specimens were analysed by grouping them in two different ways: by infection category and by specimen type (full classification can be found in section 3.2.8.2).

4.5.1 Infection category

The specimens were divided into 4 sub-groups according to the patient type: A- Congenitally or early post-natally infected infants; B- Immunocompetent patients with primary infection; C- Immunocompromised patients with primary (C_1) / or recurrent infection (C_2); D- Not known infection category.

Although all specimens were typed and used later in the glycosylation studies, the following specimens were excluded from the genotype analysis because they could not be confidently assigned to a patient group, and most importantly, statistical analysis of sub-groups with 4 cases or less was not appropriate (this is discussed in the discussion chapter); undetermined congenital or early post-natal infection (N=1), not defined primary or recurrent infection from immunocompetent patient (N=1), not defined primary or recurrent infection from an

immunocompromised patient (N=2), and unknown infection category (N=4). This resulted in the following numbers in the patient groups; congenitally or early post-natally infected infants (N=12), Immunocompetent patients with primary infection (N=7), Immunocompromised patients with primary/ or recurrent infection (N=61). (Total population for glycoprotein analysis: N=80).

The distribution of each glycoprotein genotype among each group was determined using a cross tabulation statistical test. To determine whether there was a significant correlation between the glycoprotein genotype distribution and the infection group, Pearson chi-squared statistical test was used.

4.5.1.1 Glycoprotein B

A total of 87 of 89 (97.8%) specimens were successfully gB genotyped. Of the 80 specimens included in the study a gB genotype, result was obtained for 79 (88.8%).

All gB genotypes were identified in all patient groups. gB1 was the most common (n=33, 41.8%), the other genotypes were: gB2 (n=15, 19%), gB3 (n=18, 22.8%), gB4 (n=11, 13.9%), and mixed gB genotype (n=2, 2.5%) (Table 7).

When the genotypes were analysed by infection category, gB1 (n=4, 3.3%), gB4 (n=3, 27.3%), gB2 (n=2, 16.7%) and gB3 (n=2, 16.7%) were equally distributed among infants with congenital infections (sub-group A). In the immunocompetent patients with primary infection (sub-group B), gB3 (n=4, 57.1%) was most common with gB1 (n=2, 28.6%) also found. In immunocompromised patients (sub-group C) with primary or recurrent infection (C_1), gB1 was most common (n=12, 48.0%) with gB2 (n=5, 20.5%), gB3 (n=4, 16.0%) and gB4 (n=4, 16.0%). In C_2, immunocompromised patients with recurrent infection, gB1 (n=15, 42.9%) was most common with gB2 (n=8, 22.9%) and gB3 (n=8, 22.9%) equally prevalent and gBb4 (4, 11.4%) least common (Table 7, Figure 11).

For all the above, no statistically significant relation was found with any gB genotype distribution and patient group (X^2 (12, N=79) = 15.921), p=0.19).

Table 7: gB distribution among patients with different infection categories.

gB genotypes	Congenital infection N=12	Immuno-compromised Primary infection N=25	Immuno-compromised Recurrent infection N=35	Immuno-competent Primary infection N=7	Total N=79
Mixed gB	8.3% (n=1)	0.0% (n=0)	0.0% (n=0)	14.3% (n=1)	2.5% (n=2)
gB1	33.3% (n=4)	48.0% (n=12)	42.9% (n=15)	28.6% (n=2)	41.8% (n=33)
gB2	16.7% (n-2)	20.0% (n=5)	22.9% (n=8)	0.0% (n=0)	19.0% (n=15)
gB3	16.7% (n=2)	16.0% (n=4)	22.9% (n=8)	57.1% (n=4)	22.8% (n=18)
gB4	27.3% (n=3)	16.0% (n=4)	11.4% (n=4)	0.0% (n=0)	13.9% (n=11)

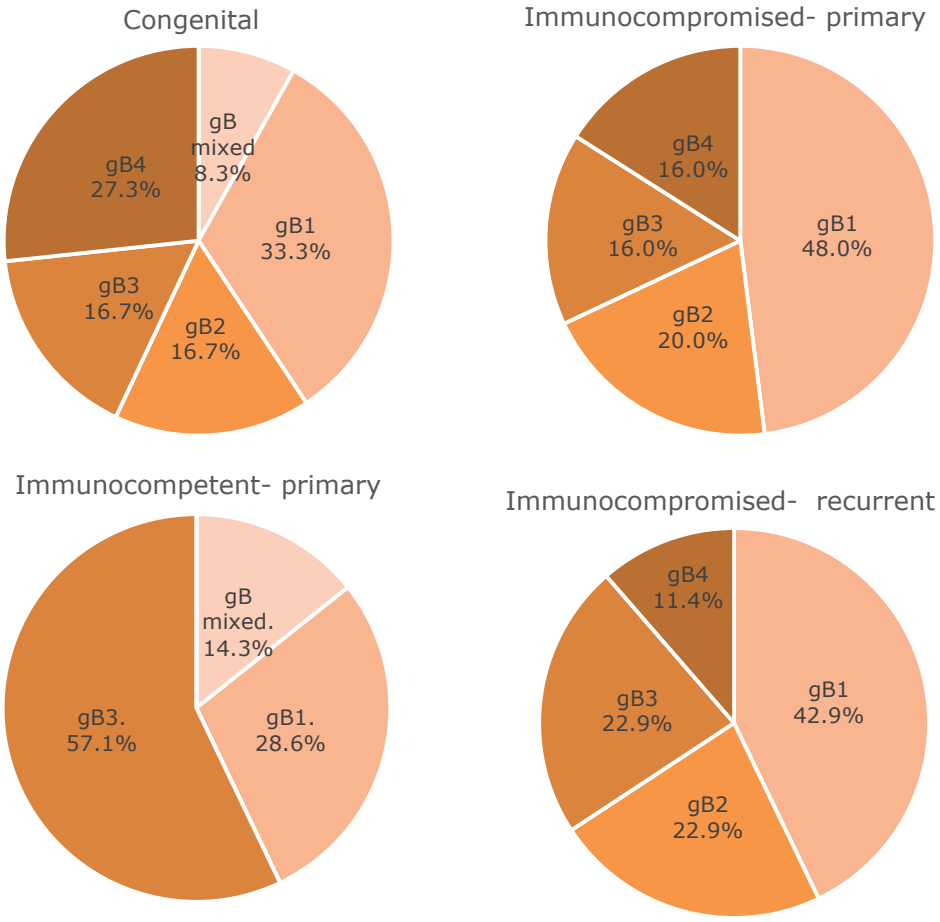

Figure 11: gB distribution among patients with different infection categories.

4.5.1.2 Glycoprotein H

A total of 84 of 89 (94.4%) specimens were successfully gH genotyped. Of the 80 specimens included in the study a gH genotype, result was obtained for 77 (8.5%).

The distribution of the gH types across all samples was as follows: gH1 (n=40, 51.9%) and gH2 (n=33, 42.9%) Mixed gH genotypes (n=4, 5.2%) (Table 8). When analysed by infection group, gH1 was the commonest genotype in sub-group A, among infants with congenital infections (n=9, 81.8%), and immunocompetent patients with primary infections (sub-group B) (n=4, 57.1%), and in immunocompromised patients (sub-group C) with primary HCMV infection (C_1) (n=18, 72.0%). Whilst gH2 was the most prevalent among immunocompromised patients with recurrent infections (C_2) (n=21, 61.8%), (Table 8, Figure 12).

The chi-squared statistical test indicated that there was a statistically significant association between infection category (X^2 (6, N=77) = 24.251), p=0.001) and the distribution of gH genotypes among HCMV patients.

Table 8: gH distribution among patients with different infection categories.

gH genotypes	Congenital infection N=11	Immuno-compromised Primary infection N=25	Immuno-compromised Recurrent infection N=34	Immuno-competent Primary infection N=7	Total N=77
Mixed gH	9.1% (n=1)	0.0% (n=0)	2.9% (n=1)	28.6% (n=2)	5.2% (n=4)
gH1	81.8% (n=9)	72.0% (n=18)	35.3% (n=12)	14.3% (n=1)	51.9% (n=40)
gH2	9.1% (n=1)	28.0% (n=7)	61.8% (n=21)	57.1% (n=4)	42.9% (n=30)

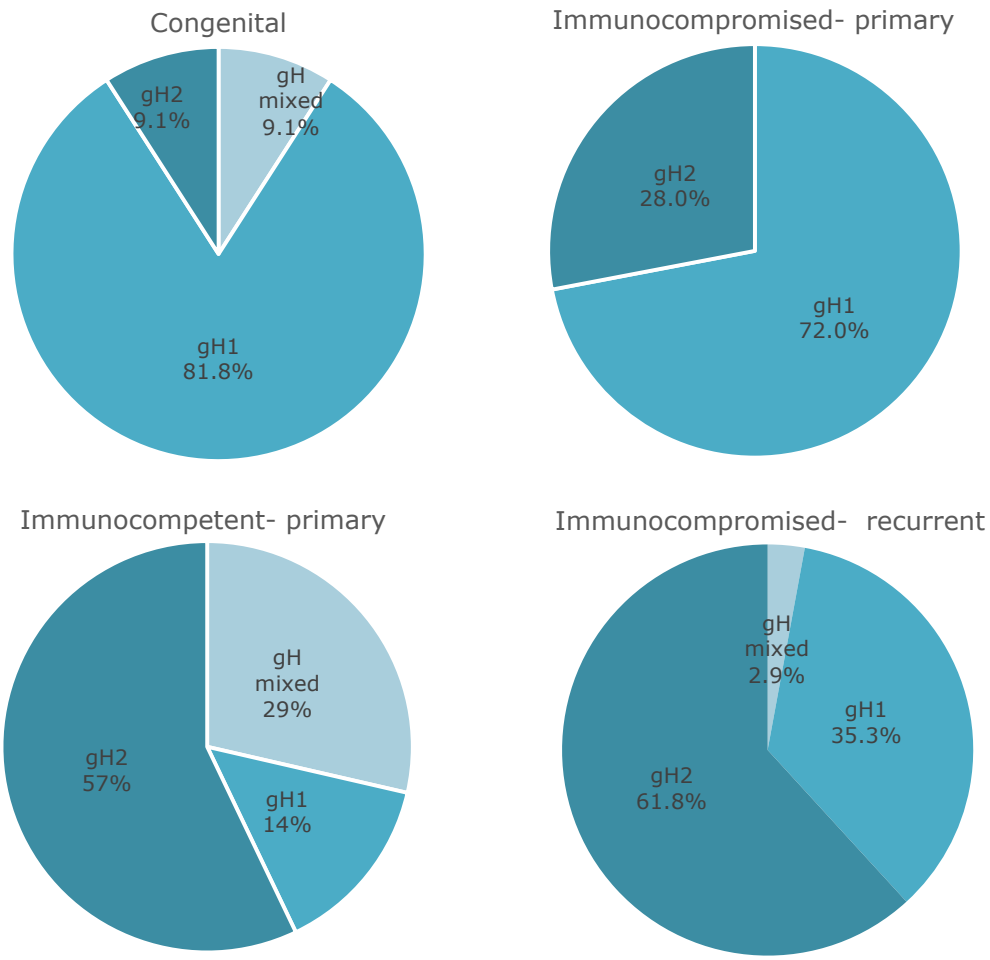

Figure 12: gH distribution among patients with different infection categories.

4.5.1.3 Glycoprotein L

A total of 83 of 89 (93.3%) specimens were successfully gL genotyped. After excluding any group of the infection categories that had 4 cases or less, the total number of gL that could be statistically analysed was reduced to 76 (85.4%).

The distribution of the genotypes among these 76 was as follows: gL1 (n=3, 3.9%), gL2 (n=17, 22.4%), gL3 (n=6, 7.9%), and gL4 (n=36, 47.4%) gL mixed genotypes (n=14, 18.4%), suggesting gL4 was the most prevalent genotype (Table 9).

Analysis of the gL types by patient group showed gL4 was the most widespread genotype among infants with congenital infections (n=7, 58.3%) (sub-group A), and immunocompetent patients (n=4, 57.1%) (sub-group B). Also,

among immunocompromised patients (sub-group C) with primary (C_1) or recurrent infections (C_2), gL4 was the most prevalent (n=12, 48%; n=13, 40.6%, respectively), followed by gL2 (n=6, 24%; n=10, 31.3%, respectively) (Table 9, Figure 13).

However, chi-squared test showed no statistically significant correlation between gL genotype and infection category (X^2 (12, N=76) = 10.556), p=0.56).

Table 9: gL distribution among patients with different infection categories.

gL genotypes	Congenital infection N=12	Immuno-compromised Primary infection N=25	Immuno-compromised Recurrent infection N=32	Immuno-competent Primary infection N=7	Total N=76
Mixed gL	25.0% (n=3)	8.0% (n=2)	21.9% (n=7)	28.6% (n=2)	18.4% (n=14)
gL1	0.0% (n=0)	8.0% (n=2)	3.1% (n=1)	0.0% (n=0)	3.9% (n=3)
gL2	8.3% (n=1)	24.0% (n=6)	31.3% (n=10)	0.0% (n=0)	22.4% (n=17)
gL3	8.3% (n=1)	12.0% (n=3)	3.1% (n=1)	14.3% (n=1)	7.9% (n=6)
gL4	58.3% (n=7)	48.0% (n=12)	40.6% (n=13)	57.1% (n=4)	47.4% (n=36)

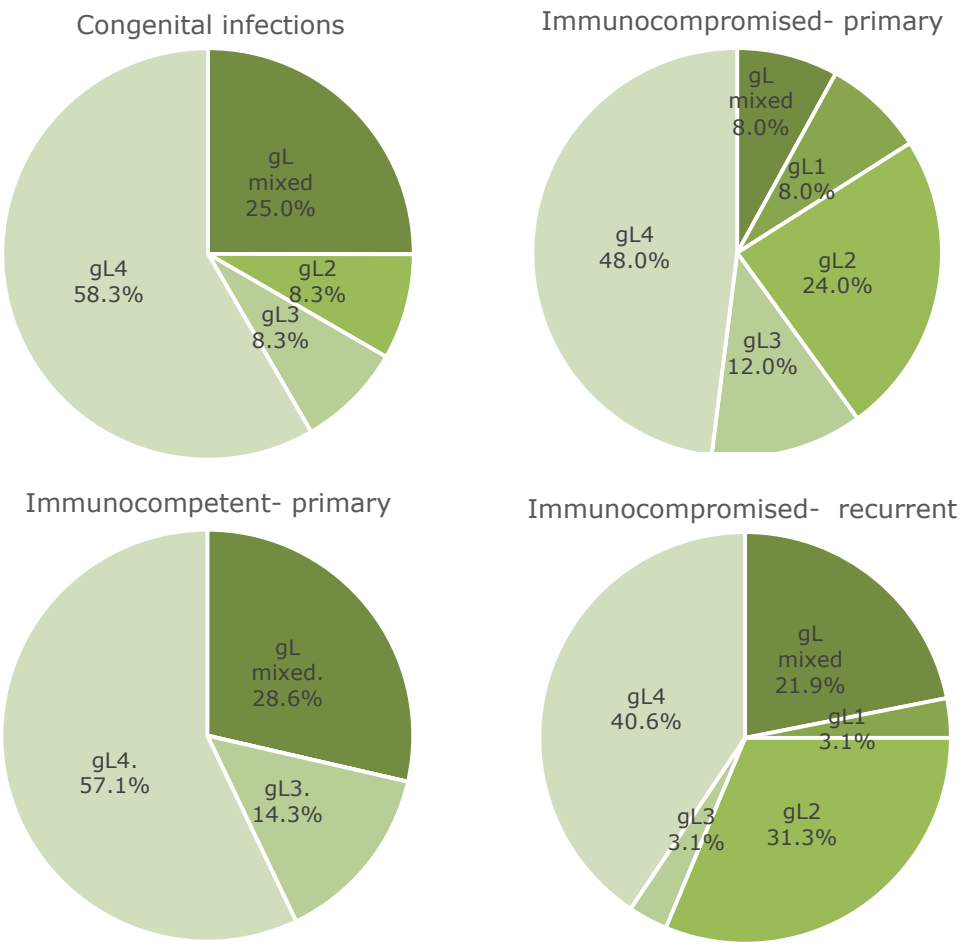

Figure 13: gL distribution among patients with different infection categories.

4.5.1.4 Glycoprotein M

A total of 74 of 89 (83.1%) specimens were successfully gM genotyped. After excluding any group of the infection categories that has 4 cases or less, the total number of gM that could be statistically analysed was reduced to 67 (75.3%).

Distribution of the gM genotypes across these 67 was as follows: gM1 (n=13, 19.4%) and gM2 (n=3, 4.5%), and gM3 (n=50, 74.6%) Mixed gM genotypes (n=1, 1.5%). Noticeably, gM3 was the most prevalent genotype followed by gM2 (Table 10).

Analysis by patient group showed; gM3 was the predominant genotype in the congenitally infected infants (sub-group A) (n=6, 66.7%), and among immunocompetent patients (sub-group B) (n=3, 60.0%) with gM1 (n=2, 40.0%).

However, numbers were very low in each type for this patient group. Also, among the immunocompromised patients (sub-group C) for both primary (C_1) (n=19, 82.6%) and recurrent (C_2) (n=22, 73.3%) gM3 was also most common (Table 10, Figure 14).

The chi-squared statistical test indicated no statistically significant association between the infection category (X^2 (3, N=71) = 5.409), p=0.79) and the distribution of gM genotypes among HCMV patients.

Table 10: gM distribution among patients with different infection categories

gM genotypes	**Congenital infection N=9**	**Immuno-compromised Primary infection N=23**	**Immuno-compromised Recurrent infection N=30**	**Immuno-competent Primary infection N=5**	**Total N=67**
Mixed gM	0.0% (n=0)	0.0% (n=0)	3.3% (n=1)	0.0% (n=0)	1.5% (n=1)
gM1	22.2% (n=2)	17.4% (n=4)	16.7% (n=5)	40% (n=2)	19.4% (n=13)
gM2	11.1% (n=1)	0.0% (n=0)	6.7% (n=2)	0.0% (n=0)	4.5% (n=3)
gM3	66.7% (n=6)	82.6% (n=19)	73.3% (n=22)	60% (n=3)	74.6% (n=50)

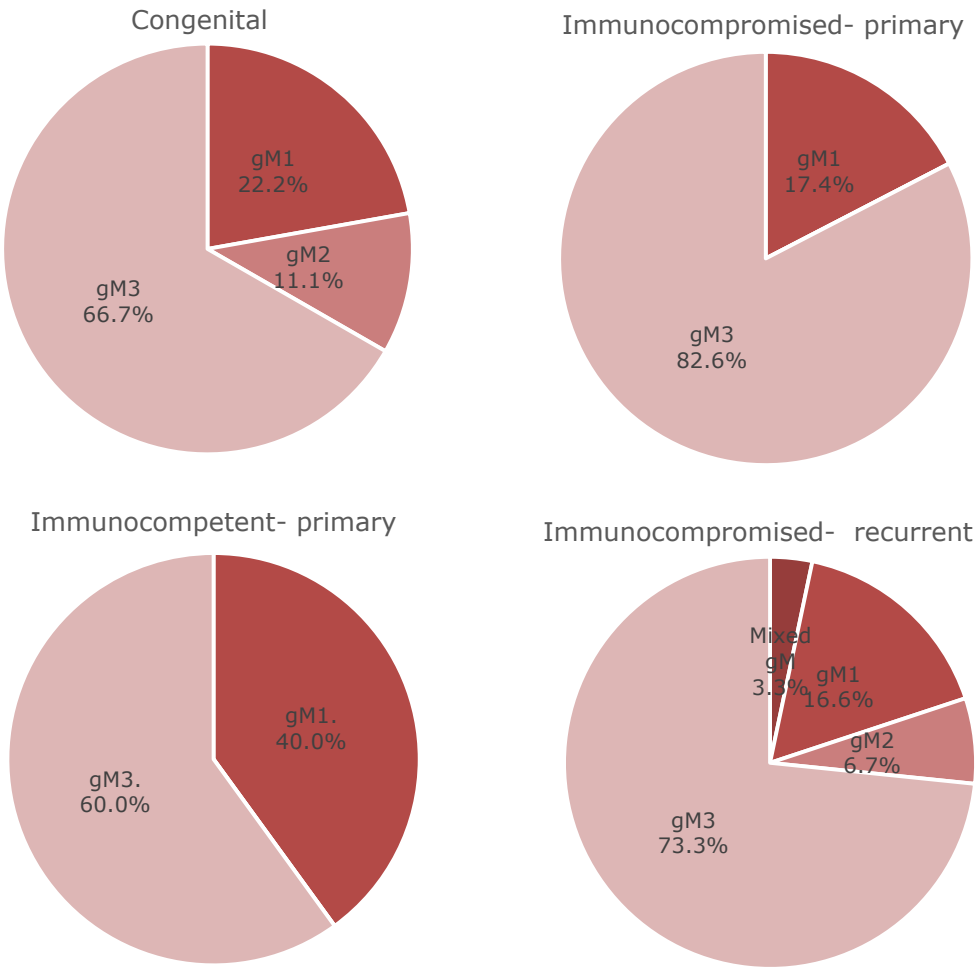

Figure 14: gM distribution among patients with different infection categories.

4.5.1.5 Glycoprotein N

A total of 74 of 89 (83.1%) specimens were successfully gN genotyped. After excluding any group of the infection categories with 4 cases or less, the total number of gN genotype that could be statistically analysed was reduced to 67 (75.3%).

The gN distribution across the whole population was as follows: gN1, n=6, 9.0%), gN3a (n=47, 70.1%), gN4a (n=5, 7.5%), gN4b (n=2, 3.0%), gN4c (n=4, 6.0%) and gN4d (n=3, 4.5%) making gN3a the most commonly found genotype (Table 11).

When differentiated by patient group; gN3a was still the most commonly found type in congenital infections (sub-group A) (n=9, 81.8%), and in immunocompetent patients (sub-group B) (n=4, 60.7%). It was also the most common (n=10, 45.5%) among immunocompromised patients (sub-group C) with primary infections (C_1), although gN1 (n=3, 13.6%), gN4a (n=3, 13.6%), and gN4b (n=2, 9.1%) were also found, but again numbers were low in each type of this group. Also, among immunocompromised patients with recurrent infection (C_2) gN3a was the most common (n= 24, 85.7%) (Table 11, figure 15).

There was no statistically significant relation between gN genotype distribution for any infection category (X^2 (15, N=67) = 16.525), p=0.35).

Table 11: gN distribution among patients with different infection categories.

gN genotypes	**Congenital infection N=11**	**Immuno-compromised Primary infection N=22**	**Immuno-compromised Recurrent infection N=28**	**Immuno-competent Primary infection N=6**	**Total N=67**
gN1	9.1% (n=1)	13.6% (n=3)	3.6% (n=1)	16.7% (n=1)	9.0% (n=6)
gN3a	81.8% (n=9)	45.5% (n=10)	85.7% (n=24)	66.7% (n=4)	70.1% (n=47)
gN4a	0.0% (n=0)	13.6% (n=3)	3.6% (n=1)	16.7% (n=1)	7.5% (n=5)
gN4b	0.0% (n=0)	9.1% (n=2)	0.0% (n=0)	0.0% (n=0)	3.0% (n=2)
gN4c	0.0% (n=0)	13.6% (n=3)	3.6% (n=1)	0.0% (n=0)	6.0% (n=4)
gN4d	9.1% (n=1)	4.5% (n=1)	3.6% (n=1)	0.0% (n=0)	4.5% (n=3)

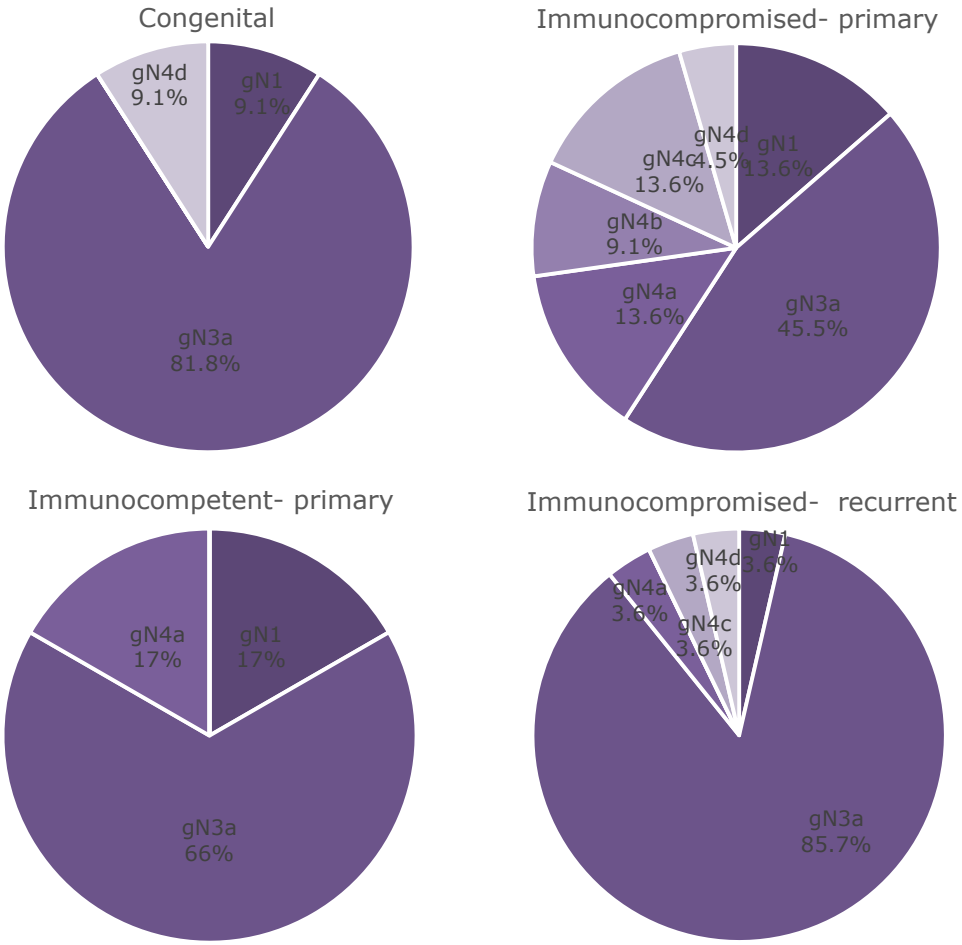

Figure 15: gN distribution among patients with different infection categories.

4.5.1.6 Glycoprotein O

A total of 71 of 89 (79.8%) specimens were successfully gO genotyped. After excluding any group of the infection categories that had 4 cases or fewer, the total number of gO that could be statistically analysed was reduced to 60 (67.4%).

The distribution of the different genotypes of gO across these 60 showed: gO1a (n=15, 25.0%) and gO1c (n=34, 56.7%), gO2a (n=2, 3.3%), gO2b (n=1, 1.7%), gO3 (n=2, 3.3%), and gO4 (n=6, 10%). gO1c was the commonest across all infection categories, followed by gO1a (Table 12).

Analysing the various patient groups showed gO1c was the most prevalent in all patient groups; congenital infections (sub-group A) (n=5, 62.5%);

immunocompetent patients with primary infection (sub- group B) (n=3, 75.0%); immunocompromised patients (sub-group C) with primary infections (C_1) ((n=10, 45.5%); and immunocompromised patients with recurrent infection (C_1) (n=19, 63.3%) (Table 12, Figure 16).

However, chi-square showed no statistically significant correlation between any gO genotype and HCMV infection category (X^2 (10, N=60) = 13.412), p=0.20).

Table 12: gO distribution among patients with different infection categories.

gO genotypes	Congenital infection N=8	Immuno-compromised Primary infection N=22	Immuno-compromised Recurrent infection N=30	Total N=60
gO1a	12.5% (n=1)	36.4% (n=8)	20.0% (n=6)	25.0% (n=15)
gO1c	62.5% (n=5)	45.5% (n=10)	63.3% (n=19)	56.7% (n=34)
gO2a	0.0% (n=0)	4.5% (n=1)	3.3% (n=1)	3.3% (n=2)
gO2b	12.5% (n=1)	0.0% (n=0)	0.0% (n=0)	1.7% (n=1)
gO3	3.2% (n=1)	0.0% (n=0)	3.3% (n=1)	3.3% (n=2)
gO4	0.0% (n=0)	13.6% (n=3)	10.0% (n=3)	10.0% (n=6)

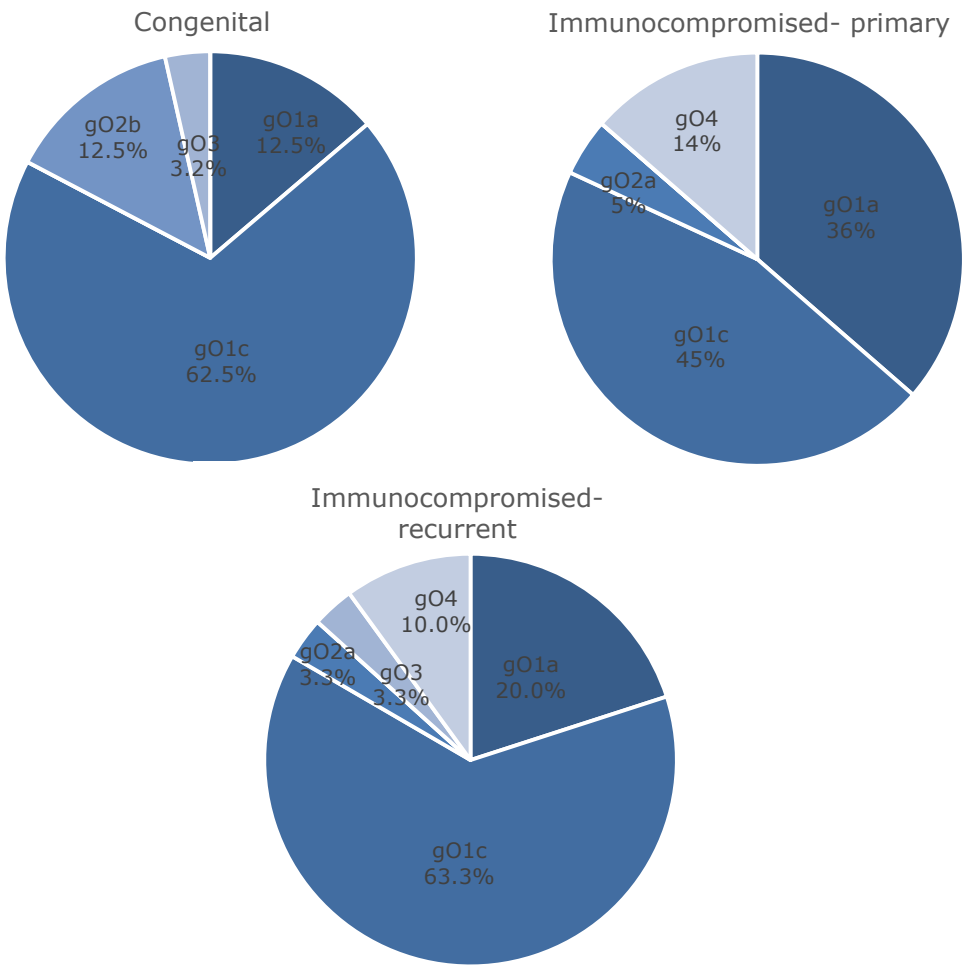

Figure 16: gO distribution among patients with different infection categories.

4.5.2 Specimen type

The distribution of the glycoprotein genotypes, and whether there is any association between HCMV glycoprotein genotypes and the specimen type in which the virus was found was also analysed. Specimens were categorised into one of 3 different sub-groups as described previously in section (3.2.8.2); sub-group A – Blood specimens; sub-group B – Urine specimens; sub-group C – respiratory specimens. However, any specimen type with 4 or fewer cases had to be excluded from the statistical analysis. This meant that respiratory specimens (N=4), which includes: Aspirate (n=1), Sputum (n=1), Saliva (n=1), and throat swab (n=1) was excluded, and only two specimen types were analysed; Blood and Urine.

2.1 Glycoprotein B

A total of 87 of 89 (97.8%) specimens were successfully gB genotyped. After excluding any specimen type with 4 or fewer cases, the total number of specimens analysed for gB was reduced to 83 (93.3%).

The distribution of gB genotypes across all sample types was as follows: gB1 (n=33, 39.8%), gB2 (n=19, 22.9%), gB3 (n=18, 21.7%), gB4 (n=11, 13.3%), mixed gB genotypes (n=2, 2.4%). The most frequent gB genotype was gB1, although the other gB genotypes were also represented (Table 13).

The most common gB genotype in blood samples (sub-group A) was (gB1) (n=31, 41.3%), followed by gB2 (n= 18, 24%), gB3 (n=15, 20.0%) and gB4 (n=9, 12%). In urine samples (sub-group B), B3 (n=3, 37.5%) was the most prevalent, although the gB types were fairly evenly distributed (n=2, 25%) (Table 13, Figure 17).

No statistically significant relationship between specimen type and gB genotype was found for any sample type (X^2 (4, N=83) = 3.059), p=0.54).

Table 13: gB distribution among different specimen types.

gB genotypes	Blood	Urine	Total
Mixed gB	2.7% (n=2)	0.0% (n=0)	2.4% (n=2)
gB1	41.3% (n=31)	25.0% (n=2)	39.8% (n=33)
gB2	24.0% (n=18)	12.5% (n=1)	22.9% (n=19)
gB3	20.0% (n=15)	37.5% (n=3)	21.7% (n=18)
gB4	12.0% (n=9)	25.0% (n=2)	13.3% (n=11)

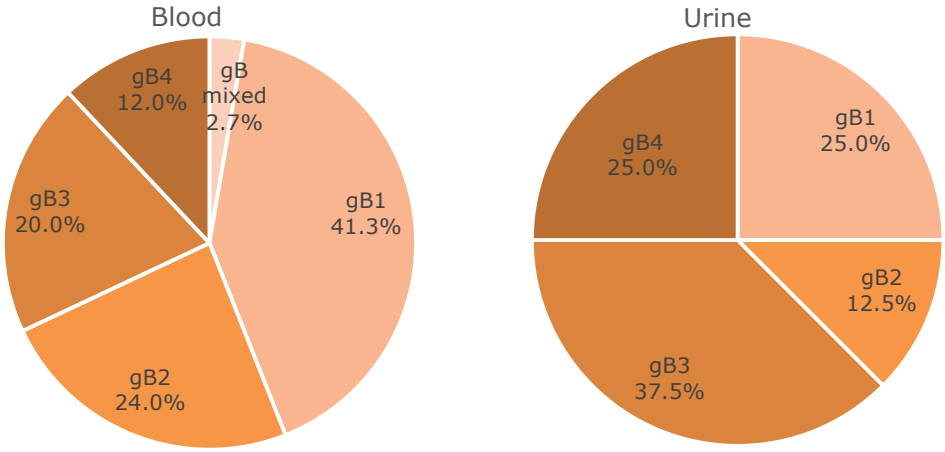

Figure 17: gB distribution among different specimen types.

4.5.2.2 Glycoprotein H

A total of 84 of 89 (94.4%) specimens were successfully gH genotyped. After excluding any specimen types that had 4 cases or less, the total number of gH samples that could be statistically analysed was reduced to 81 (91.0%).

The distribution of gH genotypes was as follows: gH1 (n=43, 53.1%), gH2 (n=34, 42%), mixed gH genotypes (n=4, 4.9%) (Table14). gH1 and gH2 were distributed almost equally among blood specimens (sub-group A) (n=36, 49.3%; n=34, 46.6%, respectively), while gH1 (100%) was the dominant genotype among urine specimens (sub-group B) (Table 14, Figure 18).

The chi-square statistical test indicated that there is a statistically significant association between specimen type (X^2 (2, N=81) = 6.734), p=0.03), and the distribution of gH genotypes among HCMV patients.

Table 14: gH distribution among different specimen types.

gH genotype	Blood	Urine	Total
Mixed gH	4.1% (n=3)	12.5% (n=1)	4.9% (n=4)
gH1	49.3% (n=36)	87.5% (n=7)	53.1% (n=43)
gH2	46.6% (n=34)	0.0% (n=0)	42.0% (n=34)

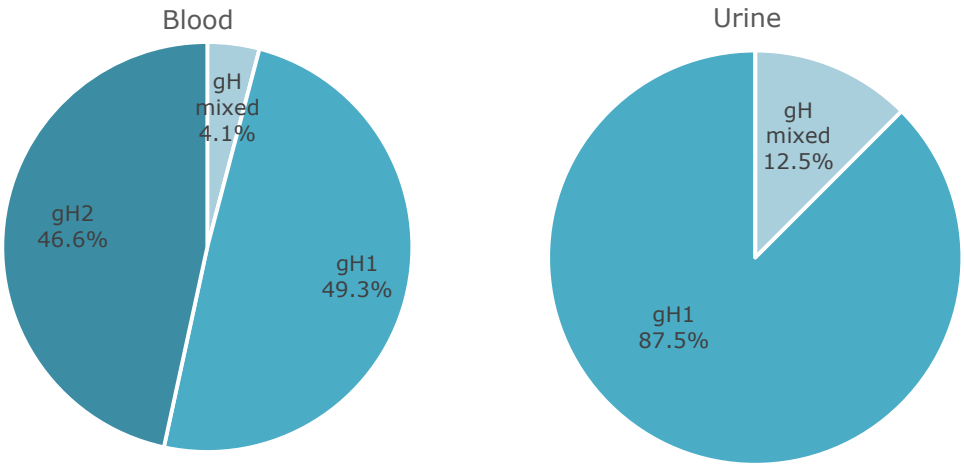

Figure 18: gH distribution among different specimen types.

4.5.2.3 Glycoprotein L

Total of 83 of 89 (93.3%) specimens were successfully gL genotyped. After excluding any group of specimen types that had 4 cases or less, the total number of gL that has been statistically analyzed was reduced to 80 (89.9%).

The distribution of gL genotypes was as follows: gL1 (n=6, 7.5%), gL2 (n=18, 22.5%), gL3 (n=7, 8.8%), gL4 (n=36, 45.0%), mixed gL genotypes (n=13, 16.3%). Thus gL 4 was the most predominant type, but all the other gL genotypes were represented at different percentages (Table 15).

In detail gL4 was most prevalent among both blood (sub-group A) and urine samples (sub-group B) (n=31, 43.1%; n=5, 62.5%, respectively), followed by gL3 (n= 5, 6.9%; n=1, 12.5%) and gL2 (n=17, 23.6; n=1, 12.5%), respectively, which were also present.

However, chi-square showed that there is no statistically significant correlation between gL genotype and specimen type (X^2 (4, N=80) = 5.794), p=0.21) (Table 15, Figure 19).

Table 15: gL distribution among different specimen types.

gL genotypes	Blood	Urine	Total
gL mixed	18.1% (n=13)	0.0% (n=0)	16.3% (n=13)
gL1	8.3% (n=6)	0.0% (n=0)	7.5% (n=6)
gL2	23.6% (n=17)	12.5% (n=1)	22.5% (n-18)
gL3	6.9% (n=5)	25% (n=2)	8.8% (n=7)
gL4	43.1% (n=31)	62.5% (n=5)	45.0% (n=36)

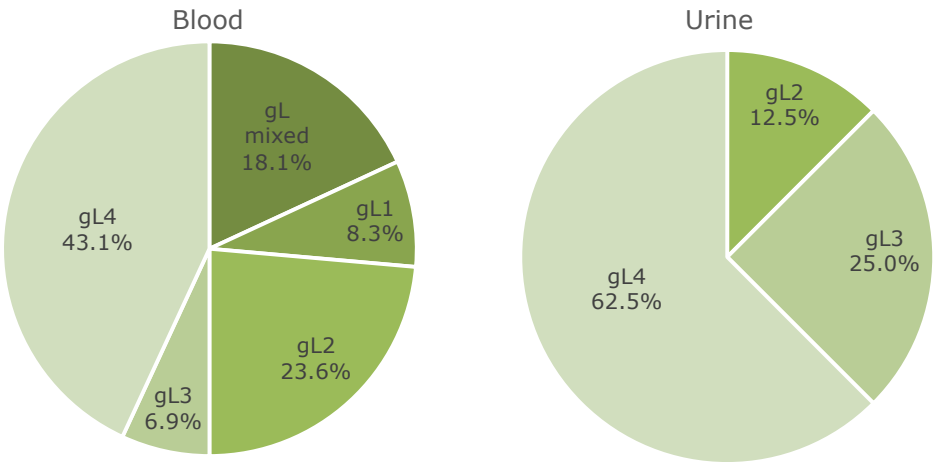

Figure 19: gL distribution among different specimen types.

4.5.2.4 Glycoprotein M

A total of 74 of 89 (83.1%) specimens were successfully gM genotyped. After excluding any group with 4 cases or less, the total number of gM specimens that could be statistically analyzed was reduced to 71 (79.8%).

The distribution of gM genotypes was as follows: gM1 (n=14, 19.7%), gM2 (n=4, 5.6%), and gM3 (n=52, 73.2%), mixed gM genotypes (n=1, 1.4%). gM3 was the most prevalent (Table 16).

Among blood specimens (sub-group A), gM3 (n=49, 77.8%) was the most predominant, followed by gM1 (n=11, 17.5%), while among urine specimens (sub-group B), gM3 and gM1 were equally represented (n=3, 37.5%) (Table 16, Figure 20).

The chi-square statistical test indicated that there is a significant relationship between the specimen type (X^2 (3, N=71) = 9.147), p=0.027), and the distribution of gM genotypes among HCMV patients.

Table 16: gM distribution among different specimen types.

gM genotypes	Blood	Urine	Total
Mixed gM	1.6% (n=1)	0.0% (n=0)	1.4% (n=1)
gM1	17.5% (n=11)	37.5% (n=3)	19.7% (n=14)
gM2	3.2% (n=2)	25% (n=2)	5.6% (n=4)
gM3	77.8% (n=49)	37.5% (n=3)	73.2% (n=52)

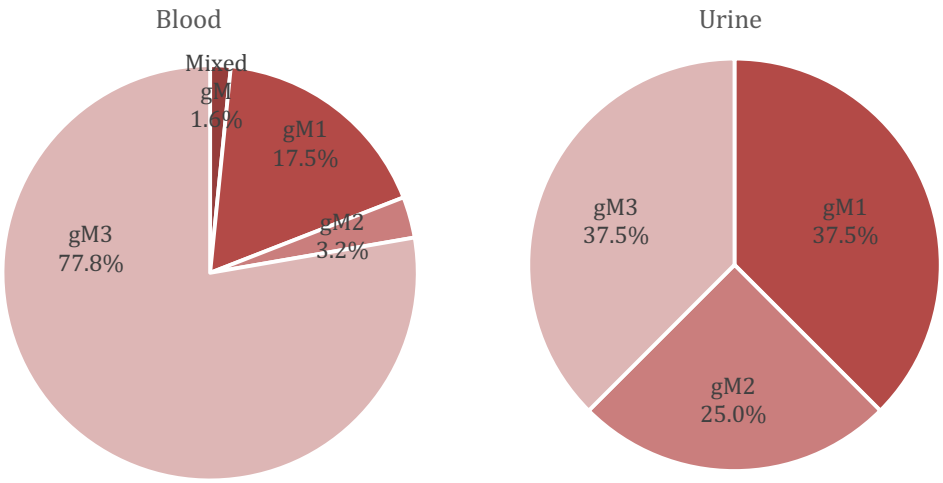

Figure 20: gM distribution among different specimen types.

4.5.2.5 Glycoprotein N

A total of 74 of 89 (83.1%) specimens were successfully gN genotyped. After excluding any group of the specimen type had 4 cases or less, the total number of gN specimens that could be statistically analyzed was reduced to to 71 (79.8%).

The distribution of gN genotypes was as follows: gN1 (n=5, 7.0%), gN3a (n=50, 70.4%), gN4a (n=6, 8.5%), gN4b (n=3, 4.2%), gN4c (n=4, 5.6%), and gN4d (n=3, 4.2%). The most common gN genotype was gN3a. Also, among blood (sub-group A) and urine samples (sub-group B), gN3a was again the most prevalent (n=43, 68.3%; n=7, 87.5%, respectively) (Table 17, Figure 21).

However, there was no statistically significant relation between specimen type and any of the gN genotypes' distribution (X^2 (5, N=71) = 2.453), p=0.78).

Table 17: gN distribution among different specimen types.

gN genotypes	Blood	Urine	Total
gN1	7.9% (n=5)	0.0% (n=0)	7.0% (n=5)
gN3a	68.3% (n=43)	87.5% (n=7)	70.4% (n=50)
gN4a	7.9% (n=5)	12.5% (n=1)	8.5% (n=6)
gN4b	4.8% (n=3)	0.0% (n=0)	4.2% (n=3)
gN4c	6.3% (n=4)	0.0% (n=0)	5.6% (n=4)
gN4d	4.8% (n=3)	0.0% (n=0)	4.2% (n=3)

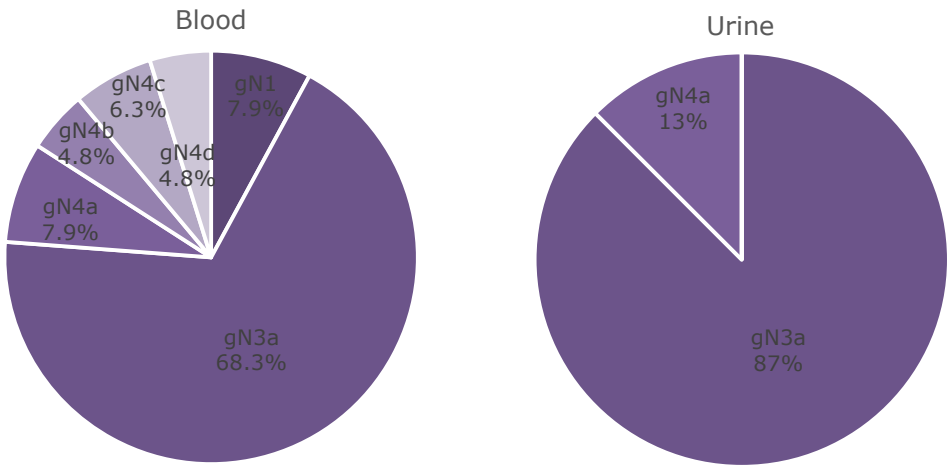

Figure 21: gN distribution among different specimen types.

4.5.2.6 Glycoprotein O

A total of 71 of 89 (79.8%) specimens were successfully gO genotyped. After excluding any group of the specimen type with 4 cases or less, the total number of gO that could be statistically analysed was reduced to 68 (76.4%).

The distribution of gO genotypes was as follows: gO1a (n=16, 23.5%), gO1c (n=41, 60.3%), gO2a (n=2, 2.9%), gO2b (n=1, 1.5%). gO3 (n=2, 2.9%), gO4 (n=5, 7.4%), mixed gO genotypes (n=1, 1.6%). Both gO1c and gO1a were the most prevalent. Also, they were the most common in blood (sub-group A) (n=37,

60.0%- n=14, 22.6%) and urine (sub-group B) (n=4, 66.7%- n=2, 33.3%) samples, respectively. In addition, among blood samples only 5 cases presented with gO4 (8.0%) (Table 18, Figure 22).

However, chi-square showed that there was no statistically significant correlation between any of gO genotypes and specimen type (X^2 (6, N=68) = 1.378), p=0.97).

Table 18: gO distribution among different specimen types.

gO genotypes	Blood	Urine	Total
Mixed gO	1.6% (n=1)	0.0% (n=0)	1.6% (n=1)
gO1a	22.6% (n=14)	33.3% (n=2)	23.5% (n=16)
gO1c	59.7% (n=37)	66.7% (n=4)	60.3% (n=41)
gO2a	3.2% (n=2)	0.0% (n=0)	2.9% (n=2)
gO2b	1.6% (n=1)	0.0% (n=0)	1.5% (n=1)
gO3	3.2% (n=2)	0.0% (n=0)	2.9% (n=2)
gO4	8.1% (n=5)	0.0% (n=0)	7.4% (n=5)

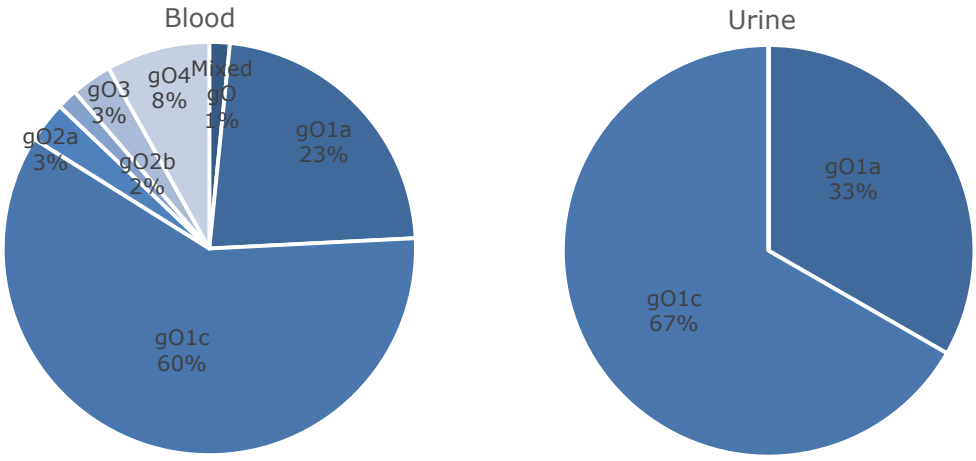

Figure 22: gO distribution among different specimen types

4.5.3 Summary

In summary, from Table 19 below we can see that in each infection category (group 1) and specimen type (group 2) there are some prevalent glycoprotein genotypes. Shared common genotypes among all categories of infection and specimen types were detected.

In general, percentages of each genotype distribution among infection category and specimen type groups are summarized and presented in Figure 23.

However, the distribution of gH was significantly influenced by the infection category type and the specimen types, while the distribution of gM was significantly affected by the specimen types only ($P<0.05$).

Table 19: The most common HCMV glycoprotein genotypes among each infection category and specimen type.

Genotypes	Infection categories				Specimen types	
	Congenital infection	Immuno-compromised Primary infection	Immuno-compromised Recurrent infection	Immuno-competent Primary infection	Blood	Urine
gB	1	1	1	3	1	3
gH	1	1	2	2	1&2	1
gL	4	4	4	4	4	4
gM	3	3	3	3	3	1&3
gN	3a	3a	3a	3a	3a	3a
gO	1a &1c	1a&1c	1a&1c	-	1a&1c	1a&1c

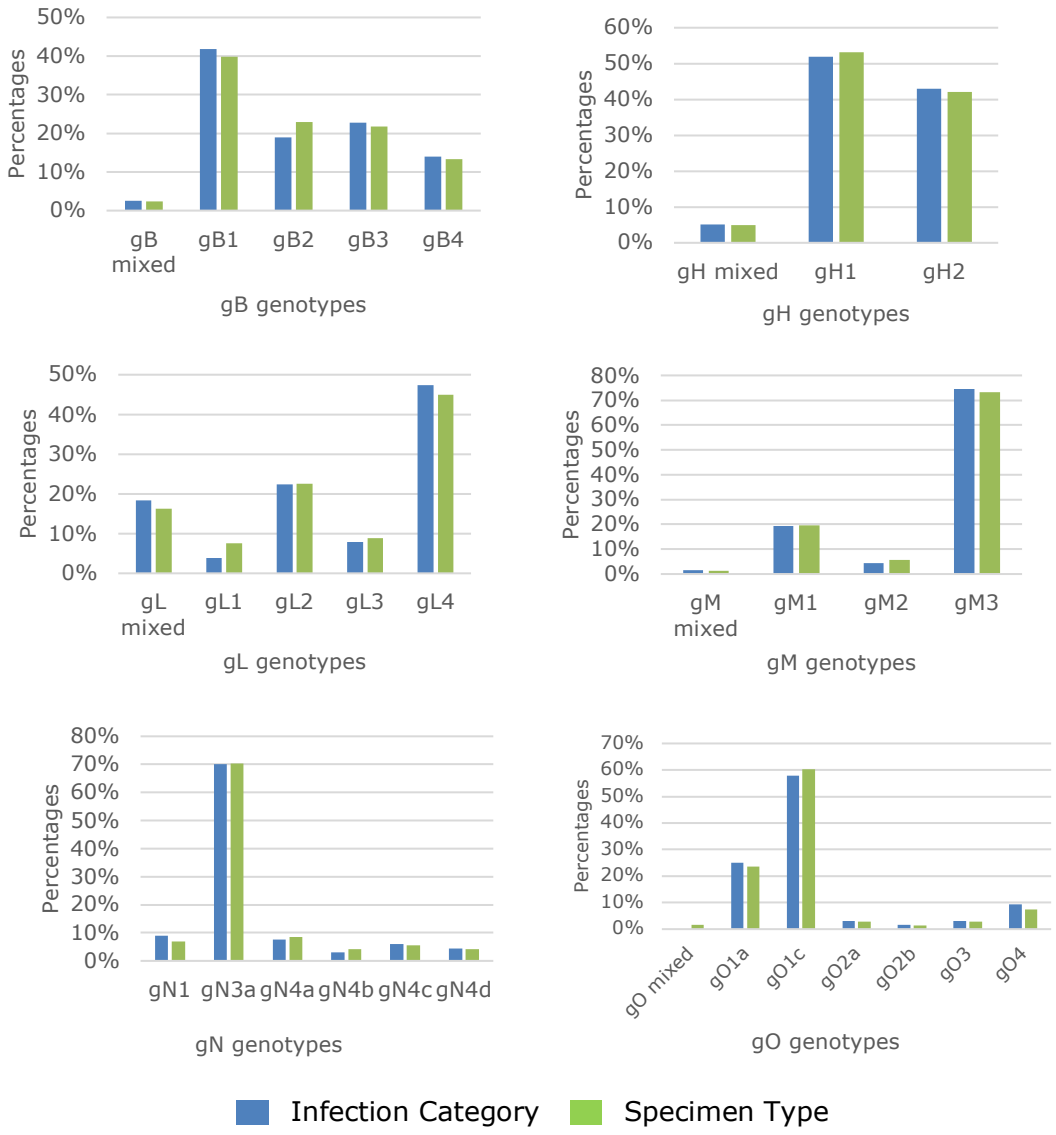

Figure 23: Percentages of each genotype distribution according to infection category and specimen type analysis.

4.6 Identification of HCMV strains growth characteristics

The growth characteristics of each viral strain were assessed in cell culture over a 4-week period post infection. A virus growth curve for all lab strains (N=5) and clinical samples (N=89), was plotted using data obtained from TCID50 assays. To validate these results, real time PCR was additionally performed with the lab strains prior to TCID50, this was not possible with the clinical samples due to the limited volume in each sample.

To standardize the conditions, $2X10^5$ cells/ml of MRC-5 cells were infected with virus at 10.000 TCID50/ml (MOI= 0.05). Duplicate wells were used for each virus dilution to increase accuracy.

4.6.1 Laboratory strains growth characteristics

4.6.1.1 Laboratory strains growth characteristics assessed by $TCID_{50}$ assay

TCID50 was carried out as explained in section (3.2.1.9). Infected wells were monitored for appearance of CPE daily and positive wells were scored at various time points; 7, 14, 21, 24 and 28 days post infection, and recorded. TCID50 values were calculated at the end of each week and the growth curve was plotted using these values.

As shown in Table 20 and Figure 24 below, the cytopathic effect started to appear from day 7 (Second week) and the virus continued to grow during weeks 3 and 4. After the 4^{th} week, CPE was seen in 100% of infected wells and destruction of the cells monolayer was noted. Although 100% CPE was seen for all lab strains by day 28, the rate of growth was faster, and the end titre was higher for AD169 and Towne strains and significantly slower and lower titre for Davis and Merlin strain with Merlin having the lowest growth rate. Toledo strain appeared to have a very low growth rate until week 3 when it then grew to reach the same titre as AD169 and Towne.

Table 20: Tracking HCMV laboratory strains growth over 4 weeks using $TCID_{50}$ values.

Laboratory strains	$TCID_{50}$/ml			
	Week 1	**Week 2**	**Week 3**	**Week 4**
AD169	10^0	$10^{2.8}$	$10^{4.4}$	$10^{11.5}$
Towne	10^0	10^3	$10^{4.4}$	$10^{11.4}$
Toledo	10^0	10^1	$10^{1.5}$	$10^{11.5}$
Davis	10^0	$10^{2.8}$	10^3	10^9
Merlin	10^0	10^0	10^1	10^9

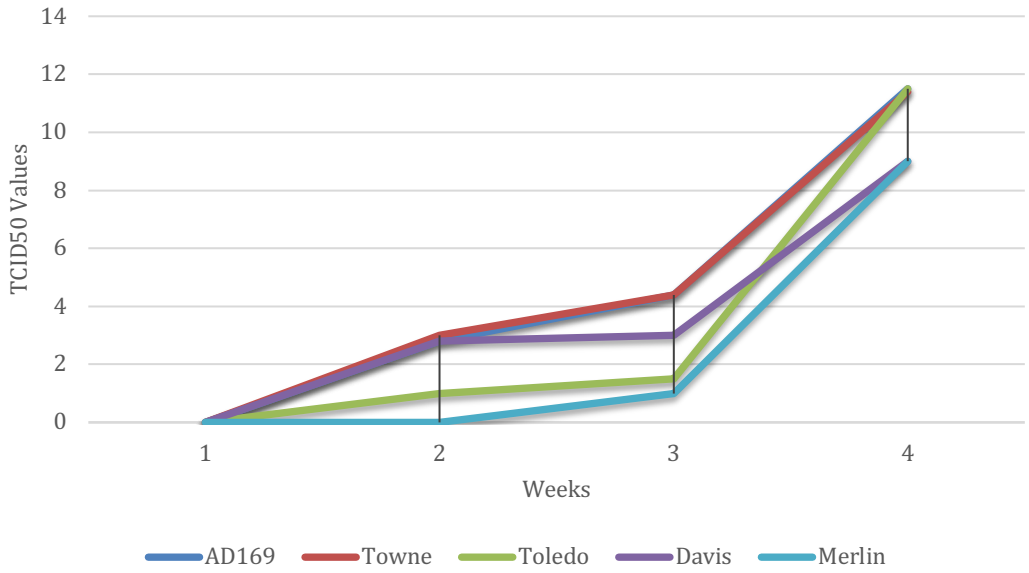

Figure 24: Laboratory strains growth characteristics over a 4-week period using $TCID_{50}$ values.

4.6.1.2 Laboratory strains growth characteristics using PCR-based $TCID_{50}$ assay

Real time PCR was performed to confirm the TCID50 results. Duplicate culture plates were set up in parallel with the TCID50 assays, but instead of observing the CPE at the set time points, the supernatants were harvested at; 24 hours, 48 hours, 4, **7**, 8, **14**, 15, **21**, 24, **28** days' post infection and then frozen at -80°C. All samples were thawed at the same time and viral DNA was extracted on

the same day. PCR was performed as described previously in section (3.2.2). For HCMV PCR standards, a 1:10 dilution series with 7 dilution steps was used. CT-values were plotted against the logarithm of the dilution factors (DF) to draw the PCR standard curve, from which the concentration of each sample was determined (Figures 25, 26).

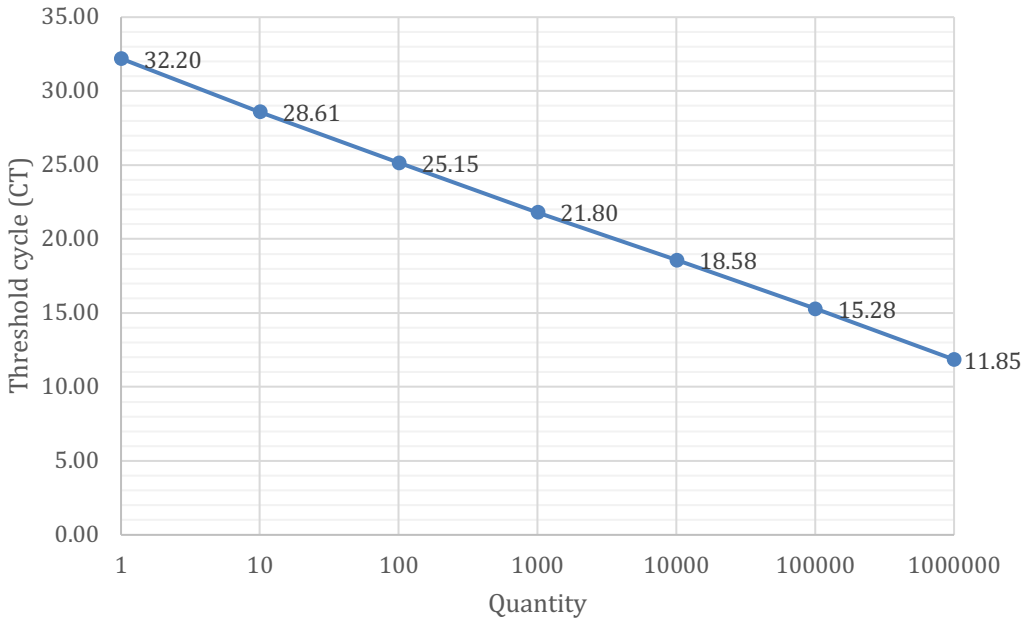

Figure 25: Standard curve for HCMV real time PCR, seven 1:10 serial dilutions of the standards were used, The Y-axis presents the CT values corresponding to the number of DNA copies of each sample.

As shown in Figure 26 below, no growth was seen in the first week of infection (first week). AD169 growth started to appear from day 7 (Second week) and continued to grow during weeks 2 and 3, in the 4th week, the virus DNA concentration was gradually decreasing. Regarding the other lab strains, Towne, Toledo, Davis and Merlin growth characteristics were similar, their viral load started to increase by day 21 (third week) and reached the peak during the fourth week until day 28 when started to decrease again. However, Merlin strain viral load was significantly lower than the others. With the exception of Towne, this pattern mirrors the TCID50 growth curves shown in Figure 24.

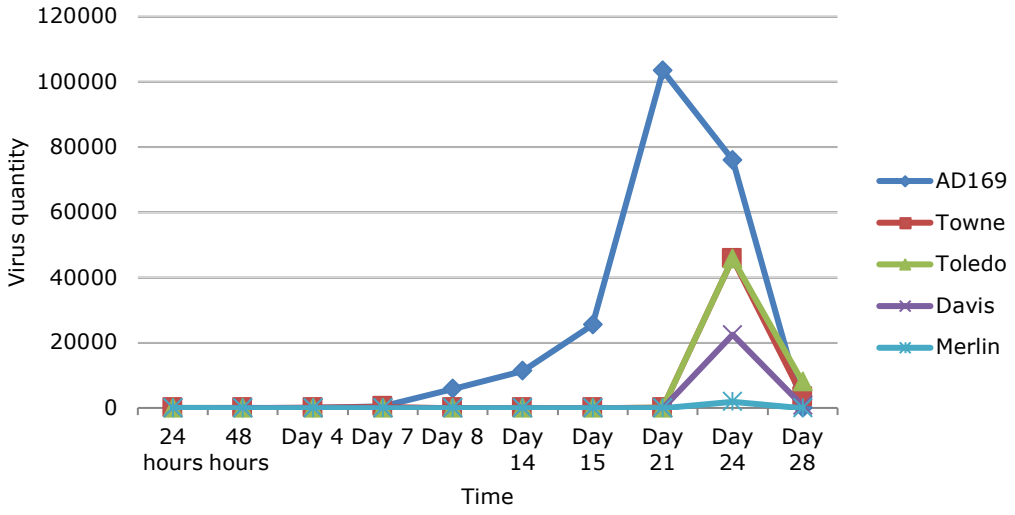

Figure 26: Laboratory strains growth characteristics over 4 weeks assayed by PCR, The Y-axis presents the number of DNA copies of each sample.

4.6.1.3 Effect of glycoprotein genotypes on laboratory strains growth characteristics

The association between each glycoprotein genotype and the growth of HCMV lab strains was analysed, however, due to the small number of the samples (N=5) statistical analysis was not possible, therefore, observational data of the virus growth, presented in Table 20 and Figure 24 above, and the genotyping profile of each strain in Table 21 below is presented. As observed, AD169 grows faster and higher, gB2, gL1, gN1 and gO1a are all specific to this strain and could be responsible for its growth characteristic. Also, in case of Merlin strain, which grows slowest; gL4, gN4c and gO5 could all be responsible. However, this has to be confirmed with further analysis of a larger samples size.

Table 21: HCMV laboratory strains glycoproteins genotyping profile.

Lab strains	Genotyping Profile					
	gB	**gH**	**gL**	**gM**	**gN**	**gO**
1-AD	2	1	1	1	1	1a
2-Tn	1	2	2	2	4b	4
3-Td	3	1	3	2	4d	1c
4-D	1	1	3	3	3b	2a
5-M	1	2	4	1	4c	5

4.6.2 HCMV growth characteristics in clinical samples

The large number of clinical samples (N=89) assayed prevented individual analysis. Instead, the mean of the TCID50 values for each week was calculated to acquire a general picture of the virus growth across all clinical samples over 4 weeks of infection. The same calculation was applied to the laboratory strains. A comparison between both groups was done using a line chart to represent the mean of TCID50 values and the standard deviation of the duplicates (Figure 27).

Surprisingly, the clinical samples started to develop CPE a week earlier than the lab strains, which suggests that the eclipse phase for the lab strains was longer than for the clinical samples. However, by the end of the experiment the lab strains had reached a considerably higher average titre than the clinical strains (Figure 27). The complete $TCID_{50}$ values for clinical strains for 4 in 4 weeks of infection can be found in the appendices section (Appendix 2).

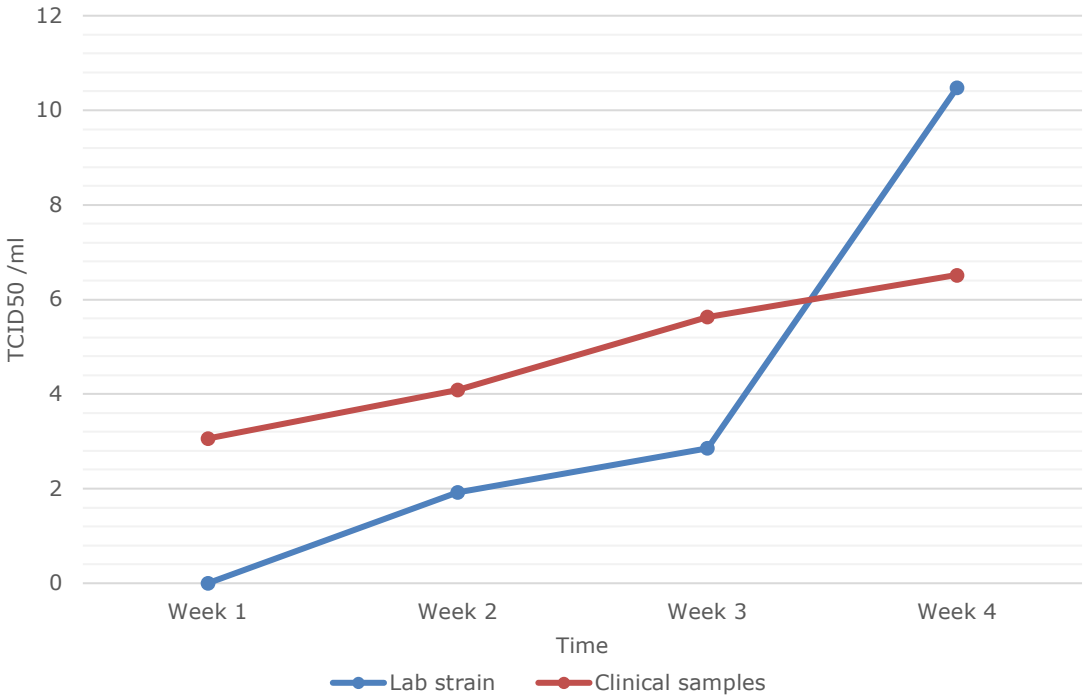

Figure 27: Growth curves of laboratory strains and clinical strains over 4 weeks of HCMV infection using the average of $TCID_{50}$ values.

4.6.2.1 Effect of glycoprotein genotypes on clinical strains growth characteristics

To determine whether glycoprotein genotype has any effect on viral growth characteristics in culture, an analysis of variance (ANOVA) was conducted for all the clinical strains. The total number of each strain and the number of glycoproteins successfully genotyped is shown in sections 4.5.1 and 4.5.2 above.

Any subtype that had less than 4 cases was excluded from any further analysis, these were: mixed gB genotypes (n=3), mixed gM genotypes (n=1), gN4b and (n=3), gN4d (n=3), mixed gO genotypes (n=1), gO2a (n=2), gO2b (n=1), and gO3 (n=2).

There were no significant differences between growth curves of each genotype over time as shown in Figure 28 below. This was statistically confirmed by one-way ANOVA test, which showed no statistically significant effect of any of the glycoprotein genotypes on the virus growth across all 4 weeks of the viral infection

(P>0.05). Detailed F & P values for each week for all glycoprotein genotypes can be found in the appendices section (Appendix 3).

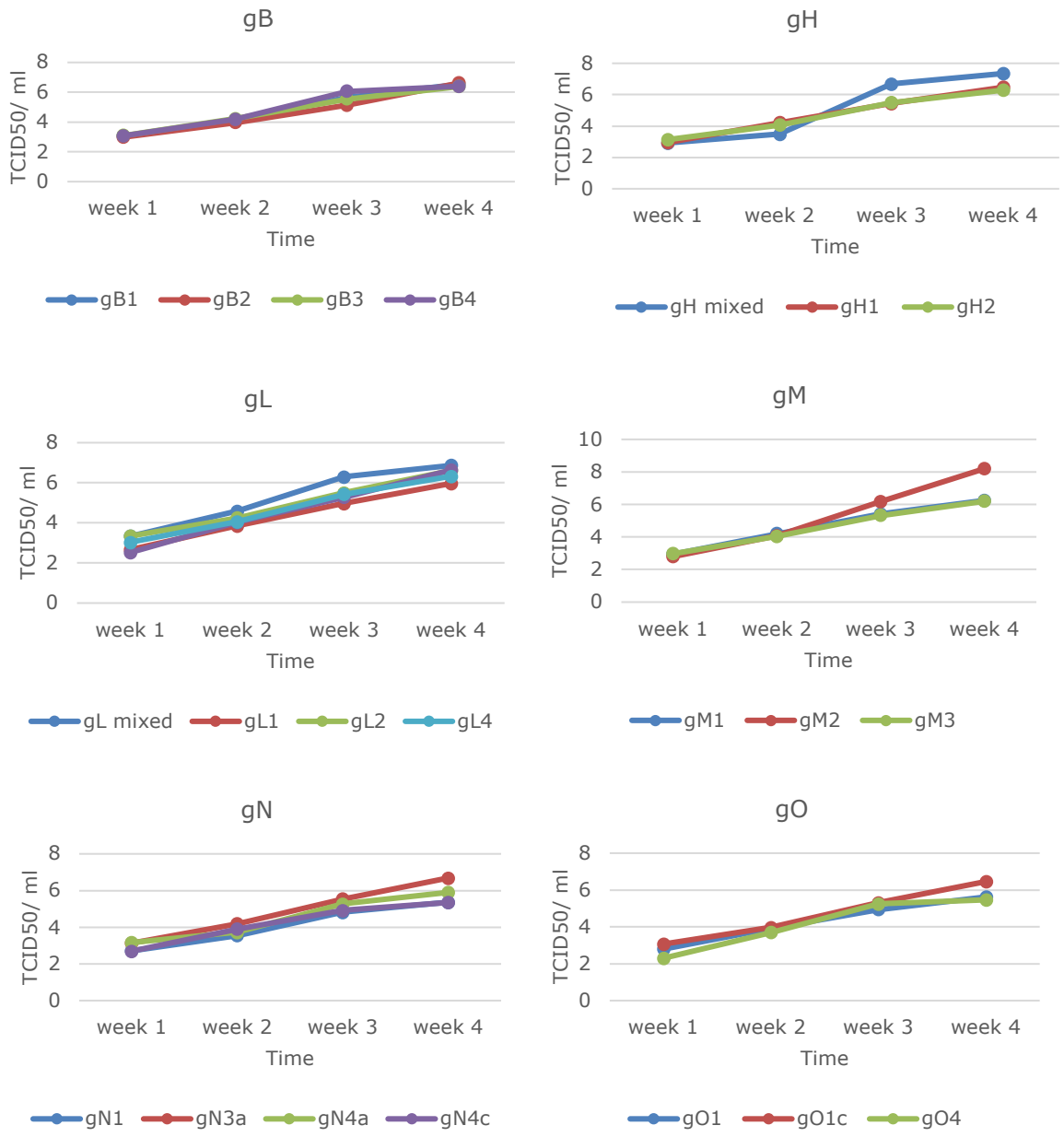

Figure 28: The effect of the glycoprotein genotypes on HCMV growth over 4 weeks' post infection, using the mean of $TCID_{50}$ values. No statistically effect has been identified according to ANOVA statistical analysis.

4.6.3 HCMV strains growth characteristics analysed by patient group

The growth characteristics of individual virus strains were analysed according to the patient group that they were collected from**.** One-way ANOVA was

carried out for all clinical samples after excluding samples that belong to infection categories with less than 4 cases (N=85), (the excluded infection categories are: unconfirmed congenital or early post-natal (N=1), not defined primary/recurrent (N=1) and not defined primary/recurrent (N=2)).

The overall outcomes of one-way ANOVA revealed that there was a statistically significant effect of patient group on the growth of HCMV over the second, third and fourth weeks of infection ((F (4, 80) =1.402, p = .003), (F (4, 80) = 11.346, p=.000), F (4, 80) = 18.502, p=.000), respectively).

Following the ANOVA test results which showed there was an effect correlated with patient group, the data was subjected to the Bonferroni post hoc test to confirm which specific infection category has a significant effect on the viral growth. It showed that in the second week of infection the growth rate of the viral strains derived from congenitally infected patients was significantly higher than those taken from the immunocompromised patients with primary and recurrent infections. During the 3^{ed} and 4th weeks, the congenital strains had a significantly higher growth rate than the strains derived from any other patient group. (Figure 29, Table 22), complete tables of ANOVA and post hoc results can be found in the appendices section (Appendix 4).

Table 22: Mean, Standard deviation (SD), and P values of congenital infection samples over 4 weeks post infection.

Time	Mean TCID50 for viral strains from congenitally infected patients	SD	Sig. (P value)
week1	3.5	2.63	0.23 (Not significant)
week2	5.44	1.45	0.003 (Significant)
week3	8.08	1.57	0.00 (Significant)
week4	9.28	0.79	0.00 (Significant)

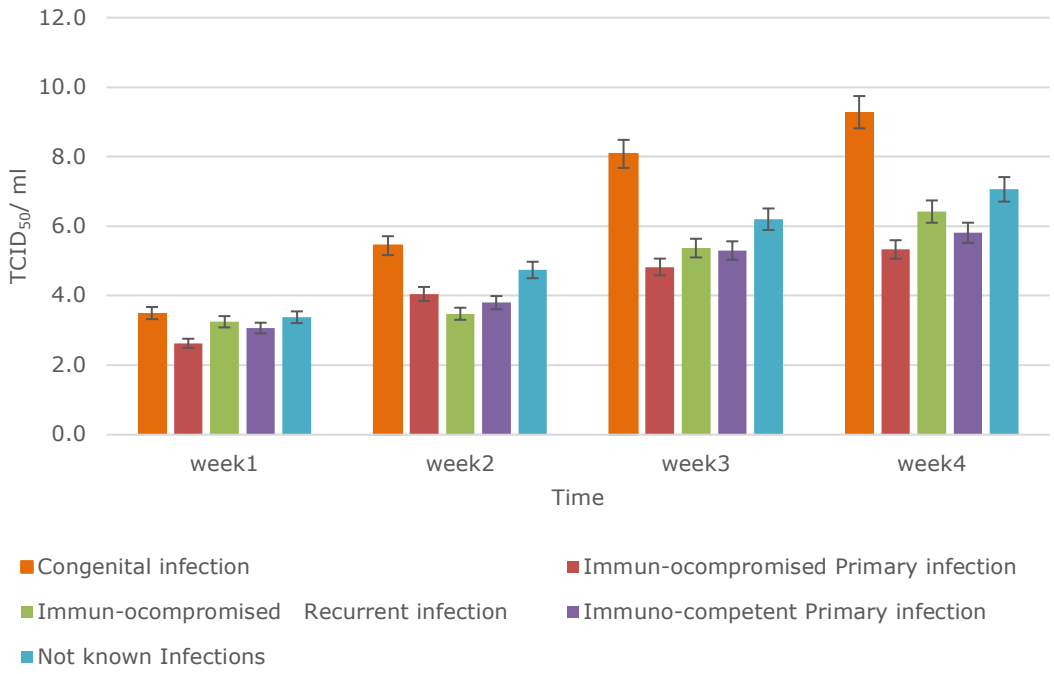

Figure 29: HCMV strains growth over 4 weeks post infection, according to patient group, using the mean TCID50 values for each week for each infection category.

4.6.4 Effect of specimen type on HCMV strains growth characteristics

To study the effect of the specimen type from which the strain was isolated on virus growth over time (4 weeks post infection), the one-way ANOVA test has been used to analyse the data for all clinical samples (N=89), These are: blood specimens (N=89), urine specimens (N=8), and respiratory specimens (N=4).

The outcomes of the one-way ANOVA revealed that there was a statistically significant effect of specimen type on the growth of HCMV over the fourth week of infection only ((F (2, 86) =5.197, p = 0.007) (Figure 30).

Since the ANOVA test showed an overall significant result, the Bonferroni post hoc test was performed to confirm which specific specimen type had a significant effect on the final stages of the viral growth. This showed that strains isolated from urine specimens had a significantly higher growth rate compared to blood specimens (Table 23). The growth rate for the urine specimens was higher

than for the strains from the respiratory samples as well but this was not statistically significant. Complete tables of ANOVA results can be found in the appendices section (Appendix 5).

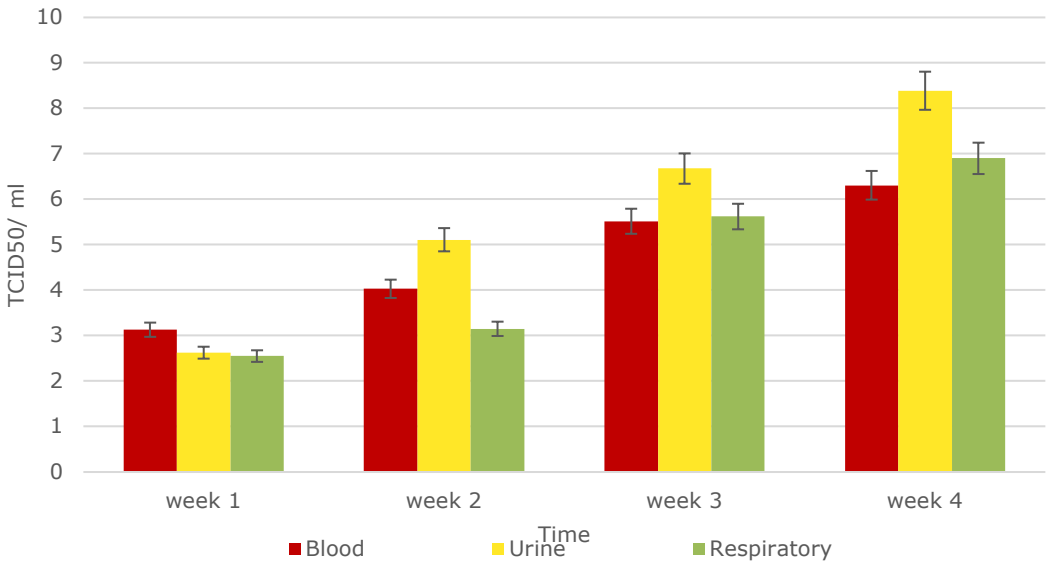

Figure 30: Effect of specimen types of on HCMV growth over 4 weeks' post infection, using the mean of $TCID_{50}$ values of each week for each specimen type.

Table 23: Mean, standard deviation (SD), and P values of Urine specimens over 4 weeks post infection.

Time	Mean	SD	Sig. (P value)
week1	2.63	0.72	0.38
week2	5.11	1.49	0.08
week3	6.68	1.53	0.22
week4	8.39	1.82	0.007

4.6.5 Summary

The results of studying the growth characteristics of the HCMV strains (lab and clinical strains) showed that the clinical samples started to develop CPE a week earlier than the lab strains, which suggests that the eclipse phase for the lab strains was longer than for the clinical samples. However, whilst the lab strains started their growth later in week 2, by the 3rd week their titre was much higher than that for the clinical samples. These results have been confirmed using real time PCR, where similar results were obtained.

Regarding the effect of the glycoprotein genotype on the virus growth, it was observed that in laboratory strains that AD169 grows faster and higher, gB2, gL1, gN1 and gO1a are all specific to this strain and could be responsible for its growth characteristic. Also, in case of Merlin strain, which grows slowest; gL4, gN4c and gO5 could all be responsible for its slow growth. However, this must be confirmed with further analysis of a larger samples size. Moreover, there was no statistically significant effect of any of the glycoprotein genotypes on the virus growth in clinical samples across all 4 weeks of the viral infection ($P>0.05$) as confirmed by one way ANOVA statistical test.

However, the alternative hypothesis that HCMV infection category and specimen type may influence the viral growth characteristic over time has been accepted and confirmed. ANOVA revealed that both congenital infections and urine specimens has a statistically significant effect of the virus higher growth rate in the last three weeks or only in the last week of infection respectively, ($P<0.05$).

4.7 Analysis of the relationship between HCMV glycoprotein genotypes and glycosylation patterns.

To determine whether the mutations in individual glycoprotein genes that lead to the various glycoprotein types affect and influence the way that the

glycoprotein is glycosylated, the laboratory and clinical strains were all tested using ELLA (Enzyme linked-lectin assay). This assay uses a panel of 20 lectins chosen according to their sugar specificity as described previously (Table 3). All laboratory (N=5) and clinical strains (N=89), plus non-infected control cells were tested with each lectin in duplicate to ensure the reliability of the assay. The average optical density (O.D.) for each lectin was calculated and the values for the uninfected controls were subtracted from the virus infected test samples to give a + or – value depending on whether the virus infected cells bound more or less of a particular lectin than the controls.

The effect of HCMV glycoprotein genotype on the virus glycoprotein glycosylation pattern and whether this was statistically significant or not was tested. The data was then analysed by infection category, and specimen type.

4.7.1 Laboratory strains glycoproteins glycosylation

Initially, a paired t test was conducted to test the null hypothesis (H_0) that there is no difference in the lectins binding profile before and after the cells are infected by HCMV. Due to the wide variation of lectin specificities, the null hypothesis was examined for each lectin separately.

The results showed that there was a statistically significant change in (45%, N=9) of the lectins' binding profile between non-infected and infected cells, a significant increase after infection was seen with 2 lectins (22.2%) [LCA (t (4) = 0.498, p=0.43) and UEA (t (4) 15.811, p = 0.000)], and a significant decrease was seen with 7 lectins (77.8%), [GSL-11 (t (4) 4.951, p = 0.008), GNL (t (4) 4.843, p = 0.008), BPL (t (4) 7.306, p = 0.002), EEL (t (4) 14.154, p = 0.000), WFA t (4) 6.957, p = 0.002), PTL (t (4) 60.249, p=0.000), and PSA (t 4) 3.624, p = 0.022. Sugar specificity, mean and standard deviation of these lectins are shown in Table 24 and Figure 31 below. The results for the other lectins was either not statistically

significant or there was no change in lectin binding before and after the cells were infected by HCMV.

Table 24: Lectins that had a statistically significant difference in their binding profile (N=9), before and after laboratory strains HCMV infection.

Lectins	Before infection	After infection	Sig (P)	Sugar specificity
LCA	M=0.43, SD=0.00	M=0.80, SD=0.29	0.43	αMan, αGlc
GSL II	M=0.20, SD=0.00	M=0.10, SD=0.04	0.01	α or βGlcNAc
GNL	M=0.38, SD=0.00	M=0.23, SD=0.07	0.01	αMan
BPL	M=0.52, SD=0.00	M=0.29, SD=0.07	0.00	Galβ3GalNAc
UEA	M=0.13, SD=0.00	M=0.80, SD=0.01	0.00	αFuc
EEL	M=0.20, SD=0.00	M=0.10, SD=0.06	0.00	Galα3Gal
WFA	M=0.53, SD=0.00	M=0.25, SD=0.09	0.00	GalNAc
PTL	M=0.41, SD=0.00	M=0.08, SD=0.01	0.00	GalNAc, Gal
PSA	M=0.53, SD=0.00	M=0.84, SD=0.09	0.22	αMan, αGlc

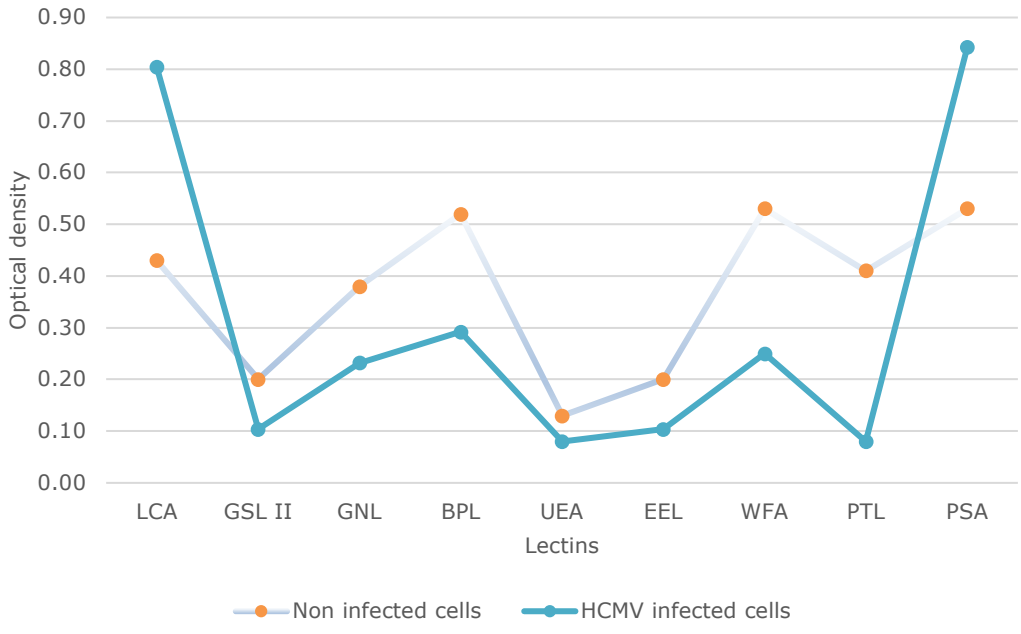

Figure 31: Lectins binding profile comparison between non-infected and HCMV infected cells in laboratory strains.

4.7.1.1 Glycoprotein genotypes effect on glycosylation patterns of laboratory strains

Next, to determine if any specific glycoprotein genotype had a significant effect on lectin binding in the HCMV infected cells, an analysis of variance (ANOVA) was conducted for all the genotypes except gH, which had only 2 groups to compare, therefore, independent samples T test was used. The complete glycoprotein genotype profile for all laboratory strains is summarized in Table 21. The genotypes, and the number of cases for each, included in the analysis according to the laboratory strains genotypes profile are presented in the table below (Table 25).

Table 25: Glycoprotein genotypes quantities included in ANOVA analysis for Laboratory strains.

Genotype	N	Genotype	N	Genotype	N	Genotype	N
gB1	3	gM1	2	gL4	1	gN4c	1
gB2	1	gM2	2	gN1	1	gO1a	1
gB3	1	gM3	1	gN4b	1	gO4	1
gH1	3	gL1	1	gN4d	1	gO1c	1
gH2	2	gL2	1	gL4	1	gO2a	1
		gL3	2	gN3	1	gO5	1

The independent-samples t-test used to compare the effect of gH1 and gH2 on the lectin binding showed a statistically significant effect between gH1 and gH2 genotypes with LCA, GSL II, GNL, EEL, WFA, PHA-E, MAA II, and PSA lectins. It revealed that gH2 was significantly associated with increased binding of the above-mentioned lectins, while gH1 with decreased binding (Table 26, Figure 32). In addition, ANOVA results showed that there was a statistically significant association between increased binding of PHA-lectin and gM1 genotype, while gM3 was associated with a decreased binding of the same lectin ($F(2, 4) = 34.748$, $p=0.03$) (Table 26, Figure 32). Although ANOVA results were significant, it was not possible

to perform the post hoc Bonferroni test, to determine the most significant gM genotype, because at least 1 group of gM has fewer than 2 cases (Table 26).

Table 26: Mean, standard deviation (SD), and P values of the overall statistical significant lectins binding profiles influenced by some HCMV genotypes in Laboratory strains.

Genotypes	Lectins	Mean	SD	Sig. (P value)	Sugar Specificity of lectins
gH1	LCA	0.73	0.19	0.03	αMan, αGlc
gH2		0.91	0.47		
gH1	GSL II	0.07	0.01	0.001	α or βGlcNAc
gH2		0.13	0.07		
gH1	GNL	0.21	0.02	0.002	αMan
gH2		0.27	0.11		
gH1	EEL	.09	0.01	0.005	Galα3Gal
gH2		0.12	0.02		
gH1	WFA	0.24	0.04	0.003	GalNAc
gH2		0.26	0.17		
gH1	PHA-E	0.34	0.06	0.47	Galβ4GlcNAcβ2Manα6 (GlcNAcβ4) (GlcNAcβ4Manα3) Manβ4
gH2		0.42	0.14		
gH1	MAA II	0.57	0.13	0.02	Neu5Acα3Galβ4GalNAc
gH2		0.67	0.44		
gH1	PSA	0.79	0.07	0.002	αMan, αGlc
gH2		0.92	0.35		
gM1	PHA-L	1.28	0.05	0.03	Galβ4GlcNAcβ6(GlcNAcβ 2Manα3) Manα3
gM2		0.92	0.07		
gM3		0.69			

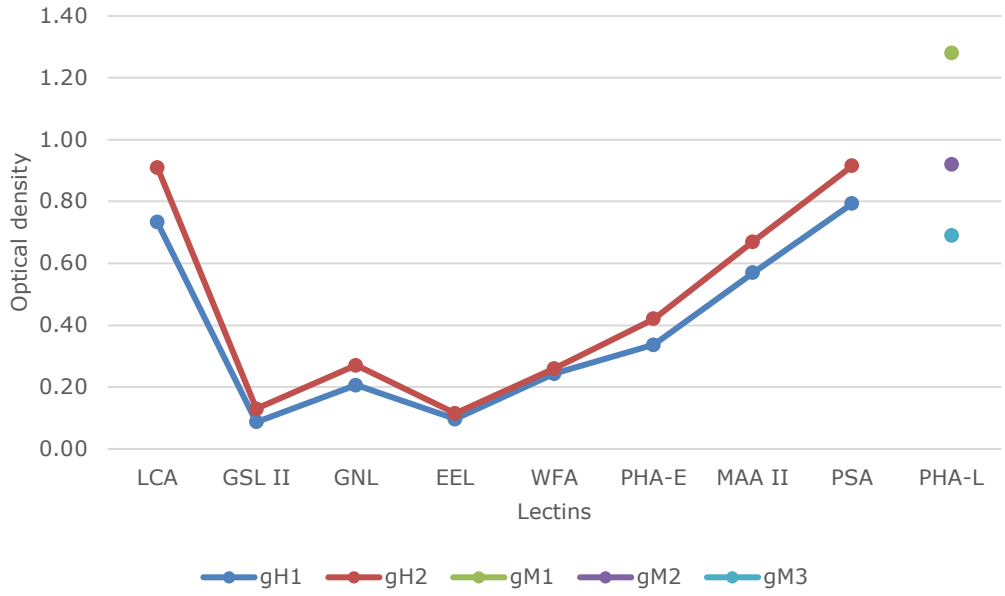

Figure 32: The significant effect of gH and gM genotypes on some of the lectins binding profile.

4.7.2 Glycoproteins glycosylation of the clinical strains

A paired t-test was performed to examine whether any significant difference in lectin binding occurred following infection of cells with HCMV strains derived from the clinical samples. Again, due to the wide variation of lectin specificities, this was done for each lectin separately.

The results showed that there was a statistically significant change in 70% (N=14) of the lectins binding between non-infected and infected cells. For 13 of these (92.9%), a statistically significant increase in binding was seen [SBA (t (88) 4.92, p=0.000), ECL (t (88) 6.281, p=0.000), LCA (t (88) 10.240, p=0.000), WGA (t (88) 8.318, p=0.000), EBL (t (88) 7.848, p=0.000), PHA-L (t (88) 2.924, p=0004), LEL (t (88) 7.299, p=0.000), BPL (t (88) 4.134, p=0.000), UEA samples (t (88) 8.205, p=0.000), PHA-E (t (88) 6.796, p=0.000), AAL (t (88) 5.830, p=0.000), and GSL-L (t (88) 4.962, p=0.000)] whilst for 1 lectin (7.1%) a statistically significant decrease was seen [WFA (t (88) 3.722, p=0.000. A list of these lectins and their specificities is shown in (Table 27) below. In the other lectins

there was either no change in the lectin binding or the change was not statistically significant, (Figure 33).

Table 27: Lectins that had a statistically significant difference in their binding profile, before and after clinical specimens HCMV infection.

Lectins	Before infection	After infection	Sig (P)	Sugar Specificity
SBA	M=0.16, SD=0.00	M=0.22, SD=0.12	0.00	α> βGalNAc
ECL	M=0.43, SD=0.00	M=0.56, SD=0.19	0.00	Galβ4GlcNAc
LCA	M=0.43, SD=0.00	M=0.70, SD=0.25	0.00	αMan, αGlc
WGA	M=0.49, SD=0.00	M=0.76, SD=0.31	0.00	GlcNAc
EBL	M=0.60, SD=0.00	M=0.85, SD=0.30	0.00	Neu5Acα6Gal/GalNAc
PHA-L	M=0.92, SD=0.00	M=0.1.08, SD=0.51	0.00	Galβ4GlcNAcβ6(GlcNAc β 2Manα3)Manα3
LEL	M=0.46, SD=0.00	M=0.67, SD=0.27	0.00	(GlcNAc) 2-4
BPL	M=0.52, SD=0.00	M=0.63, SD=0.25	0.00	Galβ3GalNAc
UEA	M=0.13, SD=0.00	M=0.24, SD=0.12	0.00	αFuc
WFA	M=0.53, SD=0.00	M=0.44, SD=0.24	0.00	GalNAc
PHA-E	M=0.39, SD=0.00	M=0.55, SD=0.22	0.00	Galβ4GlcNAcβ2Manα6 (GlcNAcβ4) (GlcNAcβ4Manα3) Manβ4
ALL	M=0.52, SD=0.00	M=0.68, SD=0.26	0.00	Galβ3GalNAc
AAL	M=0.62, SD=0.00	M=0.79, SD=0.29	0.00	Fucα6GlcNAc
GSL I	M=0.21, SD=0.00	M=0.27, SD=0.11	0.00	αGal, αGalNAc

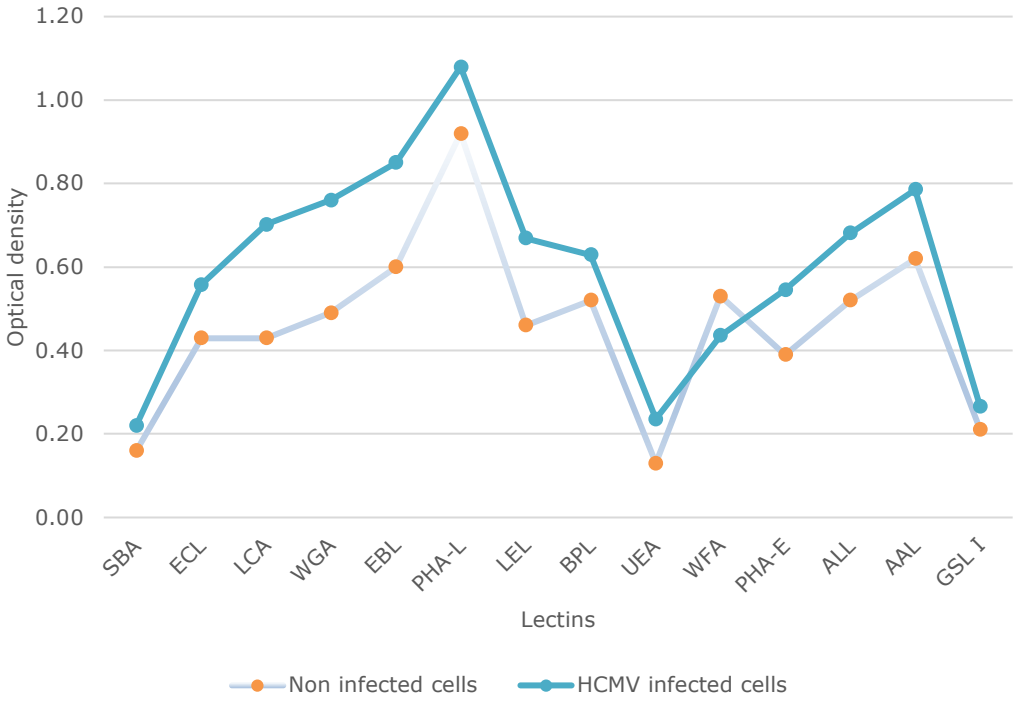

Figure 33: Lectins binding comparison between non-infected and HCMV infected cells in clinical specimens.

4.7.2.1 Glycoprotein genotypes effect on glycosylation patterns of clinical samples

To determine if any specific glycoprotein genotype had a significant effect on the glycoprotein glycosylation, an analysis of variance (ANOVA) was conducted for all the clinical strains. The total number of each glycoprotein successfully genotyped was stated previously in (Sections 4.5.1 and 4.5.2).

Any subtype that had less than 4 cases was excluded from any further analysis, these were: mixed gB genotypes (n=3), mixed gM genotypes (n=1), gN4b (n=3), gN4d (n=3), mixed gO genotypes (n=1), gO2a (n=2), gO2b (n=1), and gO3 (n=2). The genotypes included in the analysis according to the clinical samples genotypes profile are presented in the table below (Table 28).

The general outcomes of the one-way ANOVA revealed that there was a statistically significant effect of some of the glycoprotein genotypes on binding of

some of the lectins, and these were: gB on the PHA-L binding profile (F (3, 80) = 2.754, p=0.048), gH (F (2, 81) =4.136, p=0.019) and gN (F (3, 67)= 5.189, p=0.003) on GSL II binding profile, gL on PTL binding profile (F (4, 78) = 3.608, p=0.009), and gO on GNL binding profile (F (2, 62)= 7.254, p=0.001 (Figure 34).

Table 28: Glycoprotein genotypes quantities included in ANOVA analysis for clinical samples.

Genotype	N	Genotype	N	Genotype	N	Genotype	N
gB1	35	gH2	36	gM1	16	gL4c	4
gB2	19	Mixed gL	14	gM2	4	gO1a	17
gB3	19	gL1	6	gM3	53	gO1c	42
gB4	11	gL2	18	gN1	6	gO4	6
Mixed gH	4	gL3	8	gN3a	52		
gH1	44	gL4	37	gN4a	6		

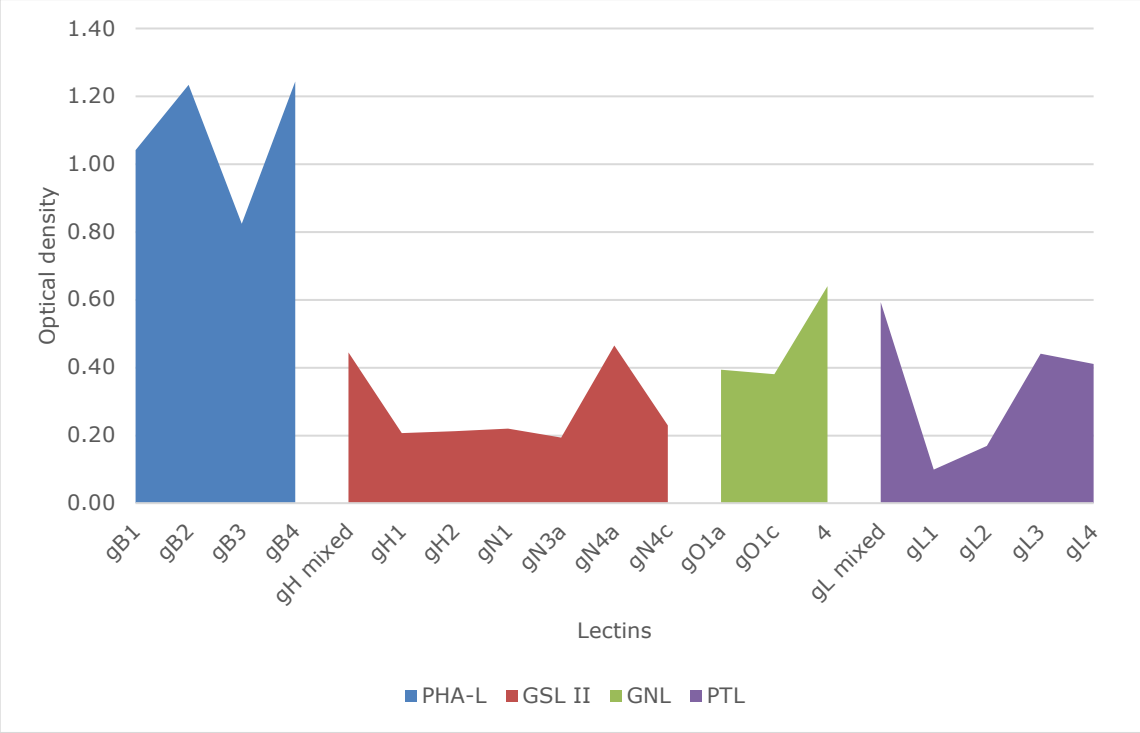

Figure 34: Lectin binding that was significantly affected by HCMV glycoprotein genotypes (ANOVA results)

Since ANOVA analysis showed overall some significant results, Bonferroni test was performed to confirm which genotype/s had a significant effect on the lectin binding. The test showed that although gB3 was associated with a lower glycosylation pattern of PHA-L binding, the association was not statistically significant (p=0.075). Still, the influence of gH mixed genotype was significantly higher compared to gH1 (p=0.017) and gH2 (p=0.02) on GSL II binding profile. Moreover, gN3a was associated significantly with the low binding profile of lectin GSL II (p=0.001), whereas gN4a had a higher binding profile (p= 0.001). Also, the effect of gO4, which was associated with the higher binding profile of lectin GNL was significant compared to gO1a (p=0.005) and gO1c (p=0.001). Lastly, the PTL binding was higher and was affected significantly by gL mixed genotype compared to gL2 (p=0.018) (Table 29, Figure 35). Complete tables of ANOVA and post hoc results can be found in the appendices section (Appendix 6).

Table 29: Mean, standard deviation (SD), and P values of the statistically significant lectin-binding influenced by HCMV genotypes in clinical samples.

Genotypes	Lectins	Mean	SD	Sig. (P value)	Sugar Specificity of lectins
Mixed gH	GSL II	0.45	0.51	0.019	α or βGlcNAc
gH1		0.21	0.12		
gH2		0.21	0.14		
gN3a	GSLII	.19	.11	0.003	α or βGlcNAc
gN4a		.47	.43		
gO1a	GNL	.39	.12	0.001	αMan
gO4		.64	.34		
Mixed gL	PTL	0.59	0.16	0.009	GalNAc, Gal
gL2		0.17	0.16		

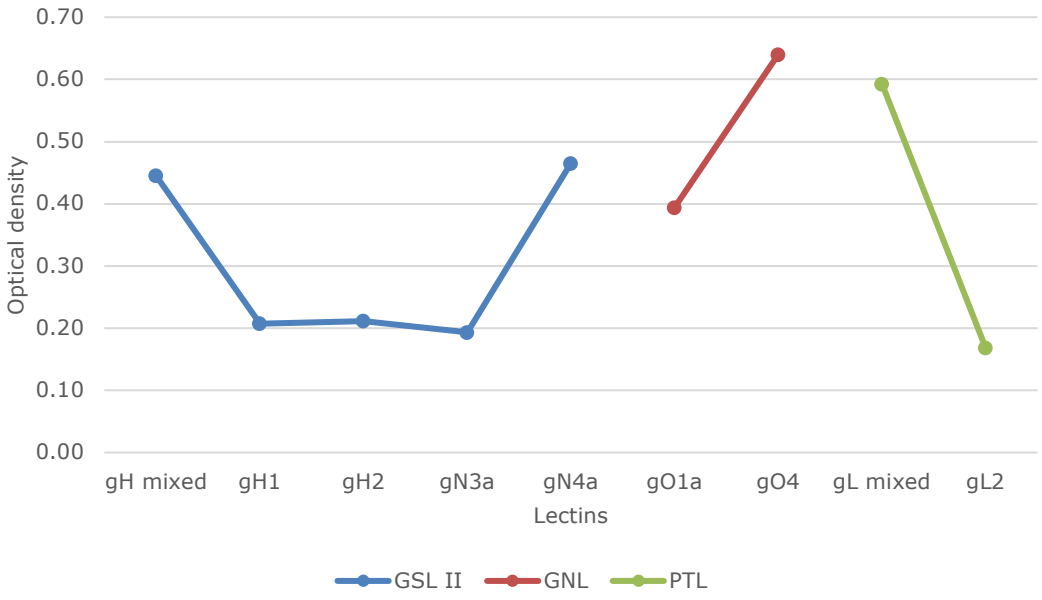

Figure 35: Lectin binding profiles that were significantly affected by HCMV glycoprotein genotypes (Bonferroni results).

4.7.3 Effect of the infection category on the glycosylation patterns of HCMV glycoprotein genotypes

To study the influence of type of HCMV infection on HCMV glycoprotein genotype glycosylation pattern, One-way ANOVA was carried out for all clinical samples after excluding samples that belonged to infection categories with less than 4 cases (N=85) (Table 30), (the excluded infection categories are: unconfirmed congenital or early post-natal (N=1), not defined primary/recurrent (N=1) and not defined primary/recurrent (N=2)).

The Results of one-way ANOVA showed that there was no statistically significant effect of any of the infection categories on the lectin binding profile. Complete tables of ANOVA and post hoc results can be found in (Appendix 7).

Table 30: Infection categories and their number of cases, included in ANOVA analysis for clinical samples.

Infection category	N
congenital infection	12
Not known infection	5
Primary infections from Immunocompetent patients	7
Primary infections from immunocompromised patients	26
Recurrent Infections from immunocompromised patients	35

4.7.4 Effect of specimen types on the glycosylation patterns of HCMV glycoprotein genotypes

To study the effect of the specimen type on the glycosylation pattern of HCMV glycoproteins, One-way ANOVA was done for all clinical samples (N=89), These are: Blood specimens (N=77), urine specimens (N=8), and respiratory specimens (N=4).

The general outcomes of the one-way ANOVA revealed that there was a statistically significant effect of specimen type on GNL lectin binding profile ($F(2, 86) = 10.297$, $p=0.000$) (Table 31).

Table 31: Mean, standard deviation (SD), and P values of overall statistical significance.

Specimen type	Mean	SD
Blood	0.39	0.15
Urine	0.35	0.09
Respiratory	0.81	0.59

Since ANOVA analysis showed overall significant results, Bonferroni test was performed to confirm which specimen type had a statistically significant effect on the GNL lectin binding profile. The test showed that the high lectin binding profile was affected by respiratory specimens compared to blood and urine specimens (p=0.000), also blood specimens had a significant effect compared to urine specimens (p=0.000) (Figure 36). Complete tables of ANOVA and post hoc results can be found in the appendices section (Appendix 8).

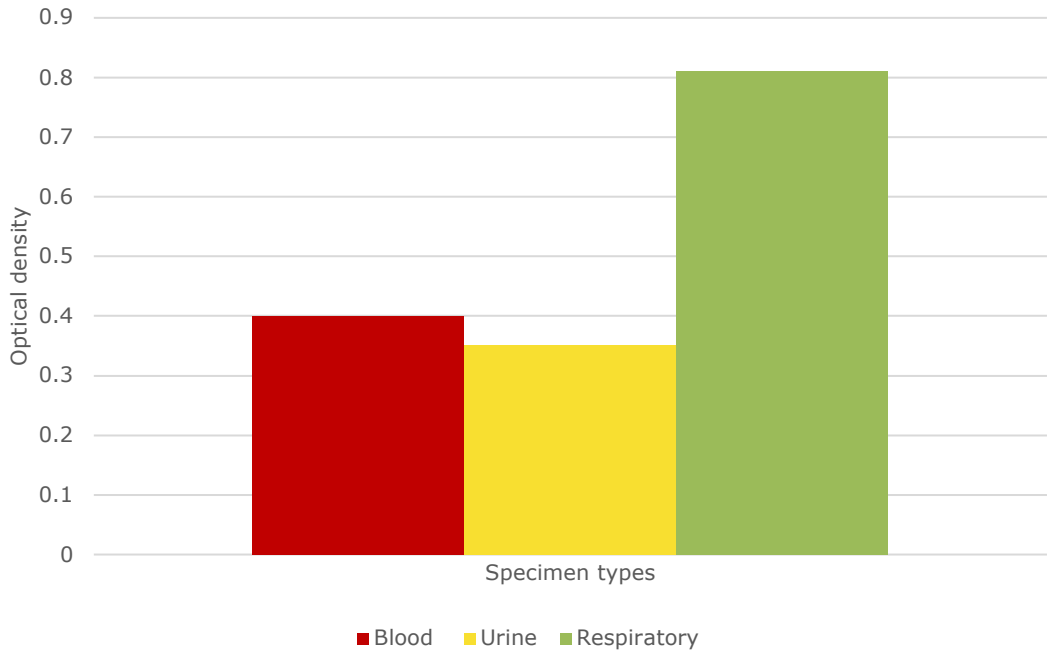

Figure 36: "GNL" lectin binding profile that was significantly affected by HCMV specimen type.

4.7.5 Summary

Investigating the changes that occur in the glycosylation patterns of HCMV glycoproteins before and after infection, and the effect of the glycoprotein genotypes, infection categories, and specimen types on the pattern of their glycosylation revealed some interesting findings.

First, the results from the laboratory strains showed a significant change in the glycosylation pattern for 9 of the 20 lectins. The glycosylation pattern was

increased in 2 of these lectins and decreased in the other 7 suggesting an overall decrease in glycosylation following infection with the lab strains. In contrast, when the same cells were infected with clinical strains of virus, the glycosylation levels increased significantly with 92.9% of the statistically significant lectin-binding showing raised levels.

Second, the effect of the genotypes profile on the glycosylation patterns of the glycoproteins in the laboratory strains, showed that both gH and gM genotypes had a significant effect. gH2 and gM1 were associated with increased glycoprotein glycosylation, while gH1 and gM3 with decreased patterns. Moreover, in the clinical strains, gH, gL, gN and gO genotypes significantly affected the glycosylation patterns; gH mixed genotypes, gL mixed genotypes, gN4a and gO4, were all associated with increased binding and therefore upregulated glycosylation, whereas gH1, gH2, gL2, gN3a and gO3a were associated with the decreased patterns.

Finally, clinical strains derived from respiratory samples showed a statistically significant increase in glycosylation levels compared to the other specimen types. No difference in glycosylation patterns was seen with strains derived from any patient population (infection category).

Moreover, individual lectins showed significant binding profiles presumably related to their different sugar specificities, this point will be discussed in detail in the discussion chapter.

CHAPTER 5

5. Discussion

HCMV envelope glycoproteins (gB, gH, gL, gM, gN and gO) have significant functions in the early communication with, attachment to, and penetration of, the host cell by the virus. They are also implicated in the regulation of the host immune response to infection. The glycoproteins are post-translationally processed and adapted in the secretory section of the host cell, where N-linked and O-linked glycan modifications influence their biological properties (Bagdonaite *et al*., 2016). Genetic polymorphisms in the HCMV genome frequently occur in about 75% of the strains that are naturally circulating in the population (Sijmons *et al*., 2015; Renzette *et al*., 2015). It was reported by Renzette *et al*., in 2011, that the intra-host genomic variability of HCMV is analogous to that of RNA viruses, which are highly mutable, and this variability is seen at the amino acid level as well as the nucleotide level of the genome.

Although there is little data in the literature concerning the glycosylation of HCMV glycoproteins, it is well established that for many viruses, including herpesviruses, alterations in the glycan of the glycoprotein can substantially alter the functioning of the protein (Sodora *et al*., 1991). A study conducted by Serafini-Cessi *et al*., (1983) showed that reduction in HSV glycoprotein glycosylation resulted in low infectivity of HSV-1, and alterations to the ability of the virus to bring about fusion with the host cell membrane. Vigerust and Shepherd, (2007) reviewed the importance of glycosylation in many viruses, such as HIV, HCV and influenza, and discussed its effects on glycoprotein stability and antigenicity, and the subsequent influence on viral virulence and pathogenicity. Also, Medina *et al*., (2013) and Hartshorn *et al*., (2008) have revealed that the ability of the influenza virus (H1N1) to escape from the host immune response, its virulence and pathogenicity is influenced by the virus hemagglutinin glycosylation. Moreover, the glycosylation of protein E in New York strain of West Nile virus was found to be the

cause of the virus neuroinvasiveness (Shirato *et al*., 2004). Additionally, Cook and Lee, (2013) reported that glycosylation of Ebola virus envelope glycoprotein plays an essential role in immune evasion by the virus and disease pathogenesis, as the viral receptor binding sites are hidden under layers of glycans, which makes them difficult to be identified by neutralizing antibodies. In the case of VZV, Yamagishi *et al*., (2008) reported that glycosylation of the gM protein initiates gene expression at the viral envelope leading to a direct role in the spread of the virus between cells. Recent work by Fontes-Garfias *et al*., (2017) have proved that the glycosylation of Zika virus envelope protein E is vital for the virus infection. The hypothesis for the current study was prompted by earlier work in this laboratory which suggested that changes in HCMV glycoprotein gene sequences exert reproducible changes in the carbohydrate binding of the glycoprotein as evidenced by lectin staining studies (Abdrhman, 2001; Khodari, 1999).

The aim of this project was to determine whether there was an association between HCMV glycoprotein polymorphisms and their glycosylation patterns, whether mutations or combinations of mutations of glycoproteins resulted in changes in glycosylation, and whether this had any influence on the viral characteristics such as replication and virulence. To investigate these aims, a cell culture model was developed to firstly isolate the virus from patient samples, each isolate was then assayed by $TCID_{50}$ to measure its growth characteristics in culture. Finally, a quantitative lectin-based assay, Enzyme Linked Immunosorbent Assay (ELLA), was developed and used to study the glycosylation patterns of the virus glycoproteins associated with each strain. In addition, PCR/ RFLP was carried out on each sample before it was put into a culture to identify the individual glycoprotein genotype profile of the viral strain. The *in vitro* data was analysed and correlated with the genotype data, and with the patients' sample data (infection category and specimen type) to identify any significant outcomes.

Clinical samples included in this project were obtained from two different sites (Public Health England, North West Regional Virus Laboratory, Manchester Royal Infirmary, Central Manchester University Hospitals, NHS Foundation Trust, UK., and Nova Medical School, Faculty of Medical Sciences, University of Lisbon, Lisbon, Portugal). These samples were unlinked, anonymised and labelled only with the infection category and the sample type. The infection categories involved were immunocompetent, immunocompromised (primary and recurrent) and congenital infections, while the samples types were blood, urine and respiratory specimens (Full categorisation of clinical samples used can be found in section (3.2.8.2)).

All samples (N=114) were confirmed as HCMV positive using phosphoprotein PCR and then stored frozen. All samples were subjected to cell culture and the HCMV-specific cytopathic effect was seen in N=89 samples, however, 22% (N=25) of the samples (Urine specimens) were negative in culture, no CPE was seen within 4 weeks of inoculation. This could be a result of a loss of infectious virus titre, which can result from many factors such as the sample storage and transport conditions. The effect of different storage conditions (4°C and -70°C) on stability of HCMV infectivity in urine samples has been assessed in previous studies which reported that the virus infectivity decreased after each storage interval (Stagno *et al*., 1980; Ross *et al*., 2011; Ross *et al*., 2014). Only those samples for which a positive culture result was obtained were included in the study. The flowchart shown in Figure 37 below details how many samples were included at each stage of the study.

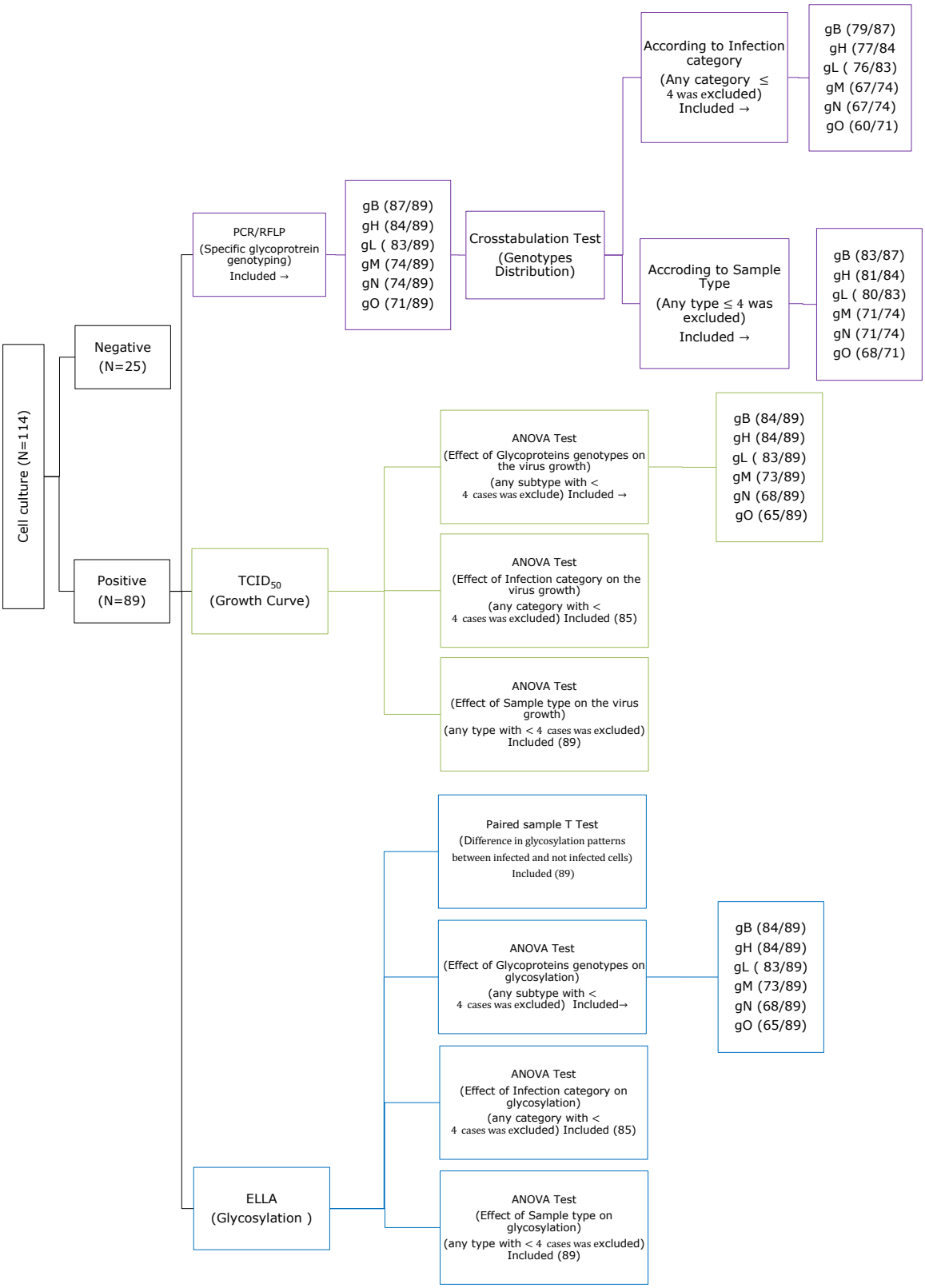

Figure 37: Flowchart presenting the number of samples included in each assay and statistical test analysis.

5.1 Distribution of HCMV genotypes in the study population

Glycoprotein genotype profiles were identified for all culture-positive HCMV samples using PCR/RFLP assays. RFLP is an established technique that can be used to characterise variations in homologous DNA sequences. The DNA sample is digested by restriction enzymes at specific recognition sites along the genome sequence; the resulting restriction fragments are then separated according to size by gel electrophoresis. Where mutations have caused insertions/deletion or alterations in restriction sites along the DNA the resulting fragments will differ in size between glycoproteins carrying different mutations allowing their differentiation. Identification of HCMV glycoprotein genotypes has been studied using RFLP in many epidemiological studies and offers reliable differentiation of the closely related HCMV subpopulations. It is also inexpensive, rapid, and requires limited specialised equipment and skills (Chou *et al*., 1990, Chou *et al*., 1992, Pignatelli *et al*., 2001, Rasmussen *et al*., 2002, Pignatelli *et al*., 2003).

Although all 114 clinical samples were reported HCMV positive using a sensitive screening PCR assay directed against the phosphoprotein gene (P2/724 primers), when the samples were tested with the individual specific glycoprotein PCR assays, 22% (25) were negative for all glycoproteins. These samples were excluded. Other samples were negative for one or more of the glycoproteins, but if they gave a positive result with at least one assay, they were included. The number of samples positive with each glycoprotein PCR was as follows: gB (87/89), for gH (84/89), gL (83/89), gM (74/89), gN (74/89), gO (71/89) (Figure 37, Purple). There are several possible explanations for being unable to genotype almost one-quarter of the samples. It is possible that the DNA in the samples had deteriorated between the first screening assay and the glycoprotein testing. This is unlikely, as some samples were retested with the P2/724c primers and gave a positive result (data not shown). A more likely explanation is due to the sensitivity of the PCR assays. Previous data from this lab suggested that the P2/724c assay has approximately 10-fold higher sensitivity than the individual glycoprotein assays.

Thus, the probable reason for non-amplification in the 22% negative samples is that the viral load in these samples was very low and although amplifiable by the screening assay, the viral copy number was below the detection threshold for the glycoprotein assays. This low viral load/copy number explanation is supported by the fact that most of the same samples could not be grown in culture. However, whilst most of the remaining 89 samples could be genotyped for most glycoproteins, some could not be genotyped for all. The individual assay sensitivities are comparable, so this is unlikely to be due to a viral load difference. An alternative explanation for this observation could be unexpected sequence variability that prevented efficient primer binding/amplification of some of the HCMV glycoproteins (Sowmya *et al*., 2006).

The prevalence of HCMV glycoprotein genotypes among the various infection categories and sample types was determined using a Cross-tabulation statistical test, and the statistical significance was determined using the Pearson chi-square test. The total number of each glycoprotein genotype included in the statistical analysis was determined after excluding any group within the infection categories that had 4 or fewer cases. This was necessary because the chance of detecting a true difference between the groups included in the study is reduced when the sample size is small (Button *et al*., 2013). Although, there is no a definite minimum number of samples that should be in the statistical analysis, the smaller the sample size, the more considered to be problematic. Therefore, samples with 4 or fewer cases were excluded from the statistical analysis, which helped in obtaining more accurate results. The excluded groups were: unconfirmed congenital infection or early post-natal (N=1), not defined primary or recurrent infection in an immunocompetent patient (N=1), not defined primary or recurrent infection in an immunocompromised patient (N=2), and "Not known" infections (N=4). This gave the following numbers of patients for analysis in each group: congenital infections (N=12), immunocompromised primary and recurrent infections (N=61), and immunocompetent primary infection (N=7). Accordingly, the total number of each

genotype involved in the statistical analysis was: gB=79, gH=77, gL=76, gM=67, gN=67, gO=60. Likewise, any group among the sample types that had 4 or fewer cases was excluded. In this case; 4 respiratory specimens (aspirate (n=1), sputum (n=1), saliva (n=1), and throat swab (n=1)) were excluded, but, 8 urines and 77 blood specimens were included. Accordingly, the total number of glycoprotein genotypes included was: gB=83, gH=81, gL=80, gM=71, gN=71, gO=68 (Figure 37, Purple).

The distribution of each glycoprotein genotype within each infection category and specimen type showed that only gH distribution was significantly influenced by both the infection category type and the specimen type, while the distribution of gM was significantly affected by the specimen type only ($P<0.05$).

gH1 was found to be the most widespread genotype among infants with congenital infections (81.8%) followed by gH2 and mixed gH genotypes, which were equally distributed (9.1%). This finding is in agreement with a study conducted by Mujtaba *et al*., (2016), where they proposed that gH1 was the most common genotype (63.7%) followed by gH2 (36.3%). By contrast, gH2 was found to be more prevalent than gH1 in about 60% of HCMV positive newborns (Paradowska *et al*., 2014).

Furthermore, in this project, gH1 was also most prevalent among immunocompromised patients with primary HCMV infection (72.0%) followed by gH2 (28%) with no mixed infections. In contrast, gH2 was most prevalent among immunocompromised patients with recurrent infections (61.8%), followed by gH1 (35.3%) and mixed gH genotypes (2.9%) (Figure 12). Similar results were reported in a recent study by Nahar *et al*., (2018) in which gH2 (75%) was the most prevalent among immunocompromised patients with ulcerative colitis with HCMV reactivation, followed by gH1 (12.5%).

In the immunocompetent patients with primary infection gH2 was the most common (57%), followed by mixed gH genotypes (29%) and gH1 (14%) (Figure

12). A study conducted by Görzer *et al*., (2010) looked at gH and gB genotypes in immunocompetent individuals and found that during primary infection, only a single genotype of either of these glycoproteins was present, whereas, in patients with recurrent infection, mixed genotypes were found. They concluded that mixed infections in immunocompetent individuals were a result of serial reinfection rather than primary co-infection with multiple strains. The data obtained in the present study do not accord with this explanation as mixed gH genotypes were found in 29% of patients with a primary infection.

The distribution of gH appeared to correlate with specimen type. Both gH1 and gH2 were found in approximately equal ratios among the blood specimens (49.3%, 46.6%, respectively), in contrast, gH1 was the significantly dominant genotype among urine specimens (87.5%), followed by mixed gH genotypes (12.5%) and in fact no single gH2 genotypes were found in the urine specimens (Figure 18). A study conducted by Li *et al*., (2015) also found gH1 to be most prevalent (71.43%) in urine samples of children with HCMV infection, although gH2 was found in 27.59% of their samples (Total=203). This is interesting, as in *Li et al*.'s study the samples were taken from children with CMV infections – almost exclusively post-natally acquired, whereas in the present study most samples are from congenitally infected infants. It might be speculated that gH1 is more common in congenital infection although this idea must be treated with caution due to the low number of urine specimens tested in this study (N=8).

Glycoprotein M has not previously been considered in the studies on HCMV glycoprotein distribution, and there is no previous literature to be considered. The reason for this lack of interest in gM is likely to be the highly conserved nature of this glycoprotein which is the most abundant protein in the viral envelope and which forms a complex with glycoprotein N, glycoprotein complex II (gCII). This complex is known to be essential for viral infectivity and is involved in attachment of the virus to the cell and fusion of the cell and viral membranes allowing the viral core to enter the cell. It is thus essential for viral replication and highly conserved.

However, a paper published some time ago by Hayajneh *et al*., (2001), reported that although the transmembrane and cytosolic portions of gM are remarkably highly conserved the N-glycosylated subdomain did show considerable diversity among primary isolates of the virus, but as this diversity did not appear to alter the overall basic charge of the glycoprotein they concluded that the basic subdomain was not essential for gM function *in vivo* and little attention has been paid to this glycoprotein since. In this study we report that distinct different types of gM do exist and that gM3 is the predominant genotype among all infection categories: infants with congenital infections (66.7%), immunocompromised patients with primary infection (82.6%), immunocompromised patients with recurrent infection (73.3%), and among immunocompetent post-natal patients (60.0%), (Figure 14). gM3 is also most commonly found among the blood specimens (77.8%) although, in urine specimens, gM3 and gM1 were equally distributed (37.5% each) (Figure 20). The significance of this finding is not clear, but it would be interesting to carry out a more extensive and more detailed study to determine whether gM type correlates with disease severity in any patient group.

gB1 was the most commonly found genotype among congenitally infected patients (33.3%), followed by gB4 (27.3%), and gB2 and gB3 (both 16.7%) (Figure 11). Several previous studies reported similar findings, reporting gB1 to be the predominant genotype found in samples taken from congenitally infected infants (Arista *et al*., 2003; Barbi *et al*., 2001; Chen *et al*., 2016; Mujtaba *et al*., 2016; Woo *et al*., 1997; Yamamoto *et al*., 2007; Zhang *et al*., 2011). Furthermore, Lukcsi *et al*., (2001) found that gB1 was not only predominant but was the only genotype present among 12 HCMV positive amniotic fluid samples tested.

Similarly, in this study gB1 was the dominant genotype amongst immunocompromised patients with either primary (48%) or recurrent infection (42.9%), followed by gB2 (20%, 22.9%), gB3 (16%, 22.9%), and gB4 (16%, 11.4%), respectively. The observation that gB1 is the most commonly found genotype in immunocompromised patient populations has been reported many

times: Vilas Boas *et al*., (2003) found that gB1 was the most common genotype among central nervous system disease in AIDS patients; Coaquette *et al*., (2004) examined the relationship between the gB genotypes and the incidence of HCMV related disease in immunocompromised patients and found that gB1 was the most common (28.9%) genotype among immunocompromised patients while gB4 was the least common genotype (2%); Fan *et al*., 2009 found gB1 (63.1%) to be common among immunocompromised patients; Jiang *et al*., (2017) explored the genetic polymorphisms of HCMV glycoprotein and looked for correlation with viral load in individuals with acquired immunodeficiency syndrome (AIDS). The authors were able to establish that gB1 (87%) was the dominant glycoprotein genotype in HCMV infected individuals with AIDS, but they found no correlation between the gB genotype and viral load; Dieamant *et al*., (2013) investigated the prevalence of HCMV genotypes in allogeneic hematopoietic stem cell transplantation patients, gB1 was again found to be the most common genotype (39%). Moreover, a very recent study conducted by Nahar *et al*., (2018) reported that gB1 was the most common type among HCMV positive patients suffering from ulcerative colitis.

In our study, in contrast, immunocompetent individuals with HCMV primary infection gB3 (57.1%) was the commonest genotype, followed by gB1 (28.6%) and mixed gB genotypes (14.3%). There are few reports on CMV genotypes in immunocompetent patients, but a study by Kashiwagi *et al*., (2002) looked at 19 immunocompetent Japanese patients and found that gB3 was not associated with symptomatic disease, whereas gB1 was (Kashiwagi *et al*., 2002). Another study from Taiwan found gB1 to be present in both immunocompromised and immunocompetent groups, but significantly associated only with the immunocompromised group, whilst gB3 was found much more commonly in the immunocompetent group (Wu *et al*., 2011). In a study by Oka *et al*., in 2015, gB1 was found most commonly (77.8%) in the eye compartment among immunocompetent patients with HCMV endotheliitis and iridocyclitis, while gB3 was found in 22.2% of the patients.

When gB genotype was analysed according to the specimen type, in this study, gB1 (41.3%) was found to be the most common genotype in blood specimens followed by gB2 (24%) and gB3 (20%) then gB4 12%, whilst gB3 (37.5%) was the most common type in urine samples, followed by gB1 (25%) and gB4 (25%), then gB2 (12.5%) (Figure 17). However, these results must be treated with caution as the numbers of urine specimens in each group are very low. As reported above, most of the studies conducted on immunocompromised patients, particularly AIDS patients, that used blood samples found gB1 to be most common in accordance with our findings. In contrast to these results, an earlier study by Gilbert *et al*., (1999) found gB2 to be the most frequent in blood specimens taken from AIDS patients. In our study, gB3 was more common in urine specimens, whereas in a study by Paca-Uccaralertkun *et al*., (2013) gB1 was most prevalent in HCMV positive urine samples taken from children aged 1 month to 5 years living in a children's home. The urine specimens we tested although small in number, mostly came from congenitally infected infants, in contrast, the study by Paca-Uccaralertkun tested urine from children living in an orphanage setting. This setting suggests the children were most likely post-natally infected as HCMV is frequently acquired in a childcare environment where the children live in close association.

The gB findings overall are interesting. In summary, gB1 appears to be the most common genotype in immunocompromised and congenital patients whilst gB3 is more common in immunocompetent patients, except in a few cases where gB1 is associated with disease in immunocompetent patients. This finding is seen in our study and many other published studies. gB is an important glycoprotein that is essential for entry into the host cell and cell-cell spread, it is highly conserved between herpes virus species and facilitates entry into the host cell by binding to heparin sulphate receptor and bringing about membrane fusion (Vanarsdall and Johnson 2012). It is interesting to speculate that gB3 is the normally circulating form of the glycoprotein found in the setting of a functioning immune response and that when the immune response is weakened or removed the virus evolves away from gB3 towards gB1, explaining why gB1 is more common in

immunocompromised patient groups. The finding in our study and others that gB1 also predominates in congenitally infected patients may also be explained by a lack of immune surveillance in the foetus and may reflect a change occurring after the virus has crossed from the mother into the foetus rather than being preferentially transmitted over other gB genotypes. However, although interesting, there is little evidence presented in the literature that the gB genotype affects the outcome in any patient group.

Analysis of the gL glycoprotein data shows gL4 was the most prevalent genotype among all patient groups. For congenitally infected infants 58.3% showed the gL4 genotype, followed by mixed gL infection (25%), and gL2 and gL3 (8.3%). Among the immunocompromised patients with either primary or recurrent infections, gL4 was present in 48% and 40.6% respectively, followed by gL2 (24%), gL3 (12%), gL1 (8%) and the mixed gL genotypes (8%), and among immunocompetent post-natal patients with primary infection it was present in 57.1%, followed by mixed gL infection (28.6%) and gL3 (14.3%). (Figure 13). When analysed by specimen type, gL4 was again the most prevalent among blood samples, (43.1%), followed by gL2 (23.6%), mixed gL genotypes (18.1%), gL1 (8.3%), and gL3 (6.9%), and amongst urine samples (62.5%), followed by gL3 (25%) and gL2 (12.5%) (Figure 19). There is almost no other published literature regarding the distribution of gL genotypes. An early study by Rasmussen *et al*., (2002) looked at gL in clinical isolates of HCMV, and although all 4 genotypes were identified, the study reported very low levels of genome variability between the types (only 2% variation), which may explain the lack of further studies on this glycoprotein. Although our study does not report any significant findings for gL4, it is the first to describe the distribution of gL genotypes among different infection categories and sample types and to show the predominance of this genotype.

For glycoprotein gN, gN3a was by far the most common type in congenital infections (81.8%), followed by gN1 and gN4d, which were equally distributed (9.1%), although this distribution was not statistically significant (Figure 15). This

gN3a genotype has previously been shown among congenitally infected infants but equally distributed with gN3b, gN3c and gN4a (Mujtaba *et al*., 2016) In the immunocompromised patient group gN3a was also found most commonly, being present in 45.5% of samples from patients with primary infection and 85.7% of patients with recurrent infection. Xia and Zhang, (2011), found similar results among hematopoietic stem cell transplant patients. A study conducted by Nahar *et al*., (2018) reported that gN3a and gN3b were equally distributed among immunocompromised patients with recurrent HCMV infection. Whilst another study suggested that gN4a was the most common among HCMV positive AIDS patients (Jiang *et al*., 2017). In our study, gN3a was also the most prevalent among immunocompetent patients (60.7%).

Regarding the distribution of gN glycoprotein genotypes according to sample type, gN3a again was the most prevalent among blood and urine samples. In blood samples, (Figure 21). These results support the results found within the infection categories, where gN3a was constantly found to be prevalent.

Lastly, the gO genotype was the only genotype where distribution was studied for only three infection categories. This was because the immunocompetent patients' category, included only 4 cases, and so was excluded as this made it statistically invalid. Among the three remaining groups; congenitally infected infants; immunocompromised primary; and immunocompromised recurrent infection patients, gO1c was the most prevalent at; 62.5%, 45%, 63.3%, respectively. The gO1a genotype was the second most common in all patient types; 12.5%, 36%, 20% respectively. It is interesting to note that gO3 was present among congenital infections (3.2%) and immunocompromised recurrent infections (3.3%) only, and, gO2b (12.5%) was only present among congenital infections (Figure 16). These findings are not dissimilar to the reports in the literature. A recent study by Jiang *et al*., (2017) found that gO1a was the most dominant (49.2%) among infected AIDS patients, and Chen *et al*., in 2016 reported that gO1

was the dominant genotype (37.1%) among a total of 1709 HCMV infected children with respiratory symptoms.

Regarding specimen types, the distribution of gO genotypes was consistent with their distribution within the infection categories. gO1c followed by gO1a were the most prevalent in blood (60%, 23%) and urine (67%, 33%), respectively. In addition, gO4 (8%), gO3 (3%), gO2a (3%), gO2b (2%), and mixed gO genotypes (1%) were all found only among blood samples, but the number of urine specimens was low (Figure 22).

Of relevance to the data reported here is a study published by Görzer *et al*., in 2015, where it was reported that mixed HCMV genotype variations are hardly ever found in urine samples of congenitally infected newborns, even using very sensitive HCMV genotyping diagnostic methodologies. These researchers tested urine samples collected from 17 congenitally infected neonates and used deep sequencing for gO genotyping and quantitative real-time PCR assays for gB and gH genotyping. No mixed gO genotype was identified for any of the urine specimens, and only one mixed gB genotype and one mixed gH genotype was found. This seems to agree with our data, where although only eight urine samples, (and only 5 of these from congenitally infected infants) were genotyped using PCR/RFLP as previously explained no mixed genotype for gO or gB and only one mixed genotype for gH was found. The same study by Görzer *et al*., (2015) however, found no predominance of a specific gB, gO or gH type in the urine specimens. This is in contrast to our study where gB3 (37.5%), gO1c (67%), and gH1 (87.5%) were the most prevalent genotypes among urine specimens, with gH1 being the only genotype with a statistically significant prevalence ($P<0.05$).

To summarise, the genotype findings presented above, gH1 appears to be the most common gH type among congenital and immunocompromised patients with primary infection, whereas, gH2 is most common among immunocompetent and immunocompromised patients with recurrent infections. Also, for the group as

a whole, gH1 and gH2 were almost equally distributed within blood samples, while in urine 62.5% of the specimens were from congenital patients, gH1 was most common. gM3 was consistently the most common genotype among all the infection categories studied. Similarly, gM3 was the most common within the blood specimens followed by gM1, whereas gM1 and gM3 were equally distributed in urine samples ($P<0.05$). Several previous studies of the distribution of gH genotypes have supported these findings. But regarding gM distribution, this study was the first to report significant results.

It is worth stating that gB1, gL4 gN3a and gO1a/gO1c although not statistically significant, were the most common genotypes identified in all patient groups. Many studies agree with our finding of gB1 predominance although the literature is more divided concerning the gN and gO results, and no previously published research has focused on the distribution of gL or gM genotypes.

All of the above discussion has focused on the individual glycoproteins and their various polymorphic forms. However, as described in chapter 2 (section 2.1.5.3), HCMV glycoproteins exist in three complexes; glycoprotein complex I which consists of gB; glycoprotein complex II, comprising gM and gN; and glycoprotein complex III which exists in two forms; a trimer of gH/gL/gO and a pentameric form where gO is down-regulated/lost and replaced by a trimeric protein complex coded for by UL128-131a (Ryckman *et al*., 2008). Recently it has been shown that the gH/gL/gO complex is required for entry into fibroblasts whilst gH/gL/UL128-131 is needed for entry into epithelial and endothelial cells (Vanarsdall and Johnson 2012). The laboratory strain AD169 uses the gH/gL/gO form allowing it to grow in fibroblast cell lines but has lost the expression of the pentameric complex gH/gL/UL128-131 which would enable it to infect endothelial and epithelial cells (Ryckman *et al*., 2008). Although if this complex is artificially reintroduced into AD169, it can infect these cell types. In contrast, clinical isolates of HCMV can express both forms of gCIII. This explains why HCMV is able to infect endothelial, epithelial and myeloid cell types in vivo and the pentamer complex is

thus essential for efficient viral growth and spread in the host. Circulating strains express both forms of gCIII but different strains express different ratios of each and the basis for the type of expression is not yet understood (Zhou *et al*., 2013). Although we did not test for the presence of the pentameric complex in this study it is worth considering the data when the glycoproteins are grouped as complexes.

The glycoprotein distribution results considered by complex suggest the following combinations are most frequent in congenital infection and in immunocompromised patients with primary infection; gCI (gB1), gCII (gM3/gN3a), gCIII (gH1/gL4/gO1a or gO1c). While for immunocompromised patients with recurrent infection, the first 2 complexes remain the same, but gC III changes to gH2/gL4/gO1a or gO1c. However, these results are not statistically significant.

The main limitation of the data presented here is the relatively low numbers of samples included in some of the groups and whilst it is encouraging to note the broad agreement with previous studies, the main aim of this thesis is to consider the effect that the glycoprotein polymorphisms have on the characteristics of the viral strains. In the next part of the discussion, the results of the glycoprotein distribution will be correlated with the results of assays that measure the growth characteristics and glycoprotein glycosylation patterns of the various glycoprotein types.

5.2 Growth characteristic of HCMV glycoprotein genotypes

The growth of the various HCMV strains in cell culture over four weeks post infection was assessed. This was done for all the laboratory strains (N=5) and any of the clinical samples where HCMV CPE was produced (N=89). Growth was compared using a TCID50 assay. TCID50 measures the infectious virus titre by quantifying the amount of virus required to kill 50% of the infected hosts or to produce a cytopathic effect (CPE) in 50% of inoculated tissue culture cells. Although it is generally considered a labour-intensive and time-consuming assay, due to the necessity for an extended incubation period because of the slow growth of many

HCMV strains, it is widely accepted and has a relatively small error rate of about 35% (Pankaj, 2013; Virocyt, 2013). The visual observation of CPE produced by the laboratory strains was confirmed and validated using PCR-based TCID50, in which the infection was measured using a quantified viral load instead of just visual inspection of the virus CPE in plates. This method has been reported to be easier and more robust than the standard TCID50 assay (Gustafsson *et al*., 2012). Unfortunately, it was not possible to extend this method to confirm the clinical samples results due to the limited volume available for each sample.

HCMV is never fully eliminated from the body and remains in the latent state for life. In primary infection. HCMV infects epithelial and myeloid (monocyte, macrophage and dendritic) cells, as well as fibroblasts and endothelial cells (Mattes *et al*., 2000; Knipe and Howley, 2013). This is in contrast to *in vitro* cell culture, where HCMV only replicates in fibroblasts. As explained above this is due to the loss of the UL128-131a component of gCIII and its replacement with gO which is known to occur in laboratory strains and likely happens after only a few passages in clinical isolates as they adapt to grow in culture. Therefore, in a laboratory setting, HCMV can only be successfully isolated in fibroblasts. The HCMV cytopathic effect (CPE), can typically be detected after 3 to 4 days incubation period, although sometimes this can take much longer (Sinclair, 2000). In the present study, CPE for the laboratory strains started to appear on day 7 (second week post-infection), and the virus continued to grow during weeks 3 and 4 until the cell monolayer was destroyed. In clinical strains, for which CPE started to appear after three days of infection, the maximum growth had typically completed by the end of the fourth week and remained at a steady level or rose very slowly for the remaining time of the growth curve. In contrast, although the laboratory strains took longer to initiate the growth phase, they continued to increase replication rate for the 4 weeks incubation period and rose quicker to much higher titres that the clinical isolates (Figure 27). It was a surprising finding that the growth of the clinical isolates was initially faster than that of the laboratory strains. A study by Wilkinson *et al*.,

(2015) suggested that the wild type HCMV strains mutate after being passaged in fibroblasts switching from UL128-131a to gO in gCIII. In a follow up study from the same group (Murrell *et al*.,2017), they demonstrated that clinical isolates rapidly mutate in culture and that *in vivo,* HCMV is largely cell associated and is transmitted around its host by moving directly from cell-cell and can infect a variety of cell types. In contrast, laboratory adapted strains of HCMV spread via diffusion of cell free virions and their replication is limited to fibroblasts. In our study, clinical strains were subjected to a maximum of 2 passages, and the comparatively low titre reached by week 4 suggests that although they had likely begun this process of mutation in culture they were not fully adapted.

The association between each envelope glycoprotein genotype and growth in culture was analysed. For the laboratory strains, the analysis was carried out simply by observation of the CPE compared to the individual glycoprotein types know to be carried by each strain. AD169 was the strain that grew fastest and to highest titre, whilst Merlin was the slowest growing and reached the lowest titre. Looking for unique glycoprotein types in these two strains, it can be suggested that as AD169 is the only strain to carry gB2, gL1, gN1 and gO1a that one or a combination of these glycoproteins could be associated with virulent growth in culture. In contrast, Merlin strain carried gL4, gN4c and gO5, raising the possibility that slow/poor growth in culture is associated with these genotypes. It is known that Merlin is the closest laboratory strain to wild type virus (Dolan *et al*., 2004) and these observations provide a starting point for comparative analysis of the growth characteristics of the clinical strains.

As there were very many more data points for the clinical samples than for the laboratory strains, observational analysis was neither possible nor desirable, and statistical analysis of the results was carried out as follows; after excluding any genotype that had less than 4 cases from the statistical analysis, an analysis of variance (ANOVA) test was used to compare the mean viral titre against genotype. (Figure 37 above (green & blue)). Statistically, there was no significant effect of

any of the glycoprotein genotypes on the virus growth across all four weeks of infection (P>0.05). In addition, when observational results for AD169 and Merlin were tested against the clinical strains, it can be seen from Figure 28 that there is no association in the clinical strains between faster/higher growth with gB2, gL1, gN1 or gO1 and with slower/lower growth and the presence of gL4, gN4c, or gO5 (no gO5 was present in the clinical samples). It can, therefore, be concluded that individual glycoprotein types do not have an effect on viral growth in cell culture. This is not unexpected and is in contrast to the *in vivo* growth characteristic of the virus where it is well known that the virus glycoproteins play an essential role in viral replication and virulence.

The rate of growth in culture was also analysed by infection categories and sample type for all the clinical samples (N=89), (Figure 37 (blue)). Analysis of the results showed that the growth of the virus strains from congenitally infected patients was significantly higher than viral strains from all other infection categories (Figure 29). Moreover, it was shown that strains isolated from urine specimens grew faster than the other strains during the last three weeks of the infection (P<0.05) (Figure 30). It was reported by Ross *et al*., in 2014 that a significant amount of HCMV was found in urine specimen from congenitally infected babies. This could explain the higher rate of growth from the congenital patients, simply because a higher viral titre was used as the inoculum (Arav-Boger, 2015; Pugel and Cekinovic, 2011).

In this project, no association between a specific glycoprotein and viral growth was seen. The most likely explanation for this is that HCMV reproduction *in vitro* is not representative of the virus replicative process in vivo (Tabata *et al*., 2015). The recent study mentioned above (Murrell *et al*., 2017) used bacterial artificial chromosome technology to construct an artificial virus that has the characteristics of wild-type virus but is able to grow in culture. They used this artificial virus to demonstrate that wild-type virus spreads directly from cell-cell, rather than by releasing the virus into the media to infect new cells. In the *in vivo*

situation, the role of the virion glycoproteins in transmission and spread is likely to be quite different from their role in cell culture of the adapted strain. For this reason, it is not valid to make any conclusions from the lack of association between viral glycoprotein types and transmissibility in culture.

5.3 Glycosylation patterns of HCMV glycoprotein genotypes

In recent years, a considerable amount of attention has been given to saccharides found covalently attached to proteins, called glycans. They can have a linear or branched structure and consist of monosaccharide units linked together by glycosidic bonds. These vital biological molecules are abundant, widely present in all living organisms, and have diverse biological functions. Glycans participate in various physiological and pathological processes, including intermolecular and cell–cell recognition events, cell cycle, cell differentiation and apoptosis, and host–pathogen interactions and inflammation. They also have a critical role in the maintenance of cells and tissue structure. The mechanism by which glycans perform these diverse functions typically involves interaction with other polymers (e.g., proteins, saccharides, lectins) in an enzymatic process called glycosylation. It is one of the most common post-translational modifications, and it is estimated that approximately 70% to 80% of all human proteins are glycosylated. Glycoproteins, which are products of glycosylation (glycoconjugates), are formed by attaching glycans to proteins. Most molecules involved in the immune response, including cellular receptors, cytokines and antibodies, are glycosylated, and the interaction between an antigen and its ligand whether on an antibody or T cell is influenced by the carbohydrate structure of both. Thus, it is feasible, even probable that alteration of the glycan portion of the viral glycoprotein will have a significant effect on its function including its ability to infect, transmit and cause disease. Two main types of glycosylation occur, O-glycosylation and N-glycosylation depending on the type of glycans being used in the process. N-linked glycans principally act as signals for cell surface recognition phenomena, whilst O-linked glycans confer certain physicochemical properties on proteins. Herpes virus glycoproteins are known to be

glycosylated (Gantt *et al*., 2015; Dall'Olio *et al*., 1987; Serafini-Cessi *et al*., 1983) and glycoprotein B of HCMV has been shown to carry both N-linked (Britt and Vugler 1989) and more recently numerous O-linked glycosylation sites (Bagdonaite *et al*., 2016).

The major aim of this project was to determine whether the glycoprotein polymorphisms affected the way in which the glycoproteins were glycosylated. If this were shown to be the case, a direct link between the viral genotypes and altered viral characteristics can be made for the first time. As the way that a protein is glycosylated is dependent on the cell in which it is produced, it would not be possible in the culture system used in this project to draw direct assumptions about how the functions may be altered, but showing a direct link between the known glycoprotein types and differences in the way they are glycosylated would provide evidence that the glycoprotein types may confer functional differences on the particular strains.

To investigate this idea, a quantitative lectin-based assay, Enzyme Linked Immunosorbent Assay (ELLA) (Figure 8), which is similar to the standard ELISA assay, but using a panel of 20 lectins in place of antibody, was carried out, to determine whether there is an association between the individual glycoprotein or a combination of glycoprotein polymorphisms and their glycosylation. ELLA has been recently developed from a previously used technique described in the literature by Leathem and Brooks (1998). It adopts the same principle (protein- glycan interaction) but gives quantitative results instead of the qualitative data, which simplifies the interpretation of the data. The method was improved and optimised by the Microbiology/Virology Unit at the University of Manchester under the supervision of Dr Carol Yates (Phung, 2011; Bala, 2010). The fast turnaround time, minimum sample consumption and low cost are some advantages of the assay.

Primarily, all non-infected (control) and HCMV infected cells showed high optical density (OD) readings. To expose the source of the high OD readings, the assay was repeated with the addition of some extra controls: including infected and

non-infected MRC-5 cells, growth medium (MEM) only and some blank wells. Each was tested under four different conditions: addition of biotinylated lectins, avidin/peroxidase (Av/Po) and the TMB substrate, addition of the biotinylated lectins and the TMB substrate only, addition of the conjugate Av/Po only, and lastly, addition of the unaccompanied substrate. The repeated assay showed high O.D readings with the uninfected MRC-5 cells, MEM and the blank wells treated with Av/Po, while the other wells with no conjugate added presented low OD readings. This clearly suggested that the Av/Po conjugate might be being degraded or that nonspecific binding of the avidin- biotin had occurred. Also, due to the presence of biotin (other than the biotin used for biotinylated of lectins) within the cells, the growth medium used was examined and seemed not to contain biotin as reported in the company's catalogue (Sigma-Aldrich) (Leathem and Brooks, 1998; Marttila *et al*., 2000).

Accordingly, the method was slightly amended to have better results and reach the study goals. First: Streptavidin was used instead of Av/Po. It is isolated from *Streptomyces avidinii*; and lacks glycosylation because there is no carbohydrate within its structure, which is beneficial in reducing the level of the non-specific binding of the conjugate especially with lectins. Second: The endogenous biotin was blocked to reduce the chance of the non-specific binding occurrence. This was done by adding streptavidin and biotin as an additional step before adding the biotinylated lectins.

Moreover, a higher concentration of the Blocking reagent H_2O_2 (1%) was tested because of the low OD readings of 0.3 that had been found with the non-infected and infected cells, which might be due to the non-specific binding of the substrate to the endogenous peroxidase. Using a higher concentration did not show any real differences in the results. However, the other modifications did significantly improve the assay. A schematic diagram presenting the method of ELLA used in this project can be found in section (3.2.7) (Figure 8).

Lectins (from the Latin word 'legere' meaning 'to choose') are a large family of proteins of non-immune origin isolated from natural sources. These carbohydrate-binding proteins are able to bind both free glycans and glycans attached to glycoconjugates, such as glycoproteins. Naturally, glycans and lectins have significant roles in the function of cells and organs, not only in humans and animals but also in viruses. Viral pathogens utilise glycans and lectins that are encoded by their own genome or that of the host cell to undergo duplication and multiplication. Recent progress in glycobiological research suggests that glycans and lectins intervene in vital interactions in the virus-host relationship, controlling viral multiplication and/or launch of the immune system (Van Breedam *et al*., 2014).

The lectins used in this project were chosen according to their diverse sugar specificity (Table 3) to cover all the protein/glycan sites that could be present within the virus structure. Lectins have complex specificities that can recognise not only different monosaccharides within the glycan chain, such as mannose, N-acetylglucosamine, sialic acid (SA) or galactose, but they can also identify different linkages between saccharide monomers or glycan branching. A good example that shows their high specificity is a glycan terminated in sialic acid (SA) and linked to galactose via either an α2-6 or α2-3 glycosidic bond, in which, lectin *sambucus nigra* agglutinin (SNA) can recognise only the α2-6 linkage, whereas *Maackia amurensis* agglutinin (MAA) lectin can recognise only the α2-3-linkage. This selective recognition of lectins plays an important role in the host-species barrier. For instance, selective recognition of the type of linkage between sialic acid (SA) to galactose, explains why the influenza virus which infects humans, does not infect birds, and vice versa. Human influenza virus specifically recognises α2-6-linked SA, while avian influenza recognises only α2-3-linked SA on the surface of the avian tissue, and not α 2-6 linked SA present in human tissues, hence, preventing these viruses from cross-species invasion (Belicky *et al*., 2016).

Studying the glycosylation patterns of HCMV glycoproteins, then, investigating the effect of the glycoprotein genotype polymorphism on the virus glycosylation was of high importance in this project. This was initiated by finding out if there is a difference in the glycoprotein glycosylation patterns between laboratory strains and clinical samples. These strains were cultured and infected under standard laboratory conditions as previously explained. The results from the laboratory strains were compared with the results of the clinical samples and evaluated using a paired sample T-test. In this case, the paired sample T-test provides an adequate statistical method to compare two independent sets of data with different sample sizes. As a large amount of data was acquired in this study, to make sure that any correlation between the variables did not occur by chance ($P<0.05$), only statistically significant results were considered.

This comparison of HCMV glycoprotein glycosylation patterns between non-infected and infected cells in both laboratory strains and clinical samples revealed that the glycoprotein glycosylation levels were decreased significantly in cells infected with laboratory strains (77.8%) compared to controls, while in cells infected with clinical samples it was significantly increased (92.9%). The decreased levels of the viral glycosylation induced by the laboratory strains compared to the clinical strains might be explained by two theories; The first is that due to the repeated passage in tissue culture, a reduction in some of the viral glycoprotein synthesis in the laboratory strains could occur and this would have a direct effect on the glycosylation levels. Moffat *et al*., showed this, in 1998, when they compared the protein synthesis of VZV clinical isolates with a VZV laboratory strain and found that after 21 days of infection the level of viral protein synthesis in the laboratory strain was significantly lower (Moffat *et al*., 1998). An alternative theory is that as the laboratory strains have adapted to infect and grow within cell culture, the immune pressure that they would have been subjected to *in vivo* was removed, and as glycoprotein glycosylation forms part of the viral mechanism to evade the immune system and increase virulence, removal of this pressure is likely to lead to

a decreased level of glycosylation. For example, it is well known that N-linked glycans are important elements in the proteins' receptor recognition within the cells (Guseva *et al*., 2010). Also, some of the most abundant neutralizing antibodies contain N-linked glycan as the main part of their structure (Zhang *et al*., 2016). Kropff *et al*., (2012) produced recombinant viruses expressing gN proteins with reduced glycan alteration; they reported that the widespread glycosylation of gN might influence the way the virus avoids neutralization by antiviral antibodies, whereas, the recombinant viruses with under-glycosylated gN were considerably more vulnerable to neutralization by a wide range of antibodies. Immunization of mice with viruses without glycan alterations provoked considerably elevated antibody titres versus the homologous virus; yet, the neutralization titres against the fully glycosylated virions were not enhanced. Thus, as described above, laboratory strains, although they are affordable and convenient, are not representative of wild-type *in vivo* infection.

The lectin panel used in this study was chosen for varying specificity of binding to the glycan sugar moieties. Whether or not a significant change in lectin binding to the cells occurred after infection with the virus was analysed using a paired t-test against the null hypothesis that there was no change due to infection. This analysis showed that for the laboratory strains a significant change in binding occurred with 9 (45%) of the lectins: for 2 of these lectins a significant increase in binding was seen, and for 7 a significant decrease occurred after infection (Figure 31). When the lectin panel was used to test the clinical strains a more marked response to infection was seen with 14 (70%) of the lectins showing an altered binding level and for most of these (13/14) it was a significant increase in binding that was seen (Figure 33).

As we have seen, HCMV carries a number of highly polymorphic, and heavily glycosylated envelope proteins. This study is the first report to describe HCMV glycosylation for the 6 major envelope glycoproteins, the first to show that the

different glycoprotein types differ in the way that they are glycosylated and the first to show that these differences affect the viral characteristics.

The significant results showed that decreased levels of glycosylation in laboratory strains were significantly associated with gH1 and gM3 genotypes, while gH2 and gM1 were associated with increased glycosylation levels ($P<0.05$). The lectins associated with these results were LCA, GSL II, GNL, EEL, WFA, PHA-E, MAA II, and PSA, in which the glycosylation levels of gH1 were lower than gH2, and PHA-L, in which the glycosylation levels of gM3 was lower than gM1 and gM2 (Figure 32). In the clinical samples, the test showed that although gB2 and gB4 are associated with increased glycosylation patterns, and gB3 was associated with decreased glycosylation levels (Figure 34), the association was not statistically significant ($p=0.075$). However, the influence of mixed gH genotypes on the glycosylation levels was significantly higher compared to gH1 ($p=0.02$) and gH2 ($p=0.02$) with GSL II lectin which binds specifically to the complex type N glycans (Nakamura-Tsuruta *et al*., 2006). In contrast, gN3a was associated significantly with a lower level binding of lectin GSL II ($p=0.001$) but gN4a was associated with higher binding of this same lectin ($p= 0.001$). Similarly, gO4, was associated with significantly higher binding of lectin GNL, which is a mannose binding lectin, compared to gO1a ($p=0.005$) and gO1c ($p=0.001$). Lastly, PTL binding, that has specificity to N-acetylgalactosamine, was significantly higher with gL mixed genotypes than with gL2 ($p=0.018$) which was associated with decreased glycosylation levels (Figure 35).

To conclude, increased glycosylation levels in clinical strains of HCMV were seen with mixed gH genotypes, gN4a, gO4 and mixed gL genotypes. Whilst decreased levels were associated with gH1, gH2, gN3a, gO1a and gL2. In laboratory strains the increased levels of glycosylation were seen with gH2 and gM1, while the decreased levels with gH1 and gM3. The similarity of both the clinical and laboratory strains was only in the case of gH1, which was associated with the decreased glycosylation levels in both cases.

The specific alterations in glycosylation detailed above are not directly translatable to the *in vivo* situation as it is known that the way a protein is glycosylated depends on the cell type in which it is produced. The significant finding from the data presented here is that there are definite and reproducible alterations in glycosylation associated with particular glycoprotein polymorphisms. This shows that the polymorphisms have the potential to cause alterations to the functional characteristics of the virus *in vivo*.

However, although direct comparisons cannot be made, it is interesting to note that the glycosylation patterns found here and associated with gN genotypes are consistent with the previously published literature. A study conducted by Rossini *et al*., (2005) among solid transplant patients suggested gN4 to be the more virulent form of this glycoprotein as it was associated with earlier initiation and increasing levels of HCMV antigenemia. In support of this, Pignatelli *et al*., 2010 confirmed that infection with the genotypes gN4a and gN4c resulted in 8x more sequelae than other variants; while there was a decreased sequelae risk for those infected with the gN1, gN3a variants. Likewise, gN1 and gN3 genotypes were proposed by Arcangeletti *et al*., (2015) to have lower virulence, and gN4 genotypes to have higher virulence. Our finding of increased glycosylation with HCMV gN4a genotype could be associated with virulence and pathogenicity of the virus, whereas the decreased glycosylation of gN3a may be associated with the virus having lower virulence. Burke and Heldwein (2015) reported on the crystal structure of gB and showed that it was extensively glycosylated and that the pattern of this glycosylation affected antibody recognition of the virus. The antigenic domains that elicited neutralizing antibody were the most heavily glycosylated, whereas the domains that were less antigenic were less heavily glycosylated. They suggested that HCMV gB utilises glycans to protect the neutralizing epitopes from antibody whilst revealing non-neutralizing epitopes. This glycosylation pattern has the effect of directing the immune response to generate non-neutralizing antibodies, enabling HCMV to escape clearance. Toriniwa and

Komiya in 2011 also reported that variation in glycoprotein glycosylation has a significant effect on the ability of the virus to escape the host immune response. These findings suggest that glycosylation of HCMV glycoproteins is an important mechanism for avoidance of antibody-mediated neutralization and this, in turn, facilitates HCMV pathogenicity.

As the immune system clearly plays a role in eliciting the glycosylation patterns of viral glycoproteins as illustrated by the HCMV gB example above (Burke and Heldwein 2015), it was interesting to assess whether there were differences in the glycosylation properties of viral isolates taken from different types of patients with differing immune responses. In order to study the glycosylation patterns in regard to infection category, any infection category that had less than 4 cases was excluded from the analysis of the results. Thus, five different infection categories were evaluated: congenital infection (12), primary (26) and recurrent (35) infection from immunocompromised patients, primary infection from immunocompetent patients (7) in addition a category for unknown infection types (5) was included (Total N=85). ANOVA test was performed to identify statistically significant glycosylation patterns taking the averages of each infection category. However, the results showed that there was no statistically significant association between the infection category and the viral glycoproteins glycosylation. This suggests that the significant alterations in glycosylation noted in this study were more likely to be due to the changes associated with the individual viral genotypes rather than a host effect arising in a specific patient group regardless of the glycoprotein type.

As previously discussed, the role of glycosylation mediating cellular processes is well known. Nevertheless, the glycosylation mechanism is not a tight system, and it is controlled by different enzymes. Therefore, the same glycoprotein may have different glycosylation patterns and mediate different interactions depending on the tissue localisation (Zámorová *et al*., 2017). Thus, it was important to consider whether different glycosylation patterns occurred in isolates from different locations.

This project has also studied the effect of different patients' sample types on the glycosylation pattern of HCMV glycoproteins. ANOVA was done for all clinical samples (N=89), These are: Blood specimens (N=89), urine specimens (N=8), and respiratory specimens (N=4). None of the specimen groups was excluded from the analysis because none has less than 4 cases. The results showed that there was a statistically significant increase in the glycosylation patterns of the strains came from respiratory specimens comparing to the blood and urine specimens (P=0.00); Likewise, blood specimens had a significant increase in the glycosylation patterns compared to urine specimens (p=0.00) (Figure 36). This increase was measured using GNL lectin binding profile. GNL has specificity to bind high-mannose-type glycans, so it recognises terminal mannose residues and was known to have an anti-HIV effect due to its binding to gP120 glycoprotein on HIV envelope (Hoorelbeke *et al*., 2011). Further investigations with larger sample size are also needed to confirm these results. Moreover, the potential role of the glycosylation pattern of HCMV infection from different sample types of the same patient should be an object of future studies.

5.4 Summary and conclusion

HCMV has the longest and the most complex genome (235 kb) amongst human-specific viruses and has high genetic variability that has been compared to that of an RNA virus. This, together with the clinical importance of HCMV as the most common congenital viral infection has encouraged many researchers to investigate the association between the virus glycoprotein polymorphisms and its pathogenesis. Whether an association exists is still inconclusive, many attempts have been made to try to understand the mechanisms involved in the relationship between the glycoproteins and disease. To our knowledge, this is the first study to concurrently investigate the six major HCMV envelope glycoproteins (gB, gH, gL, gM, gN, and gO), their polymorphic genotypes and their glycosylation patterns in relation to virus virulence and pathogenicity. Also, identification of the glycosylation

patterns of these glycoproteins before and after infection in patients with different infection categories and different sample types was first studied in this project.

These envelope glycoproteins are well identified and known as essential mediators for many viral activities including binding, entry into host cells, cell-cell spread and infection. The viral glycans are derived from the host cell, and because of this active cellular participation viral proteins are glycosylated in a similar manner to that of the cellular proteins themselves (Sugrue, 2007).

The data obtained from this study showed that there is a significant alteration of the glycosylation patterns of HCMV glycoprotein on the surface of the infected cells compared to the non-infected cells, this implies firstly that glycosylation of the HCMV glycoproteins plays an important role in the virus virulence and pathogenesis, as has been shown for viruses other than HCMV. We saw significantly reduced levels of glycosylation in cells infected with the laboratory strains after infection and propose that this could be a result of the prolonged absence of immune pressure in the lab-adapted strains as one of the functions of glycans is to protect antigenic sites on the viral glycoprotein from the immune response. An alternative explanation is that some of the glycoproteins are down regulated in the lab strains due to repeated passage and the switch from a cell-cell transmission route to production of large amounts of cell-free virus in the laboratory strains. This might explain why some glycoprotein types were associated with an increase in levels of glycosylation after infection. The overall 78% reduction in the glycosylation levels in the laboratory strains infected cells compared to the non-infected is explained by the virus infection of the cell taking over the host cell machinery and could be explained by either of the hypotheses above. Also, in this project we investigated the effect of the HCMV glycoprotein genotypes on glycosylation and that in contrast to the laboratory strains, glycosylation was significantly increased after infection with the clinical strains (93%). Importantly, distinct and significant differences in the glycoprotein glycosylation were found to be associated with particular glycoprotein genotypes in both laboratory and clinical

HCMV strains. Although it is not possible to make direct extrapolations between these data and the *in vivo* situation for reasons already discussed, we propose that viral strains that have these particular glycoprotein types, could be more pathogenic and harmful than others, and that this might be an explanation for the variability of the outcome in HCMV congenitally infected infants.

In the laboratory strains the increased glycosylation patterns after HCMV infection were found to be associated with gH2 and gM1 genotypes, and in clinical samples they were associated with gH mixed genotypes, gN4a, gO4 and mixed gL genotypes; While the decreased glycosylation patterns were associated with gH1 and gM3 in laboratory strains, and in clinical samples with gH1, gH2, gN3a, gO1a and gL2 ($P<0.05$). In the case of gB, although gB2 and gB4 were associated with increased glycosylation, and gB3 was associated with decreased glycosylation, the association was not statistically significant ($p=0.075$).

Concerning gN and gB genotypes, the results of glycosylation were found to be supported by a limited number of studies, as it was reported that extensive glycosylation of gN and gB could influence the way the virus avoids neutralization by antiviral antibodies. Also, data from the current project together with what was reported previously confirm that infection with gN4a and gN4c genotypes resulted in sequelae 8x more often than other variants; whilst a decreased sequelae risk was observed in those infected with the gN1, gN3a variant. We suggest that that the increased glycosylation of HCMV gN4a could be associated with the virulence and pathogenicity of the virus, whereas the decreased glycosylation of gN3a is associated with the virus being less virulent. Accordingly, glycosylation of specific HCMV glycoprotein genotypes could have advanced to direct the immune response to generation of non-neutralizing antibodies, therefore, aiding HCMV to escape clearance. These findings demonstrate that glycosylation of glycoprotein in HCMV signifies a potentially essential mechanism for avoidance of antibody-mediated neutralization, which, in turn, facilitates HCMV pathogenicity.

Despite the numerous efforts to develop an effective HCMV vaccine, to date, there is no such vaccine yet available. Opportunely, considering the association found between HCMV specific glycoproteins and their glycosylation in this project, an effort to design a new vaccine with an effective target against these glycoproteins could be a chance to solve this problem. In a study by Hoorelbeke *et al*., (2011), they found that low glycosylation of gP120 (an HIV envelope glycoprotein), which was analysed by the low affinity of the lectin used (GNL) against the mannose oligomer in the virus glycoprotein was a result of the reduced antiviral activity of this lectin. This proposes that lectins, can be used as antivirals and that they should be considered as putative vaccine targets. Thus, the significantly increased glycosylation of gH mixed genotypes (occurrence of both gH1 and gH2 simultaneously) and gN4a genotype, which was analysed using GSL II lectin indicates the inclusion of the complex type N- glycans in the structure of these glycoproteins. Likewise, the structure of the glycoprotein gO4 comprises of the mannose oligomer indicated by GNL lectin specificity. Also, the existence of the mixed gL genotypes caused a significant increase in their glycosylation levels, which suggests that their structure contains N-acetylgalactosamine glycans. Targeting these sugars molecules could lead to a novel vaccine to be developed. Targeted therapeutic inhibition of glycosylation and subsequent expression of the glycoproteins on the surface of infected cells is an important step in eliminating the virus from the host cell and in expediting recovery from infection. Gantt *et al*., (2015), for example, reported that the glycosylation of crucial HSV-1 glycoproteins gB and gC which play essential roles in virus maturation and egress stages as a result of glycan-protein triggered intercellular and cell-pathogen interactions, was decreased in the presence of the drug Nelfinavir through a process of aberrant sub-cellular localization caused by the drug's induction of stress in the endoplasmic reticulum.

Furthermore, it was found that glycoproteins of strains isolated from respiratory specimens were significantly highly glycosylated compared to the blood

and urine samples, and from blood specimens compared to the urine samples only. This was analysed using GNL lectin the carbohydrate specificity of which was discussed earlier. Interestingly, only one strain of the four respiratory specimens has gO4 included in its glycoprotein profile, five strains of a total of 77 blood samples, and none of the eight urine samples. This may support the extensive presence of mannose-binding sites within HCMV glycoprotein structure, which would have a considerable impact on the viral infectivity and virulence.

With respect to the results of the glycoprotein glycosylation patterns, which were correlated with that of the virus growth characteristics for the first time in this project, the following observations were revealed: in laboratory strains, the decreased levels of the glycoprotein glycosylation were accompanied by the viral strains' slow growth, while in clinical samples, as expected, the slow growth was accompanied by the increased levels of the glycoproteins' glycosylation.

Furthermore, correlation of the current project results revealed that the disease outcome of HCMV infection could be significantly associated with the glycoprotein genotypes polymorphisms, but this was not according to their prevalence directly; instead it is related to their glycosylation analysis. Consequently, this was related to the pathogenicity of the virus strain. This was suggested as in contrast to the high prevalence of gN3a, gH1, gH2 and gO1a, they all had significantly lower glycosylation levels, which may be associated with their low virulence. The low virulence of some of these glycoprotein types (gN3a) was supported by several other studies as discussed previously. Likewise, the genotypes: mixed gH, gN4a, gO4, and mixed gL genotypes have had a low prevalence, but significantly high glycosylation levels, possibly indicating their high virulence as was discussed earlier. Moreover, gB1 was the most common among all infection categories, but no significant glycosylation pattern associated with it was found. Similarly, gM3 was significantly the most common, but again no significant glycosylation pattern of this genotype was detected.

However, it is also not feasible to relate the virus pathogenicity and virulence to the glycoproteins through studying their growth characteristic in culture. This might be because the monolayer cell cultures have a limited capability to equal the intricate *in vivo* conditions and critical viral gene functions for virus multiplication and pathogenesis *in vivo* so that this remains unobserved *in vitro*. As a result, HCMV reproduction *in vitro* is unlikely to be representative of the virus replicative process *in vivo*.

Despite this, the growth of the virus was higher among congenitally infected infants compared to the HCMV positive immunocompromised and immunocompetent patients. Also, the strains isolated from urine specimen grew significantly better than the ones isolated from blood samples. This could be related to the virus strains being mostly present in urine specimens from congenitally infected babies and contained higher titre of virus perhaps because these strains are more virulent and so grow significantly faster. No significant association between the virus growth, the glycosylation of the virus glycoproteins and virulence of the viral strains was achieved to be able to confirm this suggestion. This study is the first to investigate such a relationship, and further research with larger sample size is needed to explore these observations further.

In conclusion, primarily, the association between HCMV glycoproteins polymorphism and the altered characteristics of the virus has been confirmed in this project. This was done through proving that a reproducible association between the glycoproteins and their glycosylation patterns exists. This is the most significant finding as regardless of the actual changes seen – which will be system dependent, for the first time a link between glycoprotein polymorphisms and the important process of glycosylation has been made. Extending this work and furthering the understanding of the mechanism by which the virus could escape the host immune response, could help to provide new strategies for both diagnosis related to pathogenesis of the different strains, and for an effective HCMV vaccine with a new therapeutic target.

5.5 Limitation of the study

- Although the ELLA method used in this study allowed a quantitative measure of viral glycosylation to be made. It relies on growth of virus in culture the possibility that the viral strains tested have been passaged more than once and mutated means that the ELLA results may differ from the true picture *in vivo*.

- As well as the problem associated with culture grown virus, the cell culture is a time-consuming, specialised and laborious method for identifying the positive HCMV isolates.

- Identifying the strains glycoproteins profile using PCR/RFLP assay caused some difficulties regarding reading some of the bands due to the presence of overlapping ones. Again, a better approach would be to sequence the genomes, but for reasons of cost and availability, this option was not possible at the beginning of this project.

- The exclusion of groups with small numbers of cases (<4) from the statistical analysis was done to reach significant and accurate results of the study. A statistical test such as ANOVA compares the mean of multiple groups and gives a result stating that one group has a significant result (such as gN), without determining which subgroup (such as gN3a) was significant compared to which subgroups of that glycoprotein. However, given that the sample size was already not large this may have masked some observations. Consequently, to have a specific genotype that is significantly related to the virus virulence and pathogenicity, a follow up statistical test (such as Bonferroni post hoc), which does not perform a comparison between subgroups with a low number of cases, had to be carried out.

5.6 Future work

- Further study with larger sample size is certainly needed to support and confirm the attained results. This would allow a larger and wider number of genotypes to be detected increasing the size of the sample groups and give more power to the findings observed in the study.

- Using a more advanced and non-cell culture dependent technique such as sequencing to analyse the virus genome and proteins directly from the clinical samples, could help in avoiding drawbacks of the RFLP method as well as allowing high throughput of samples with detailed sequence results. RFLP is only able to detect the gross mutations, but other changes in the genome were missed with this method.

- Although the use of cell culture here has revealed some interesting results, a major limitation is the inability to directly relate the results to in vivo work, due to the glycosylation being host cell dependent. There are several possibilities to overcome this limitation; firstly, the cell culture system could be adapted to use primary cell lines, e.g. neural cell lines, which may provide data which is closer to the *in vivo* situation. An alternative approach would be to identify the glycoprotein types of interest and express these in an alternative system such as a baculovirus system to produce a protein which can be examined by crystallography and study the effects the mutation has on the protein structure allowing prediction of the glycosylation pattern. Similarly, site directed mutagenesis could be used to introduce specific mutations and create a recombinant virus that displayed the genotypes of interest to see how its characteristics changed as a result of the mutation.

- Designing a prospective study would allow more patient information to be obtained; such as age, sex, clinical manifestations, control the time of specimen collection, and specimen handling to ensure that no confounding factors are introduced to the results.

- The study could be extended by investigating the interaction between the virus glycoprotein polymorphism and the host immune response, for example looking at levels of antibody and cytokine produced in response to infection which could lead to a better understanding of the virus pathogenicity and virulence.

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APPENDICES

Appendix 1: Clinical HCMV strains glycoprotein genotypes profile.

Sample No.	Genotyping Profile						Specimen type	Specimen Category
	gB	gH	gL	gM	gN	gO		
1	4	1	4	1	3a	1c	Urine	Congenital infection
10	1	2	4	3	3a	1c	Blood EDTA	
17	3	1	4	3	3a	1c	Urine	
19	1	1	2	1	3a	1c	Urine	
40	4	1	4	3	3a	1c	Urine	
66	3	1	3	2	3a		Urine	
76	Mixed	Mixed	4		3a		Blood EDTA	
79	4	1	Mixed	3	4d		Blood EDTA	
84	2	1	Mixed	3	3a		Blood EDTA	
85	1	1	4			2b	Blood EDTA	
86	1	1	4	3	1	1a	Throat swab	
87	2		Mixed		3a	3	Blood	
70	Mixed						Saliva	Unconfirmed congenital or early post-natal infection
27	3	1	4	1	3a		Blood EDTA	Immunocompetent primary infections
32	3	Mixed	4	3	4a	1a	Urine	
41	Mixed	Mixed	Mixed		3a		Blood Clot	
42	3	2	4	3	3a	1c	Blood EDTA	
53	1	2	4	3	1	1c	Blood EDTA	
61	1	2	Mixed				Blood EDTA	
67	3	2	3	1	3a	1c	aspirate	
21	2	1	1	1	3a	1c	Blood EDTA	Immunocompetent, not defined Primary or recurrent infection
5	4	2	2	1	1	1c	Blood EDTA	Immunocompromised Primary infection
9	1	1	4	3	4a	1c	Blood EDTA	
16	1	2	3	3	3a	1c	Blood EDTA	
18	1	1	4	3	3a	1c	Blood EDTA	
22	3	1	4	3	4c	1a	Blood EDTA	
23	2	1	1	3	3a	1a	Blood EDTA	
24	1	1	4	3	4b	1c	Blood EDTA	
25	2	1	2	3	3a	1a	Blood EDTA	
28	1	2	4	1	3a	1a	Blood EDTA	
31	1	2	Mixed	3	4c	4	Blood EDTA	
33	3	1	2	3	3a	1a	Blood EDTA	
37	2	1	4				Blood EDTA	
46	1	2	2	3	4d	1c	Blood EDTA	
47	1	1	4	3	4b	4	Blood Clot	
50	4	1	1	3	3a	1a	Blood EDTA	
51	1	2	Mixed	3		4	Blood EDTA	
52	1	1	4	3	1	1a	Blood EDTA	
54	4	1	2	3	3a	1c	Blood EDTA	
55	3	1	2	3	4a	1c	Blood EDTA	
58							Blood Clot	
59	2	2	4	3	3a	1c	Blood EDTA	
60	4	1	4	1	4a	1c	Blood EDTA	
64	3	1	4		4c		Blood EDTA	
74	2	1	4	1	3a		Urine	
78	1	1	3	3	1	1a	Blood EDTA	
80	1	1	3	3		2a	Blood EDTA	
3	1	1	Mixed	3	1	1a	Blood EDTA	Immunocompromised

4	4	2	4	1	4c	1a	Blood EDTA	recurrent infection
6	3	1	2	3	3a	1c	Blood EDTA	
7	4	2	1	1	3a	1a	Blood Clot	
8	3	2	2	3	3a	1c	Blood EDTA	
11	2	2	2	2	3a	1c	Blood EDTA	
12	3	1	4	3	3a	1c	Blood EDTA	
13	3	1	2	3	3a	1c	Blood EDTA	
15	3	1	2	3	3a	1c	Blood EDTA	
20	3	2	4	2	3a	1c	Blood EDTA	
29	1	2	Mixed	3	4a	1a	Blood EDTA	
30	1	2					Blood EDTA	
36	1	2	4	1	3a	1c	Blood EDTA	
38	1	2	4	3	3a	1c	Blood EDTA	
39	2	2	2	3	3a	2a	Blood EDTA	
43	4	2					Blood EDTA	
44	3	1	Mixed	3	3a	1c	Blood EDTA	
45	3	1	2	1	3a	1c	Blood EDTA	
49	2	1	3	3	3a	1c	Blood EDTA	
56	2	1	2	3	3a	1a	Blood EDTA	
57	2	2	4	3	3a	1c	Blood Clot	
62	4	1	4	3	3a	1c	Blood EDTA	
63	2	2	Mixed		3a		Blood EDTA	
65	1	2	4	3		4	Blood Clot	
68	1	1	2	3	3a	1a	Blood EDTA	
69	1	2	4	3	3a	1c	Blood EDTA	
71	1	1	4	3	4d	1c	Blood EDTA	
72	2	2	4	3	3a	1c	Blood EDTA	
73	1						Blood Clot	
75	1	2	Mixed		3a		Blood EDTA	
77	1	Mixed	2	Mix ed	3a	1c	Blood EDTA	
81	2	2	Mixed	3		3	Blood EDTA	
82	1	2	4	3		1c	Blood Clot	
88	1	2	4	3		4	Blood Clot	
89	1	2	Mixed	1	3a	4	Sputum	
26	1	2	3	1	4b	Mixe d	Blood EDTA	Immunocompromised, not defined Primary or recurrent infection
35	2	2	2	1	3a	1c	Blood EDTA	
2	1	1	3	2	3a	1a	Urine	Not known
14	2	2	4	3	4a	1c	Plasma	
34	3	1	1	3	3a	1c	Blood EDTA	
48	2	1	1	3	3a	1c	Blood EDTA	
83							Blood EDTA	

Appendix 2: Tracking HCMV clinical strains growth over 4 weeks using $TCID_{50}$ assay.

Sample No.	$TCID_{50}$/ml (Log 10^x)					
	Week 1	Week 2	Week3	Week 4	Specimen type	Specimen category
1	2.8	6.1	9.5	9.5	Urine	Congenital infection
10	5.5	5.8	8.4	9.5	Blood EDTA	
17	2.8	8.1	7.3	10.4	Urine	
19	3.3	4.6	6.4	9.5	Urine	
40	0.9	4.5	5	7	Urine	
66	2.8	4.8	8.1	9.4	Urine	
76	5	5.1	9.4	9	Blood EDTA	
79	2.1	5.1	10.4	9.5	Blood EDTA	
84	3.3	5.3	6.3	9.1	Blood EDTA	
85	6.1	7.4	8.4	9.5	Blood EDTA	
86	2.1	2.4	8.4	9.5	Blood	
87	5.4	6.1	9.4	9.5	Blood	
70	2.1	2.3	4.8	6.8	Saliva	Unconfirmed congenital or early post-natal infection
27	3.8	5.4	5.9	5.4	Blood EDTA	Immunocompetent post -natal patients
32	2.8	2.9	6.1	5.9	Urine	
41	1.1	2	4.9	5	Blood Clot	
42	2	2.9	4	6	Blood EDTA	
53	4.4	4.8	5.9	6	Blood EDTA	
61	3.3	5.1	6.5	6.6	Blood EDTA	
67	4.1	3.5	3.8	5.8	aspirate	
21	1.3	2.1	3.3	8.8	Blood EDTA	Immunocompetent, not defined Primary or recurrent infection
5	2.5	3.4	4.4	4.4	Blood EDTA	Immunocompromised Primary infection
9	2.8	2.1	3.8	5.1	Blood EDTA	
16	2.1	1.8	6.1	6.1	Blood EDTA	
18	4.4	3.4	5.9	5.1	Blood EDTA	
22	1.9	3.6	4.4	4.1	Blood EDTA	
23	1.9	3.9	4.4	4.4	Blood EDTA	
24	4	6	6	6.3	Blood EDTA	
25	1.8	2.8	2.1	4.4	Blood EDTA	
28	1.9	3	4.4	4.6	Blood EDTA	
31	2.2	3.4	5.4	5.5	Blood EDTA	
33	1.9	2.1	6.4	5.5	Blood EDTA	
37	1.8	2.3	3.3	4.8	Blood EDTA	
46	3.8	5.9	6.8	6.8	Blood EDTA	
47	2.8	3.4	6	7	Blood Clot	
50	2.3	4.1	5.6	6	Blood EDTA	
51	2.8	2.8	4.4	4.4	Blood EDTA	
52	1.4	1.5	1.5	3.5	Blood EDTA	
54	5.5	5	6.4	6.5	Blood EDTA	
55	3.3	2.5	3.4	5.5	Blood EDTA	
58	2.4	2.1	6.4	6.6	Blood Clot	
59	1.4	3.3	5.1	5.4	Blood EDTA	
60	4.1	4.9	4.9	5.1	Blood EDTA	
64	2.8	4.5	5.5	6.3	Blood EDTA	
74	2.8	4.8	5.6	5.9	Urine	
78	2.4	5.1	4.4	4.4	Blood EDTA	
80	1.6	2.8	2.9	4.9	Blood EDTA	
3	3.5	4.1	4.3	4.4	Blood EDTA	Immunocompromised

4	3.9	4.1	4.4	5.5	Blood EDTA	recurrent infection
6	5.6	5.8	6.6	9.5	Blood EDTA	
7	4.8	3.9	4	4.1	Blood Clot	
8	2.4	3	5.1	5.4	Blood EDTA	
11	2.1	2.8	6.6	7.1	Blood EDTA	
12	5.1	5.5	5.8	5.6	Blood EDTA	
13	3.3	5.9	6.3	7	Blood EDTA	
15	2.4	3	6.3	6.6	Blood EDTA	
20	3.5	3.6	4.6	6.8	Blood EDTA	
29	2.6	6.4	7.6	7.8	Blood EDTA	
30	1.9	2.5	4.5	4.6	Blood EDTA	
36	1.8	2	5.4	6.6	Blood EDTA	
38	1.4	2	2.6	3.4	Blood EDTA	
39	4.8	6.8	5.9	9.5	Blood EDTA	
43	2.8	2.8	8.1	7.5	Blood EDTA	
44	2.8	2.8	3.9	4.3	Blood EDTA	
45	2	3.3	5	5.3	Blood EDTA	
49	1.5	2.1	2.1	3.5	Blood EDTA	
56	5.1	5.8	5.1	6.1	Blood EDTA	
57	3	4.6	6.3	8	Blood Clot	
62	2.1	2.1	3.9	5.3	Blood EDTA	
63	5.1	6.6	7	7.1	Blood EDTA	
65	2	3.3	5.3	5.3	Blood Clot	
68	4.4	5.4	5.4	5.9	Blood EDTA	
69	4.4	4.4	3.4	4.5	Blood EDTA	
71	3	4.6	4.9	7	Blood EDTA	
72	2.8	3	2.8	7.3	Blood EDTA	
73	2.8	2.1	9.5	9.5	Blood Clot	
75	6.1	6.1	6.1	9.5	Blood EDTA	
77	2.8	3.9	6.3	9.5	Blood EDTA	
81	4.5	4.1	6.5	7.9	Blood EDTA	
82	3.5	4.1	6	6.8	Blood Clot	
88	2.1	4.9	4.9	5.1	Blood Clot	
89	1.9	4.4	5.5	5.5	Sputum	
26	2.9	7.1	9.5	9.5	Blood EDTA	Immunocompromised, not defined Primary or recurrent infection
35	3.2	4.5	4.5	4.5	Blood EDTA	
2	2.8	5.1	5.4	9.5	Urine	Not known
14	3.4	3.6	5.8	6	Plasma	
34	3.3	6.8	6.9	6.4	Blood EDTA	
48	2.4	2.3	5.6	6.1	Blood EDTA	
83	5	5.9	7.3	7.3	Blood EDTA	

Appendix 3: Results of One-way ANOVA, the Tables below, for each glycoprotein genotype, show that no significant differences between the growth behaviour of HCMV strains in clinical samples and the glycoprotein genotypes.

3.1 Glycoprotein B

Weeks		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
						Lower Bound	Upper Bound	
1	1.00	35	3.0514	1.24295	.21010	2.6245	3.4784	1.40
	2.00	19	3.0316	1.36180	.31242	2.3752	3.6879	1.30
	3.00	19	3.0842	1.02049	.23412	2.5923	3.5761	1.90
	4.00	11	3.0727	1.35284	.40790	2.1639	3.9816	.90
	Total	84	3.0571	1.21727	.13282	2.7930	3.3213	.90
2	1.00	35	4.1057	1.59483	.26958	3.5579	4.6536	1.50
	2.00	19	4.0421	1.54032	.35337	3.2997	4.7845	2.10
	3.00	19	4.2105	1.64212	.37673	3.4191	5.0020	2.10
	4.00	11	4.1818	1.12944	.34054	3.4230	4.9406	2.10
	Total	84	4.1250	1.51617	.16543	3.7960	4.4540	1.50
3	1.00	35	5.6629	1.81482	.30676	5.0394	6.2863	1.50
	2.00	19	5.1421	1.84701	.42373	4.2519	6.0323	2.10
	3.00	19	5.5474	1.29887	.29798	4.9213	6.1734	3.40
	4.00	11	6.0545	2.28139	.68786	4.5219	7.5872	3.90
	Total	84	5.5702	1.77729	.19392	5.1845	5.9559	1.50
4	1.00	35	6.5343	2.02266	.34189	5.8395	7.2291	3.40
	2.00	19	6.6000	1.86011	.42674	5.7035	7.4965	3.50
	3.00	19	6.3789	1.68509	.38659	5.5668	7.1911	4.10
	4.00	11	6.4000	1.84282	.55563	5.1620	7.6380	4.10
	Total	84	6.4964	1.85975	.20292	6.0928	6.9000	3.40

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
1	Between Groups	.030	3	.010	.007	.999
	Within Groups	122.956	80	1.537		
	Total	122.986	83			
2	Between Groups	.318	3	.106	.045	.987
	Within Groups	190.479	80	2.381		
	Total	190.797	83			
3	Between Groups	6.373	3	2.124	.664	.576
	Within Groups	255.803	80	3.198		
	Total	262.176	83			
4	Between Groups	.618	3	.206	.058	.982
	Within Groups	286.450	80	3.581		
	Total	287.069	83			

Multiple Comparisons							
Post hoc test: Bonferroni							
Dependent Variable	(I) gB.r	(J) gB.r	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Week1	1.00	2.00	.01985	.35328	1.000	-.9359	.9756
		3.00	-.03278	.35328	1.000	-.9886	.9230
		4.00	-.02130	.42853	1.000	-1.1807	1.1381
	2.00	1.00	-.01985	.35328	1.000	-.9756	.9359
		3.00	-.05263	.40222	1.000	-1.1408	1.0356
		4.00	-.04115	.46970	1.000	-1.3119	1.2296
	3.00	1.00	.03278	.35328	1.000	-.9230	.9886
		2.00	.05263	.40222	1.000	-1.0356	1.1408
		4.00	.01148	.46970	1.000	-1.2593	1.2822
	4.00	1.00	.02130	.42853	1.000	-1.1381	1.1807
		2.00	.04115	.46970	1.000	-1.2296	1.3119
		3.00	-.01148	.46970	1.000	-1.2822	1.2593
Week2	1.00	2.00	.06361	.43971	1.000	-1.1260	1.2532
		3.00	-.10481	.43971	1.000	-1.2944	1.0848
		4.00	-.07610	.53337	1.000	-1.5191	1.3669
	2.00	1.00	-.06361	.43971	1.000	-1.2532	1.1260
		3.00	-.16842	.50063	1.000	-1.5228	1.1860
		4.00	-.13971	.58461	1.000	-1.7213	1.4419

	3.00	1.00	.10481	.43971	1.000	-1.0848	1.2944
		2.00	.16842	.50063	1.000	-1.1860	1.5228
		4.00	.02871	.58461	1.000	-1.5529	1.6103
	4.00	1.00	.07610	.53337	1.000	-1.3669	1.5191
		2.00	.13971	.58461	1.000	-1.4419	1.7213
		3.00	-.02871	.58461	1.000	-1.6103	1.5529
Week3	1.00	2.00	.52075	.50956	1.000	-.8578	1.8993
		3.00	.11549	.50956	1.000	-1.2631	1.4941
		4.00	-.39169	.61810	1.000	-2.0639	1.2805
	2.00	1.00	-.52075	.50956	1.000	-1.8993	.8578
		3.00	-.40526	.58016	1.000	-1.9748	1.1643
		4.00	-.91244	.67748	1.000	-2.7453	.9204
	3.00	1.00	-.11549	.50956	1.000	-1.4941	1.2631
		2.00	.40526	.58016	1.000	-1.1643	1.9748
		4.00	-.50718	.67748	1.000	-2.3401	1.3257
	4.00	1.00	.39169	.61810	1.000	-1.2805	2.0639
		2.00	.91244	.67748	1.000	-.9204	2.7453
		3.00	.50718	.67748	1.000	-1.3257	2.3401
Week4	1.00	2.00	-.06571	.53922	1.000	-1.5245	1.3931
		3.00	.15534	.53922	1.000	-1.3035	1.6142
		4.00	.13429	.65408	1.000	-1.6353	1.9039
	2.00	1.00	.06571	.53922	1.000	-1.3931	1.5245
		3.00	.22105	.61393	1.000	-1.4399	1.8820
		4.00	.20000	.71691	1.000	-1.7396	2.1396
	3.00	1.00	-.15534	.53922	1.000	-1.6142	1.3035
		2.00	-.22105	.61393	1.000	-1.8820	1.4399
		4.00	-.02105	.71691	1.000	-1.9606	1.9185
	4.00	1.00	-.13429	.65408	1.000	-1.9039	1.6353
		2.00	-.20000	.71691	1.000	-2.1396	1.7396
		3.00	.02105	.71691	1.000	-1.9185	1.9606

3.2 Glycoprotein H

Weeks		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
						Lower Bound	Upper Bound	
1	Mixed	4	2.9250	1.59870	.79935	.3811	5.4689	1.10
	1.00	44	2.9568	1.22729	.18502	2.5837	3.3299	.90
	2.00	36	3.1361	1.21729	.20288	2.7242	3.5480	1.40
	Total	84	3.0321	1.22712	.13389	2.7658	3.2984	.90
2	Mixed	4	3.4750	1.33260	.66630	1.3545	5.5955	2.00
	1.00	44	4.2091	1.60085	.24134	3.7224	4.6958	1.50
	2.00	36	4.0639	1.42029	.23672	3.5833	4.5444	1.80
	Total	84	4.1119	1.50600	.16432	3.7851	4.4387	1.50
3	Mixed	4	6.6750	1.91898	.95949	3.6215	9.7285	4.90
	1.00	44	5.4318	1.86482	.28113	4.8649	5.9988	1.50
	2.00	36	5.4889	1.51785	.25298	4.9753	6.0025	2.60
	Total	84	5.5155	1.72484	.18820	5.1412	5.8898	1.50
.4	Mixed	4	7.3500	2.23383	1.11692	3.7955	10.9045	5.00
	1.00	44	6.4750	1.96186	.29576	5.8785	7.0715	3.50
	2.00	36	6.2889	1.62635	.27106	5.7386	6.8392	3.40
	Total	84	6.4369	1.82762	.19941	6.0403	6.8335	3.40

ANOVA						
Weeks		Sum of Squares	df	Mean Square	F	Sig.
1	Between Groups	.685	2	.342	.223	.801
	Within Groups	124.299	81	1.535		
	Total	124.983	83			
2	Between Groups	2.121	2	1.061	.462	.632
	Within Groups	186.127	81	2.298		
	Total	188.248	83			
3	Between Groups	5.711	2	2.856	.959	.388
	Within Groups	241.219	81	2.978		
	Total	246.930	83			
4	Between Groups	4.188	2	2.094	.621	.540
	Within Groups	273.048	81	3.371		
	Total	277.236	83			

Multiple Comparisons							
Post hoc test: Bonferroni							
Dependent Variable	(I) gH	(J) gH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
1	Mixed	1.00	-.03182	.64693	1.000	-1.6134	1.5497
		2.00	-.21111	.65289	1.000	-1.8072	1.3850
	1.00	Mixed	.03182	.64693	1.000	-1.5497	1.6134
		2.00	-.17929	.27839	1.000	-.8599	.5013
	2.00	Mixed	.21111	.65289	1.000	-1.3850	1.8072
		1.00	.17929	.27839	1.000	-.5013	.8599
2	Mixed	1.00	-.73409	.79164	1.000	-2.6694	1.2012
		2.00	-.58889	.79893	1.000	-2.5421	1.3643
	1.00	Mixed	.73409	.79164	1.000	-1.2012	2.6694
		2.00	.14520	.34067	1.000	-.6876	.9780
	2.00	Mixed	.58889	.79893	1.000	-1.3643	2.5421
		1.00	-.14520	.34067	1.000	-.9780	.6876
3	Mixed	1.00	1.24318	.90121	.515	-.9600	3.4464
		2.00	1.18611	.90952	.588	-1.0374	3.4096
	1.00	Mixed	-1.24318	.90121	.515	-3.4464	.9600
		2.00	-.05707	.38782	1.000	-1.0052	.8910
	2.00	Mixed	-1.18611	.90952	.588	-3.4096	1.0374
		1.00	.05707	.38782	1.000	-.8910	1.0052
4	Mixed	1.00	.87500	.95883	1.000	-1.4691	3.2191
		2.00	1.06111	.96767	.828	-1.3046	3.4268
	1.00	Mixed	-.87500	.95883	1.000	-3.2191	1.4691
		2.00	.18611	.41261	1.000	-.8226	1.1948
	2.00	Mixed	-1.06111	.96767	.828	-3.4268	1.3046
		1.00	-.18611	.41261	1.000	-1.1948	.8226

3.3 Glycoprotein L

Weeks		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
						Lower Bound	Upper Bound	
1	Mixed	14	3.3357	1.45158	.38795	2.4976	4.1738	1.10
	1.00	6	2.6667	1.23396	.50376	1.3717	3.9616	1.30
	2.00	18	3.3444	1.25802	.29652	2.7188	3.9700	1.80
	3.00	8	2.5250	.83452	.29505	1.8273	3.2227	1.50
	4.00	37	3.0351	1.25349	.20607	2.6172	3.4531	.90
	Total	83	3.0771	1.25468	.13772	2.8031	3.3511	.90
2	Mixed	14	4.5929	1.46627	.39188	3.7463	5.4395	2.00
	1.00	6	3.8500	1.68731	.68884	2.0793	5.6207	2.10
	2.00	18	4.2500	1.44680	.34101	3.5305	4.9695	2.10
	3.00	8	4.0375	1.80471	.63806	2.5287	5.5463	1.80
	4.00	37	4.0541	1.51979	.24985	3.5473	4.5608	1.50
	Total	83	4.1711	1.51251	.16602	3.8408	4.5014	1.50
3	Mixed	14	6.3000	1.87083	.50000	5.2198	7.3802	3.90
	1.00	6	4.9667	1.30945	.53458	3.5925	6.3409	3.30
	2.00	18	5.5000	1.26909	.29913	4.8689	6.1311	2.10
	3.00	8	5.2875	2.54077	.89830	3.1634	7.4116	2.10
	4.00	37	5.4270	1.79917	.29578	4.8272	6.0269	1.50
	Total	83	5.5434	1.76083	.19328	5.1589	5.9279	1.50
4	Mixed	14	6.8643	2.03493	.54386	5.6894	8.0392	4.30
	1.00	6	5.9667	1.68365	.68735	4.1998	7.7335	4.10
	2.00	18	6.6111	1.79013	.42194	5.7209	7.5013	4.40
	3.00	8	6.6375	2.47498	.87504	4.5684	8.7066	3.50
	4.00	37	6.3270	1.76031	.28939	5.7401	6.9139	3.40
	Total	83	6.4831	1.85425	.20353	6.0782	6.8880	3.40

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
1	Between Groups	5.737	4	1.434	.907	.464
	Within Groups	123.349	78	1.581		
	Total	129.087	82			
2	Between Groups	3.871	4	.968	.411	.800
	Within Groups	183.720	78	2.355		
	Total	187.591	82			
3	Between Groups	11.069	4	2.767	.888	.475
	Within Groups	243.175	78	3.118		
	Total	254.244	82			
4	Between Groups	5.021	4	1.255	.354	.841
	Within Groups	276.915	78	3.550		
	Total	281.936	82			

Multiple Comparisons							
Post hoc test: Bonferroni							
Dependent Variable	(I) gL	(J) gL	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
1	Mixed	1.00	.66905	.61362	1.000	-1.1037	2.4418
		2.00	-.00873	.44812	1.000	-1.3034	1.2859
		3.00	.81071	.55734	1.000	-.7995	2.4209
		4.00	.30058	.39459	1.000	-.8394	1.4406
	1.00	Mixed	-.66905	.61362	1.000	-2.4418	1.1037
		2.00	-.67778	.59281	1.000	-2.3904	1.0349
		3.00	.14167	.67915	1.000	-1.8204	2.1038
		4.00	-.36847	.55345	1.000	-1.9674	1.2305
	2.00	Mixed	.00873	.44812	1.000	-1.2859	1.3034
		1.00	.67778	.59281	1.000	-1.0349	2.3904
		3.00	.81944	.53435	1.000	-.7243	2.3632
		4.00	.30931	.36138	1.000	-.7347	1.3534
	3.00	Mixed	-.81071	.55734	1.000	-2.4209	.7995
		1.00	-.14167	.67915	1.000	-2.1038	1.8204
		2.00	-.81944	.53435	1.000	-2.3632	.7243
		4.00	-.51014	.49032	1.000	-1.9267	.9064
	4.00	Mixed	-.30058	.39459	1.000	-1.4406	.8394
		1.00	.36847	.55345	1.000	-1.2305	1.9674
		2.00	-.30931	.36138	1.000	-1.3534	.7347
		3.00	.51014	.49032	1.000	-.9064	1.9267
2	Mixed	1.00	.74286	.74887	1.000	-1.4207	2.9064
		2.00	.34286	.54690	1.000	-1.2372	1.9229
		3.00	.55536	.68019	1.000	-1.4098	2.5205
		4.00	.53880	.48156	1.000	-.8525	1.9301
	1.00	Mixed	-.74286	.74887	1.000	-2.9064	1.4207
		2.00	-.40000	.72348	1.000	-2.4902	1.6902
		3.00	-.18750	.82885	1.000	-2.5821	2.2071
		4.00	-.20405	.67544	1.000	-2.1555	1.7474
	2.00	Mixed	-.34286	.54690	1.000	-1.9229	1.2372
		1.00	.40000	.72348	1.000	-1.6902	2.4902
		3.00	.21250	.65213	1.000	-1.6716	2.0966
		4.00	.19595	.44104	1.000	-1.0782	1.4701
	3.00	Mixed	-.55536	.68019	1.000	-2.5205	1.4098
		1.00	.18750	.82885	1.000	-2.2071	2.5821
		2.00	-.21250	.65213	1.000	-2.0966	1.6716
		4.00	-.01655	.59840	1.000	-1.7454	1.7123
	4.00	Mixed	-.53880	.48156	1.000	-1.9301	.8525
		1.00	.20405	.67544	1.000	-1.7474	2.1555
		2.00	-.19595	.44104	1.000	-1.4701	1.0782
		3.00	.01655	.59840	1.000	-1.7123	1.7454
3	Mixed	1.00	1.33333	.86156	1.000	-1.1558	3.8225
		2.00	.80000	.62920	1.000	-1.0178	2.6178
		3.00	1.01250	.78255	1.000	-1.2484	3.2734
		4.00	.87297	.55403	1.000	-.7277	2.4736
	1.00	Mixed	-1.33333	.86156	1.000	-3.8225	1.1558
		2.00	-.53333	.83235	1.000	-2.9381	1.8714
		3.00	-.32083	.95358	1.000	-3.0758	2.4341

		4.00	-.46036	.77709	1.000	-2.7054	1.7847
	2.00	Mixed	-.80000	.62920	1.000	-2.6178	1.0178
		1.00	.53333	.83235	1.000	-1.8714	2.9381
		3.00	.21250	.75027	1.000	-1.9551	2.3801
		4.00	.07297	.50741	1.000	-1.3930	1.5389
	3.00	Mixed	-1.01250	.78255	1.000	-3.2734	1.2484
		1.00	.32083	.95358	1.000	-2.4341	3.0758
		2.00	-.21250	.75027	1.000	-2.3801	1.9551
		4.00	-.13953	.68845	1.000	-2.1285	1.8495
	4.00	Mixed	-.87297	.55403	1.000	-2.4736	.7277
		1.00	.46036	.77709	1.000	-1.7847	2.7054
		2.00	-.07297	.50741	1.000	-1.5389	1.3930
		3.00	.13953	.68845	1.000	-1.8495	2.1285
4	Mixed	1.00	.89762	.91939	1.000	-1.7586	3.5538
		2.00	.25317	.67143	1.000	-1.6866	2.1930
		3.00	.22679	.83508	1.000	-2.1858	2.6394
		4.00	.53726	.59122	1.000	-1.1708	2.2453
	1.00	Mixed	-.89762	.91939	1.000	-3.5538	1.7586
		2.00	-.64444	.88822	1.000	-3.2106	1.9217
		3.00	-.67083	1.01758	1.000	-3.6107	2.2690
		4.00	-.36036	.82925	1.000	-2.7561	2.0354
	2.00	Mixed	-.25317	.67143	1.000	-2.1930	1.6866
		1.00	.64444	.88822	1.000	-1.9217	3.2106
		3.00	-.02639	.80063	1.000	-2.3395	2.2867
		4.00	.28408	.54146	1.000	-1.2802	1.8484
	3.00	Mixed	-.22679	.83508	1.000	-2.6394	2.1858
		1.00	.67083	1.01758	1.000	-2.2690	3.6107
		2.00	.02639	.80063	1.000	-2.2867	2.3395
		4.00	.31047	.73466	1.000	-1.8120	2.4330
	4.00	Mixed	-.53726	.59122	1.000	-2.2453	1.1708
		1.00	.36036	.82925	1.000	-2.0354	2.7561
		2.00	-.28408	.54146	1.000	-1.8484	1.2802
		3.00	-.31047	.73466	1.000	-2.4330	1.8120

3.4 Glycoprotein M

Weeks		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
						Lower Bound	Upper Bound	
1	1.00	16	2.9437	1.00927	.25232	2.4059	3.4816	1.30
	2.00	4	2.8000	.57155	.28577	1.8905	3.7095	2.10
	3.00	53	2.9736	1.19665	.16437	2.6437	3.3034	.90
	Total	73	2.9575	1.12323	.13146	2.6955	3.2196	.90
2	1.00	16	4.1938	1.34980	.33745	3.4745	4.9130	2.00
	2.00	4	4.0750	1.06888	.53444	2.3742	5.7758	2.80
	3.00	53	4.0321	1.53093	.21029	3.6101	4.4541	1.50
	Total	73	4.0699	1.45752	.17059	3.7298	4.4099	1.50
3	1.00	16	5.4062	1.79015	.44754	4.4523	6.3602	3.30
	2.00	4	6.1750	1.52398	.76199	3.7500	8.6000	4.60
	3.00	53	5.3226	1.68393	.23131	4.8585	5.7868	1.50
	Total	73	5.3877	1.68819	.19759	4.9938	5.7816	1.50
4	1.00	16	6.2500	1.94148	.48537	5.2155	7.2845	4.10
	2.00	4	8.2000	1.44914	.72457	5.8941	10.5059	6.80
	3.00	53	6.1981	1.72052	.23633	5.7239	6.6723	3.40
	Total	73	6.3192	1.79418	.20999	5.9006	6.7378	3.40

ANOVA						
Weeks		Sum of Squares	df	Mean Square	F	Sig.
1	Between Groups	.116	2	.058	.045	.956
	Within Groups	90.722	70	1.296		

	Total	90.838	72			
2	Between Groups	.321	2	.161	.074	.929
	Within Groups	152.632	70	2.180		
	Total	152.954	72			
3	Between Groups	2.709	2	1.355	.468	.628
	Within Groups	202.490	70	2.893		
	Total	205.199	72			
4	Between Groups	15.003	2	7.502	2.422	.096
	Within Groups	216.770	70	3.097		
	Total	231.773	72			

Multiple Comparisons

Post hoc test: Bonferroni

Dependent Variable	(I) gM.r	(J) gM.r	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Week1	1.00	2.00	.14375	.63640	1.000	-1.4173	1.7048
		3.00	-.02983	.32474	1.000	-.8264	.7667
	2.00	1.00	-.14375	.63640	1.000	-1.7048	1.4173
		3.00	-.17358	.59031	1.000	-1.6215	1.2744
	3.00	1.00	.02983	.32474	1.000	-.7667	.8264
		2.00	.17358	.59031	1.000	-1.2744	1.6215
Week2	1.00	2.00	.11875	.82547	1.000	-1.9060	2.1435
		3.00	.16167	.42121	1.000	-.8715	1.1949
	2.00	1.00	-.11875	.82547	1.000	-2.1435	1.9060
		3.00	.04292	.76567	1.000	-1.8352	1.9210
	3.00	1.00	-.16167	.42121	1.000	-1.1949	.8715
		2.00	-.04292	.76567	1.000	-1.9210	1.8352
Week3	1.00	2.00	-.76875	.95077	1.000	-3.1009	1.5634
		3.00	.08361	.48515	1.000	-1.1064	1.2736
	2.00	1.00	.76875	.95077	1.000	-1.5634	3.1009
		3.00	.85236	.88191	1.000	-1.3108	3.0156
	3.00	1.00	-.08361	.48515	1.000	-1.2736	1.1064
		2.00	-.85236	.88191	1.000	-3.0156	1.3108
Week4	1.00	2.00	-1.95000	.98373	.154	-4.3630	.4630
		3.00	.05189	.50197	1.000	-1.1794	1.2832
	2.00	1.00	1.95000	.98373	.154	-.4630	4.3630
		3.00	2.00189	.91247	.095	-.2363	4.2401
	3.00	1.00	-.05189	.50197	1.000	-1.2832	1.1794
		2.00	-2.00189	.91247	.095	-4.2401	.2363

3.5 Glycoprotein N

Weeks		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
						Lower Bound	Upper Bound	
1	1	6	2.7167	1.06849	.43621	1.5954	3.8380	1.40
	3a	52	3.1346	1.38690	.19233	2.7485	3.5207	.90
	4a	6	3.1667	.55377	.22608	2.5855	3.7478	2.60
	4c	4	2.7000	.88318	.44159	1.2947	4.1053	1.90
	Total	68	3.0750	1.27693	.15485	2.7659	3.3841	.90
2	1	6	3.5500	1.40107	.57198	2.0797	5.0203	1.50
	3a	52	4.1923	1.55599	.21578	3.7591	4.6255	1.80
	4a	6	3.7333	1.63544	.66767	2.0170	5.4496	2.10
	4c	4	3.9000	.49666	.24833	3.1097	4.6903	3.40
	Total	68	4.0779	1.49924	.18181	3.7150	4.4408	1.50
3	1	6	4.8167	2.26399	.92427	2.4408	7.1926	1.50
	3a	52	5.5404	1.68328	.23343	5.0718	6.0090	2.10
	4a	6	5.2667	1.56162	.63753	3.6278	6.9055	3.40
	4c	4	4.9250	.60759	.30380	3.9582	5.8918	4.40

	Total	68	5.4162	1.67284	.20286	5.0113	5.8211	1.50
4	1	6	5.3667	2.18052	.89019	3.0784	7.6550	3.50
	3a	52	6.6865	1.93371	.26816	6.1482	7.2249	3.40
	4a	6	5.9000	1.00598	.41069	4.8443	6.9557	5.10
	4c	4	5.3500	.91469	.45735	3.8945	6.8055	4.10
	Total	68	6.4221	1.88710	.22884	5.9653	6.8788	3.40

ANOVA

Weeks		Sum of Squares	df	Mean Square	F	Sig.
Week 1	Between Groups	1.568	3	.523	.311	.818
	Within Groups	107.679	64	1.682		
	Total	109.247	67			
Week 2	Between Groups	3.192	3	1.064	.462	.710
	Within Groups	147.405	64	2.303		
	Total	150.597	67			
Week 3	Between Groups	4.058	3	1.353	.472	.703
	Within Groups	183.434	64	2.866		
	Total	187.492	67			
Week 4	Between Groups	16.553	3	5.518	1.590	.200
	Within Groups	222.044	64	3.469		
	Total	238.597	67			

Multiple Comparisons

Post hoc test: Bonferroni

Dependent Variable	(I) gN.r	(J) gN.r	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Week1	1	3a	-.41795	.55926	1.000	-1.9407	1.1048
		4a	-.45000	.74889	1.000	-2.4890	1.5890
		4c	.01667	.83728	1.000	-2.2630	2.2964
	3a	1	.41795	.55926	1.000	-1.1048	1.9407
		4a	-.03205	.55926	1.000	-1.5548	1.4907
		4c	.43462	.67304	1.000	-1.3979	2.2671
	4a	1	.45000	.74889	1.000	-1.5890	2.4890
		3a	.03205	.55926	1.000	-1.4907	1.5548
		4c	.46667	.83728	1.000	-1.8130	2.7464
	4c	1	-.01667	.83728	1.000	-2.2964	2.2630
		3a	-.43462	.67304	1.000	-2.2671	1.3979
		4a	-.46667	.83728	1.000	-2.7464	1.8130
Week2	1	3a	-.64231	.65434	1.000	-2.4239	1.1393
		4a	-.18333	.87621	1.000	-2.5690	2.2023
		4c	-.35000	.97963	1.000	-3.0173	2.3173
	3a	1	.64231	.65434	1.000	-1.1393	2.4239
		4a	.45897	.65434	1.000	-1.3226	2.2406
		4c	.29231	.78746	1.000	-1.8517	2.4364
	4a	1	.18333	.87621	1.000	-2.2023	2.5690
		3a	-.45897	.65434	1.000	-2.2406	1.3226
		4c	-.16667	.97963	1.000	-2.8339	2.5006
	4c	1	.35000	.97963	1.000	-2.3173	3.0173
		3a	-.29231	.78746	1.000	-2.4364	1.8517
		4a	.16667	.97963	1.000	-2.5006	2.8339
Week3	1	3a	-.72372	.72994	1.000	-2.7112	1.2637
		4a	-.45000	.97744	1.000	-3.1113	2.2113
		4c	-.10833	1.09281	1.000	-3.0838	2.8671
	3a	1	.72372	.72994	1.000	-1.2637	2.7112
		4a	.27372	.72994	1.000	-1.7137	2.2612
		4c	.61538	.87844	1.000	-1.7764	3.0072
	4a	1	.45000	.97744	1.000	-2.2113	3.1113
		3a	-.27372	.72994	1.000	-2.2612	1.7137
		4c	.34167	1.09281	1.000	-2.6338	3.3171
	4c	1	.10833	1.09281	1.000	-2.8671	3.0838
		3a	-.61538	.87844	1.000	-3.0072	1.7764
		4a	-.34167	1.09281	1.000	-3.3171	2.6338
Week4	1	3a	-1.31987	.80309	.631	-3.5065	.8667
		4a	-.53333	1.07540	1.000	-3.4614	2.3947
		4c	.01667	1.20233	1.000	-3.2570	3.2903
	3a	1	1.31987	.80309	.631	-.8667	3.5065
		4a	.78654	.80309	1.000	-1.4001	2.9732
		4c	1.33654	.96648	1.000	-1.2949	3.9680

	4a	1	.53333	1.07540	1.000	-2.3947	3.4614
		3a	-.78654	.80309	1.000	-2.9732	1.4001
		4c	.55000	1.20233	1.000	-2.7236	3.8236
	4c	1	-.01667	1.20233	1.000	-3.2903	3.2570
		3a	-1.33654	.96648	1.000	-3.9680	1.2949
		4a	-.55000	1.20233	1.000	-3.8236	2.7236

3.6 Glycoprotein O

Weeks		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
						Lower Bound	Upper Bound	
1	1a	17	2.7941	1.13714	.27580	2.2095	3.3788	1.40
	1c	42	3.0667	1.17487	.18129	2.7006	3.4328	.90
	4	6	2.3000	.40000	.16330	1.8802	2.7198	1.90
	Total	65	2.9246	1.12889	.14002	2.6449	3.2043	.90
2	1a	17	3.8941	1.35714	.32916	3.1963	4.5919	1.50
	1c	42	3.9619	1.52171	.23480	3.4877	4.4361	1.80
	4	6	3.7000	.78486	.32042	2.8763	4.5237	2.80
	Total	65	3.9200	1.41346	.17532	3.5698	4.2702	1.50
3	1a	17	4.9353	1.69667	.41150	4.0629	5.8076	1.50
	1c	42	5.3119	1.53356	.23663	4.8340	5.7898	2.10
	4	6	5.2500	.54681	.22323	4.6762	5.8238	4.40
	Total	65	5.2077	1.50886	.18715	4.8338	5.5816	1.50
4	1a	17	5.6235	1.79462	.43526	4.7008	6.5462	3.50
	1c	42	6.4595	1.71209	.26418	5.9260	6.9930	3.40
	4	6	5.4667	.85479	.34897	4.5696	6.3637	4.40
	Total	65	6.1492	1.70890	.21196	5.7258	6.5727	3.40

ANOVA						
Weeks		Sum of Squares	df	Mean Square	F	Sig.
1	Between Groups	3.478	2	1.739	1.381	.259
	Within Groups	78.083	62	1.259		
	Total	81.561	64			
2	Between Groups	.376	2	.188	.091	.913
	Within Groups	127.488	62	2.056		
	Total	127.864	64			
3	Between Groups	1.728	2	.864	.372	.691
	Within Groups	143.978	62	2.322		
	Total	145.706	64			
4	Between Groups	11.537	2	5.769	2.040	.139
	Within Groups	175.365	62	2.828		
	Total	186.902	64			

Multiple Comparisons							
Post hoc test: Bonferroni							
Dependent Variable	(I) gO.r	(J) gO.r	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Week1	1a	1c	-.27255	.32260	1.000	-1.0664	.5213
		4	.49412	.53290	1.000	-.8172	1.8054
	1c	1a	.27255	.32260	1.000	-.5213	1.0664
		4	.76667	.48978	.368	-.4385	1.9719
	4	1a	-.49412	.53290	1.000	-1.8054	.8172
		1c	-.76667	.48978	.368	-1.9719	.4385
Week2	1a	1c	-.06779	.41221	1.000	-1.0821	.9465
		4	.19412	.68093	1.000	-1.4814	1.8697

	1c	1a	.06779	.41221	1.000	-.9465	1.0821
		4	.26190	.62584	1.000	-1.2781	1.8019
	4	1a	-.19412	.68093	1.000	-1.8697	1.4814
		1c	-.26190	.62584	1.000	-1.8019	1.2781
Week3	1a	1c	-.37661	.43806	1.000	-1.4545	.7013
		4	-.31471	.72363	1.000	-2.0953	1.4659
	1c	1a	.37661	.43806	1.000	-.7013	1.4545
		4	.06190	.66508	1.000	-1.5746	1.6984
	4	1a	.31471	.72363	1.000	-1.4659	2.0953
		1c	-.06190	.66508	1.000	-1.6984	1.5746
Week4	1a	1c	-.83599	.48345	.266	-2.0256	.3536
		4	.15686	.79862	1.000	-1.8083	2.1220
	1c	1a	.83599	.48345	.266	-.3536	2.0256
		4	.99286	.73400	.543	-.8133	2.7990
	4	1a	-.15686	.79862	1.000	-2.1220	1.8083
		1c	-.99286	.73400	.543	-2.7990	.8133

Appendix 4: Tables of One-way ANOVA results for the relation between HCMV growth behaviour and the sample's infection category.

Weeks		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
						Lower Bound	Upper Bound	
1	congenital infection	12	3.5083	1.61946	.46750	2.4794	4.5373	.90
	Not known infection	5	3.3800	.99096	.44317	2.1496	4.6104	2.40
	Primary from Immunocompetent	7	3.0714	1.19124	.45025	1.9697	4.1731	1.10
	Primary from immunocompromised	26	2.6385	1.01314	.19869	2.2292	3.0477	1.40
	Recurrent Infections	35	3.2514	1.28186	.21667	2.8111	3.6918	1.40
	Total	85	3.0929	1.25146	.13574	2.8230	3.3629	.90
2	congenital infection	12	5.4417	1.45880	.42112	4.5148	6.3685	2.40
	Not known infection	5	4.7400	1.80083	.80536	2.5040	6.9760	2.30
	Primary from Immunocompetent	7	3.8000	1.30384	.49281	2.5941	5.0059	2.00
	Primary from immunocompromized	26	3.4808	1.24901	.24495	2.9763	3.9853	1.50
	Recurrent Infections	35	4.0514	1.43923	.24327	3.5570	4.5458	2.00
	Total	85	4.0929	1.50529	.16327	3.7683	4.4176	1.50
3	congenital infection	12	8.0833	1.57471	.45458	7.0828	9.0839	5.00
	Not known infection	5	6.2000	.84558	.37815	5.1501	7.2499	5.40
	Primary from Immunocompetent	7	5.3000	1.07238	.40532	4.3082	6.2918	3.80
	Primary from immune compromised	26	4.8269	1.38031	.27070	4.2694	5.3844	1.50
	Recurrent Infections	35	5.3714	1.53902	.26014	4.8428	5.9001	2.10
	Total	85	5.6306	1.75567	.19043	5.2519	6.0093	1.50
4	congenital infection	12	9.2833	.79296	.22891	8.7795	9.7872	7.00

	Not known infection	5	7.0600	1.45705	.65161	5.2508	8.8692	6.00
	Primary from Immunocompetent	7	5.8143	.50474	.19077	5.3475	6.2811	5.00
	Primary from immunocompromized	26	5.3308	.92985	.18236	4.9552	5.7063	3.50
	Recurrent Infections	35	6.4229	1.76403	.29818	5.8169	7.0288	3.40
	Total	85	6.4800	1.81887	.19728	6.0877	6.8723	3.40

ANOVA

Weeks		Sum of Squares	df	Mean Square	F	Sig.
Week 1	Between Groups	8.735	4	2.184	1.422	.234
	Within Groups	122.820	80	1.535		
	Total	131.556	84			
Week 2	Between Groups	34.327	4	8.582	4.401	.003
	Within Groups	156.009	80	1.950		
	Total	190.336	84			
Week 3	Between Groups	93.721	4	23.430	11.346	.000
	Within Groups	165.199	80	2.065		
	Total	258.920	84			
Week 4	Between Groups	133.542	4	33.385	18.502	.000
	Within Groups	144.354	80	1.804		
	Total	277.896	84			

Multiple Comparisons

Post hoc test: Bonferroni

Dependent Variable	(I) infectiontypewithnotknown.r	(J) infectiontypewithnotknown.r	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Week1	congenital infection	Not known infection	.12833	.65954	1.000	-1.7757	2.0324
		Primary from Immunocompetent	.43690	.58929	1.000	-1.2644	2.1382
		Primary from immunocompromized	.86987	.43242	.476	-.3785	2.1183
		Recurrent Infections	.25690	.41449	1.000	-.9397	1.4535
	Not known infection	congenital infection	-.12833	.65954	1.000	-2.0324	1.7757
		Primary from Immunocompetent	.30857	.72552	1.000	-1.7860	2.4031
		Primary from immunocompromized	.74154	.60506	1.000	-1.0053	2.4883
		Recurrent Infections	.12857	.59238	1.000	-1.5816	1.8388
	Primary from Immunocompetent	congenital infection	-.43690	.58929	1.000	-2.1382	1.2644
		Not known infection	-.30857	.72552	1.000	-2.4031	1.7860
		Primary from immunocompromized	.43297	.52761	1.000	-1.0902	1.9562
		Recurrent Infections	-.18000	.51302	1.000	-1.6611	1.3011
	Primary from immunocompromized	congenital infection	-.86987	.43242	.476	-2.1183	.3785
		Not known	-.74154	.6050	1.00	-2.4883	1.0053

		infection		6	0		
		Primary from Immunocompetent	-.43297	.52761	1.000	-1.9562	1.0902
		Recurrent Infections	-.61297	.32080	.596	-1.5391	.3132
	Recurrent Infections	congenital infection	-.25690	.41449	1.000	-1.4535	.9397
		Not known infection	-.12857	.59238	1.000	-1.8388	1.5816
		Primary from Immunocompetent	.18000	.51302	1.000	-1.3011	1.6611
		Primary from immunocompromized	.61297	.32080	.596	-.3132	1.5391
Week2	congenital infection	Not known infection	.70167	.74332	1.000	-1.4443	2.8476
		Primary from Immunocompetent	1.64167	.66415	.156	-.2757	3.5591
		Primary from immunocompromized	1.96090*	.48735	.001	.5539	3.3679
		Recurrent Infections	1.39024*	.46715	.039	.0416	2.7389
	Not known infection	congenital infection	-.70167	.74332	1.000	-2.8476	1.4443
		Primary from Immunocompetent	.94000	.81769	1.000	-1.4206	3.3006
		Primary from immunocompromized	1.25923	.68193	.685	-.7095	3.2279
		Recurrent Infections	.68857	.66764	1.000	-1.2389	2.6160
	Primary from Immunocompetent	congenital infection	-1.64167	.66415	.156	-3.5591	.2757
		Not known infection	-.94000	.81769	1.000	-3.3006	1.4206
		Primary from immunocompromized	.31923	.59464	1.000	-1.3975	2.0359
		Recurrent Infections	-.25143	.57819	1.000	-1.9207	1.4178
	Primary from immunocompromized	congenital infection	-1.96090*	.48735	.001	-3.3679	-.5539
		Not known infection	-1.25923	.68193	.685	-3.2279	.7095
		Primary from Immunocompetent	-.31923	.59464	1.000	-2.0359	1.3975
		Recurrent Infections	-.57066	.36155	1.000	-1.6145	.4731
	Recurrent Infections	congenital infection	-1.39024*	.46715	.039	-2.7389	-.0416
		Not known infection	-.68857	.66764	1.000	-2.6160	1.2389
		Primary from Immunocompetent	.25143	.57819	1.000	-1.4178	1.9207
		Primary from immunocompromized	.57066	.36155	1.000	-.4731	1.6145
Week3	congenital infection	Not known infection	1.88333	.76491	.160	-.3249	4.0916
		Primary from Immunocompetent	2.78333*	.68343	.001	.8103	4.7564
		Primary from immunocompromized	3.25641*	.50150	.000	1.8086	4.7042
		Recurrent Infections	2.71190*	.48071	.000	1.3241	4.0997
	Not known infection	congenital infection	-1.88333	.76491	.160	-4.0916	.3249
		Primary from Immunocompetent	.90000	.84143	1.000	-1.5292	3.3292
		Primary from immunocompromized	1.37308	.70173	.539	-.6528	3.3989
		Recurrent	.82857	.6870	1.00	-1.1548	2.8120

		Infections		2	0		
	Primary from Immunocompetent	congenital infection	-2.78333*	.68343	.001	-4.7564	-.8103
		Not known infection	-.90000	.84143	1.000	-3.3292	1.5292
		Primary from immunocompromized	.47308	.61190	1.000	-1.2935	2.2396
		Recurrent Infections	-.07143	.59498	1.000	-1.7891	1.6463
	Primary from immunocompromized	congenital infection	-3.25641*	.50150	.000	-4.7042	-1.8086
		Not known infection	-1.37308	.70173	.539	-3.3989	.6528
		Primary from Immunocompetent	-.47308	.61190	1.000	-2.2396	1.2935
		Recurrent Infections	-.54451	.37205	1.000	-1.6186	.5296
	Recurrent Infections	congenital infection	-2.71190*	.48071	.000	-4.0997	-1.3241
		Not known infection	-.82857	.68702	1.000	-2.8120	1.1548
		Primary from Immunocompetent	.07143	.59498	1.000	-1.6463	1.7891
		Primary from immunocompromized	.54451	.37205	1.000	-.5296	1.6186
Week4	congenital infection	Not known infection	2.22333*	.71502	.026	.1591	4.2876
		Primary from Immunocompetent	3.46905*	.63886	.000	1.6247	5.3134
		Primary from immunocompromized	3.95256*	.46880	.000	2.5992	5.3060
		Recurrent Infections	2.86048*	.44936	.000	1.5632	4.1578
	Not known infection	congenital infection	-2.22333*	.71502	.026	-4.2876	-.1591
		Primary from Immunocompetent	1.24571	.78655	1.000	-1.0250	3.5165
		Primary from immunocompromized	1.72923	.65596	.101	-.1645	3.6230
		Recurrent Infections	.63714	.64222	1.000	-1.2169	2.4912
	Primary from Immunocompetent	congenital infection	-3.46905*	.63886	.000	-5.3134	-1.6247
		Not known infection	-1.24571	.78655	1.000	-3.5165	1.0250
		Primary from immunocompromized	.48352	.57199	1.000	-1.1678	2.1348
		Recurrent Infections	-.60857	.55618	1.000	-2.2142	.9971
	Primary from immunocompromized	congenital infection	-3.95256*	.46880	.000	-5.3060	-2.5992
		Not known infection	-1.72923	.65596	.101	-3.6230	.1645
		Primary from Immunocompetent	-.48352	.57199	1.000	-2.1348	1.1678
		Recurrent Infections	-1.09209*	.34779	.024	-2.0961	-.0880
	Recurrent Infections	congenital infection	-2.86048*	.44936	.000	-4.1578	-1.5632
		Not known infection	-.63714	.64222	1.000	-2.4912	1.2169
		Primary from Immunocompetent	.60857	.55618	1.000	-.9971	2.2142
		Primary from immunocompromized	1.09209*	.34779	.024	.0880	2.0961

Appendix 5: Tables of One-way ANOVA results for the relation between HCMV growth behaviour and the sample's specimen type.

Dependent Variable	(I) spec.type.r	(J) spec.type.r	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval
						Lower Bound
Week1	respiratory	blood	-.58247	.63715	1.000	-2.1382
		Urine	-.07500	.76083	1.000	-1.9327
	blood	respiratory	.58247	.63715	1.000	-.9733
		Urine	.50747	.46152	.824	-.6194
	Urine	respiratory	.07500	.76083	1.000	-1.7827
		blood	-.50747	.46152	.824	-1.6344
Week2	respiratory	blood	-.88117	.77135	.769	-2.7646
		Urine	-1.96250	.92108	.108	-4.2115
	blood	respiratory	.88117	.77135	.769	-1.0022
		Urine	-1.08133	.55873	.169	-2.4456
	Urine	respiratory	1.96250	.92108	.108	-.2865
		blood	1.08133	.55873	.169	-.2829
Week3	respiratory	blood	.10812	.91130	1.000	-2.1170
		Urine	-1.05000	1.08820	1.000	-3.7071
	blood	respiratory	-.10812	.91130	1.000	-2.3332
		Urine	-1.15812	.66010	.249	-2.7699
	Urine	respiratory	1.05000	1.08820	1.000	-1.6071
		blood	1.15812	.66010	.249	-.4537
Week4	respiratory	blood	.59221	.89906	1.000	-1.6030
		Urine	-1.48750	1.07358	.508	-4.1089
	blood	respiratory	-.59221	.89906	1.000	-2.7874
		Urine	-2.07971*	.65124	.006	-3.6698
	Urine	respiratory	1.48750	1.07358	.508	-1.1339
		blood	2.07971*	.65124	.006	.4896

ANOVA

Weeks		Sum of Squares	df	Mean Square	F	Sig.
1	Between Groups	2.959	2	1.479	.958	.388
	Within Groups	132.754	86	1.544		
	Total	135.712	88			
2	Between Groups	12.165	2	6.082	2.689	.074
	Within Groups	194.564	86	2.262		
	Total	206.729	88			
3	Between Groups	9.720	2	4.860	1.539	.220
	Within Groups	271.571	86	3.158		
	Total	281.291	88			
4	Between Groups	31.945	2	15.973	5.197	.007
	Within Groups	264.324	86	3.074		
	Total	296.269	88			

Post hoc test: Bonferroni

Dependent Variable	(I) spec.type.r	(J) spec.type.r	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval
						Lower Bound
Week1	respiratory	blood	-.58247	.63715	1.000	-2.1382
		Urine	-.07500	.76083	1.000	-1.9327
	blood	respiratory	.58247	.63715	1.000	-.9733
		Urine	.50747	.46152	.824	-.6194
	Urine	respiratory	.07500	.76083	1.000	-1.7827
		blood	-.50747	.46152	.824	-1.6344
Week2	respiratory	blood	-.88117	.77135	.769	-2.7646
		Urine	-1.96250	.92108	.108	-4.2115
	blood	respiratory	.88117	.77135	.769	-1.0022
		Urine	-1.08133	.55873	.169	-2.4456
	Urine	respiratory	1.96250	.92108	.108	-.2865
		blood	1.08133	.55873	.169	-.2829
Week3	respiratory	blood	.10812	.91130	1.000	-2.1170
		Urine	-1.05000	1.08820	1.000	-3.7071
	blood	respiratory	-.10812	.91130	1.000	-2.3332

		Urine	-1.15812	.66010	.249	-2.7699
	Urine	respiratory	1.05000	1.08820	1.000	-1.6071
		blood	1.15812	.66010	.249	-.4537
Week4	respiratory	blood	.59221	.89906	1.000	-1.6030
		Urine	-1.48750	1.07358	.508	-4.1089
	blood	respiratory	-.59221	.89906	1.000	-2.7874
		Urine	-2.07971*	.65124	.006	-3.6698
	Urine	respiratory	1.48750	1.07358	.508	-1.1339
		blood	2.07971*	.65124	.006	.4896

Appendix 6: Tables of One-way ANOVA results for the relation between HCMV glycoprotein genotypes and the glycosylation of the glycoproteins.

6.1 Lectins' identification number in the following ANOVA Tables

Lectins order	Lectins Abbreviation
Lectin 1	SBA
Lectin 2	ECL
Lectin 3	LCA
Lectin 4	WGA
Lectin 5	GSL II
Lectin 6	EBL
Lectin 7	PHA-L
Lectin 8	GNL
Lectin 9	LEL
Lectin 10	BPL
Lectin 11	UEA
Lectin 12	EEL
Lectin 13	WFA
Lectin 14	PHA-E
Lectin 15	ALL
Lectin 16	PTL
Lectin 17	MAA II
Lectin 18	AAL
Lectin 19	GSL-I
Lectin 20	PSA

6.2 Glycoprotein B

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Lectin1b	1.00	35	.2154	.08396	.01419	.1866	.2443	.10	.45
	2.00	19	.2111	.09746	.02236	.1641	.2580	.09	.42
	3.00	19	.2116	.17836	.04092	.1256	.2975	.09	.88
	4.00	11	.2636	.11775	.03550	.1845	.3427	.11	.42
	Total	84	.2199	.11753	.01282	.1944	.2454	.09	.88
Lectin2b	1.00	35	.5751	.21116	.03569	.5026	.6477	.33	1.17
	2.00	19	.5700	.17211	.03948	.4870	.6530	.33	1.01
	3.00	19	.4995	.14860	.03409	.4278	.5711	.29	.80
	4.00	11	.5609	.23956	.07223	.4000	.7218	.20	1.01

	Total	84	.5550	.19320	.02108	.5131	.5969	.20	1.17
Lectin3b	1.00	35	.6903	.18163	.03070	.6279	.7527	.34	1.08
	2.00	19	.7232	.25353	.05816	.6010	.8454	.32	1.22
	3.00	19	.6263	.27562	.06323	.4935	.7592	.35	1.48
	4.00	11	.8000	.38709	.11671	.5399	1.0601	.39	1.47
	Total	84	.6976	.25438	.02775	.6424	.7528	.32	1.48
Lectin4b	1.00	35	.7483	.24571	.04153	.6639	.8327	.36	1.40
	2.00	19	.7821	.28936	.06638	.6426	.9216	.40	1.20
	3.00	19	.7163	.42759	.09810	.5102	.9224	.30	2.27
	4.00	11	.7882	.33283	.10035	.5646	1.0118	.41	1.48
	Total	84	.7539	.31074	.03390	.6865	.8214	.30	2.27
Lectin5b	1.00	35	.2360	.14093	.02382	.1876	.2844	.08	.78
	2.00	19	.1711	.06082	.01395	.1417	.2004	.09	.31
	3.00	19	.2547	.27573	.06326	.1218	.3876	.07	1.20
	4.00	11	.2082	.09704	.02926	.1430	.2734	.08	.32
	Total	84	.2219	.16585	.01810	.1859	.2579	.07	1.20
Lectin6b	1.00	35	.8360	.27021	.04567	.7432	.9288	.43	1.57
	2.00	19	.8884	.31707	.07274	.7356	1.0412	.44	1.44
	3.00	19	.8068	.35774	.08207	.6344	.9793	.35	1.57
	4.00	11	.8236	.26526	.07998	.6454	1.0018	.50	1.25
	Total	84	.8396	.29795	.03251	.7750	.9043	.35	1.57
Lectin7b	1.00	35	1.0409	.45644	.07715	.8841	1.1976	.17	1.78
	2.00	19	1.2332	.55710	.12781	.9646	1.5017	.37	2.50
	3.00	19	.8232	.44608	.10234	.6082	1.0382	.18	1.91
	4.00	11	1.2436	.57423	.17314	.8579	1.6294	.64	2.41
	Total	84	1.0617	.50976	.05562	.9510	1.1723	.17	2.50
Lectin8b	1.00	35	.4337	.20037	.03387	.3649	.5025	.15	1.14
	2.00	19	.3779	.13256	.03041	.3140	.4418	.16	.58
	3.00	19	.3542	.13696	.03142	.2882	.4202	.12	.63
	4.00	11	.4273	.12305	.03710	.3446	.5099	.24	.64
	Total	84	.4023	.16527	.01803	.3664	.4381	.12	1.14
Lectin9b	1.00	35	.6331	.20665	.03493	.5622	.7041	.34	1.20
	2.00	19	.7374	.31425	.07209	.5859	.8888	.36	1.54
	3.00	19	.6711	.32195	.07386	.5159	.8262	.32	1.71
	4.00	11	.6836	.32647	.09844	.4643	.9030	.35	1.35
	Total	84	.6719	.27543	.03005	.6121	.7317	.32	1.71
Lectin10b	1.00	35	.6077	.24747	.04183	.5227	.6927	.15	1.26
	2.00	19	.6368	.19227	.04411	.5442	.7295	.26	.96
	3.00	19	.5747	.25123	.05764	.4536	.6958	.27	1.11
	4.00	11	.6609	.24672	.07439	.4952	.8267	.36	1.12
	Total	84	.6138	.23432	.02557	.5630	.6647	.15	1.26
Lectin11b	1.00	35	.2400	.13309	.02250	.1943	.2857	.12	.62
	2.00	19	.2268	.09328	.02140	.1819	.2718	.08	.46

	3.00	19	.2116	.11644	.02671	.1555	.2677	.09	.47
	4.00	11	.2782	.15138	.04564	.1765	.3799	.10	.58
	Total	84	.2356	.12346	.01347	.2088	.2624	.08	.62
Lectin12b	1.00	35	.1900	.12412	.02098	.1474	.2326	.07	.71
	2.00	19	.1589	.05311	.01219	.1333	.1845	.10	.26
	3.00	19	.1968	.29460	.06759	.0548	.3388	.07	1.39
	4.00	11	.1791	.08479	.02556	.1221	.2361	.09	.32
	Total	84	.1831	.16374	.01787	.1476	.2186	.07	1.39
Lectin13b	1.00	35	.4263	.13534	.02288	.3798	.4728	.24	.84
	2.00	19	.4068	.10889	.02498	.3544	.4593	.17	.65
	3.00	19	.4747	.46520	.10673	.2505	.6990	.23	2.35
	4.00	11	.4255	.11835	.03568	.3459	.5050	.29	.68
	Total	84	.4327	.24347	.02656	.3799	.4856	.17	2.35
Lectin14b	1.00	35	.5354	.19820	.03350	.4673	.6035	.26	.98
	2.00	19	.5442	.19219	.04409	.4516	.6368	.21	1.02
	3.00	19	.4958	.25994	.05963	.3705	.6211	.26	1.39
	4.00	11	.6164	.21082	.06356	.4747	.7580	.37	1.10
	Total	84	.5390	.21294	.02323	.4928	.5853	.21	1.39
Lectin15b	1.00	35	.6823	.29545	.04994	.5808	.7838	.11	1.30
	2.00	19	.6753	.18611	.04270	.5856	.7650	.39	1.06
	3.00	19	.6505	.26547	.06090	.5226	.7785	.33	1.47
	4.00	11	.7127	.27583	.08317	.5274	.8980	.39	1.31
	Total	84	.6775	.26088	.02846	.6209	.7341	.11	1.47
Lectin16b	1.00	35	.4017	.33969	.05742	.2850	.5184	.08	1.40
	2.00	19	.4363	.59946	.13753	.1474	.7252	.07	2.41
	3.00	19	.3084	.27540	.06318	.1757	.4412	.07	.79
	4.00	11	.2355	.23602	.07116	.0769	.3940	.07	.88
	Total	84	.3667	.39121	.04269	.2818	.4516	.07	2.41
Lectin17b	1.00	35	.7871	.22373	.03782	.7103	.8640	.40	1.20
	2.00	19	.9247	.34784	.07980	.7571	1.0924	.38	1.52
	3.00	19	.8568	.39169	.08986	.6681	1.0456	.41	2.15
	4.00	11	.8855	.29005	.08745	.6906	1.0803	.46	1.36
	Total	84	.8469	.30539	.03332	.7806	.9132	.38	2.15
Lectin18b	1.00	35	.7549	.30087	.05086	.6515	.8582	.22	1.91
	2.00	19	.8668	.30381	.06970	.7204	1.0133	.42	1.51
	3.00	19	.6842	.21337	.04895	.5814	.7871	.31	1.19
	4.00	11	.8982	.30948	.09331	.6903	1.1061	.46	1.44
	Total	84	.7830	.29059	.03171	.7199	.8460	.22	1.91
Lectin19b	1.00	35	.2751	.11014	.01862	.2373	.3130	.12	.57
	2.00	19	.2553	.07784	.01786	.2177	.2928	.11	.41
	3.00	19	.2621	.13049	.02994	.1992	.3250	.08	.57
	4.00	11	.2609	.11104	.03348	.1863	.3355	.12	.46

	Total	84	.2658	.10738	.01172	.2425	.2891	.08	.57
Lectin20b	1.00	35	.4971	.15487	.02618	.4439	.5503	.14	.84
	2.00	19	.5589	.22306	.05117	.4514	.6665	.25	1.14
	3.00	19	.4911	.27610	.06334	.3580	.6241	.15	1.44
	4.00	11	.5764	.19906	.06002	.4426	.7101	.32	1.00
	Total	84	.5201	.20761	.02265	.4751	.5652	.14	1.44

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lectin1b	Between Groups	.025	3	.008	.583	.628
	Within Groups	1.122	80	.014		
	Total	1.146	83			
Lectin2b	Between Groups	.077	3	.026	.684	.565
	Within Groups	3.021	80	.038		
	Total	3.098	83			
Lectin3b	Between Groups	.226	3	.075	1.172	.326
	Within Groups	5.145	80	.064		
	Total	5.371	83			
Lectin4b	Between Groups	.056	3	.019	.188	.905
	Within Groups	7.959	80	.099		
	Total	8.015	83			
Lectin5b	Between Groups	.079	3	.026	.951	.420
	Within Groups	2.204	80	.028		
	Total	2.283	83			
Lectin6b	Between Groups	.069	3	.023	.252	.860
	Within Groups	7.299	80	.091		
	Total	7.368	83			
Lectin7b	Between Groups	2.019	3	.673	2.754	.048
	Within Groups	19.549	80	.244		
	Total	21.568	83			
Lectin8b	Between Groups	.097	3	.032	1.188	.320
	Within Groups	2.170	80	.027		
	Total	2.267	83			
Lectin9b	Between Groups	.136	3	.045	.587	.626
	Within Groups	6.161	80	.077		
	Total	6.297	83			
Lectin10b	Between Groups	.065	3	.022	.385	.764
	Within Groups	4.492	80	.056		
	Total	4.557	83			
Lectin11b	Between Groups	.033	3	.011	.715	.546
	Within Groups	1.232	80	.015		
	Total	1.265	83			
Lectin12b	Between Groups	.017	3	.006	.199	.897
	Within Groups	2.209	80	.028		
	Total	2.225	83			
Lectin13b	Between Groups	.048	3	.016	.264	.851
	Within Groups	4.872	80	.061		
	Total	4.920	83			
Lectin14b	Between Groups	.102	3	.034	.745	.528
	Within Groups	3.661	80	.046		
	Total	3.764	83			
Lectin15b	Between Groups	.028	3	.009	.135	.939
	Within Groups	5.621	80	.070		
	Total	5.649	83			
Lectin16b	Between Groups	.389	3	.130	.842	.475
	Within Groups	12.314	80	.154		
	Total	12.703	83			

Lectin17b	Between Groups	.258	3	.086	.921	.435
	Within Groups	7.483	80	.094		
	Total	7.741	83			
Lectin18b	Between Groups	.493	3	.164	2.016	.118
	Within Groups	6.516	80	.081		
	Total	7.009	83			
Lectin19b	Between Groups	.006	3	.002	.159	.923
	Within Groups	.951	80	.012		
	Total	.957	83			
Lectin20b	Between Groups	.098	3	.033	.751	.525
	Within Groups	3.480	80	.043		
	Total	3.577	83			

Multiple Comparisons

Post hoc test: Bonferroni

Dependent Variable	(I) gB.r	(J) gB.r	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Lectin1b	1.00	2.00	.00438	.03375	1.000	-.0869	.0957
		3.00	.00385	.03375	1.000	-.0874	.0951
		4.00	-.04821	.04093	1.000	-.1590	.0625
	2.00	1.00	-.00438	.03375	1.000	-.0957	.0869
		3.00	-.00053	.03842	1.000	-.1045	.1034
		4.00	-.05258	.04487	1.000	-.1740	.0688
	3.00	1.00	-.00385	.03375	1.000	-.0951	.0874
		2.00	.00053	.03842	1.000	-.1034	.1045
		4.00	-.05206	.04487	1.000	-.1734	.0693
	4.00	1.00	.04821	.04093	1.000	-.0625	.1590
		2.00	.05258	.04487	1.000	-.0688	.1740
		3.00	.05206	.04487	1.000	-.0693	.1734
Lectin2b	1.00	2.00	.00514	.05537	1.000	-.1447	.1549
		3.00	.07567	.05537	1.000	-.0741	.2255
		4.00	.01423	.06717	1.000	-.1675	.1959
	2.00	1.00	-.00514	.05537	1.000	-.1549	.1447
		3.00	.07053	.06304	1.000	-.1000	.2411
		4.00	.00909	.07362	1.000	-.1901	.2083
	3.00	1.00	-.07567	.05537	1.000	-.2255	.0741
		2.00	-.07053	.06304	1.000	-.2411	.1000
		4.00	-.06144	.07362	1.000	-.2606	.1377
	4.00	1.00	-.01423	.06717	1.000	-.1959	.1675
		2.00	-.00909	.07362	1.000	-.2083	.1901
		3.00	.06144	.07362	1.000	-.1377	.2606
Lectin3b	1.00	2.00	-.03287	.07226	1.000	-.2284	.1626
		3.00	.06397	.07226	1.000	-.1315	.2595
		4.00	-.10971	.08766	1.000	-.3469	.1274
	2.00	1.00	.03287	.07226	1.000	-.1626	.2284
		3.00	.09684	.08227	1.000	-.1257	.3194
		4.00	-.07684	.09608	1.000	-.3368	.1831
	3.00	1.00	-.06397	.07226	1.000	-.2595	.1315
		2.00	-.09684	.08227	1.000	-.3194	.1257
		4.00	-.17368	.09608	.446	-.4336	.0862
	4.00	1.00	.10971	.08766	1.000	-.1274	.3469
		2.00	.07684	.09608	1.000	-.1831	.3368
		3.00	.17368	.09608	.446	-.0862	.4336
Lectin4b	1.00	2.00	-.03382	.08988	1.000	-.2770	.2093
		3.00	.03197	.08988	1.000	-.2112	.2751
		4.00	-.03990	.10902	1.000	-.3349	.2551
	2.00	1.00	.03382	.08988	1.000	-.2093	.2770
		3.00	.06579	.10233	1.000	-.2111	.3426
		4.00	-.00608	.11950	1.000	-.3294	.3172
	3.00	1.00	-.03197	.08988	1.000	-.2751	.2112
		2.00	-.06579	.10233	1.000	-.3426	.2111
		4.00	-.07187	.11950	1.000	-.3952	.2514
	4.00	1.00	.03990	.10902	1.000	-.2551	.3349
		2.00	.00608	.11950	1.000	-.3172	.3294
		3.00	.07187	.11950	1.000	-.2514	.3952
Lectin5b	1.00	2.00	.06495	.04730	1.000	-.0630	.1929
		3.00	-.01874	.04730	1.000	-.1467	.1092
		4.00	.02782	.05738	1.000	-.1274	.1831
	2.00	1.00	-.06495	.04730	1.000	-.1929	.0630
		3.00	-.08368	.05386	.745	-.2294	.0620
		4.00	-.03713	.06289	1.000	-.2073	.1330

	3.00	1.00	.01874	.04730	1.000	-.1092	.1467
		2.00	.08368	.05386	.745	-.0620	.2294
		4.00	.04656	.06289	1.000	-.1236	.2167
	4.00	1.00	-.02782	.05738	1.000	-.1831	.1274
		2.00	.03713	.06289	1.000	-.1330	.2073
		3.00	-.04656	.06289	1.000	-.2167	.1236
Lectin6b	1.00	2.00	-.05242	.08608	1.000	-.2853	.1805
		3.00	.02916	.08608	1.000	-.2037	.2620
		4.00	.01236	.10441	1.000	-.2701	.2948
	2.00	1.00	.05242	.08608	1.000	-.1805	.2853
		3.00	.08158	.09800	1.000	-.1836	.3467
		4.00	.06478	.11444	1.000	-.2448	.3744
	3.00	1.00	-.02916	.08608	1.000	-.2620	.2037
		2.00	-.08158	.09800	1.000	-.3467	.1836
		4.00	-.01679	.11444	1.000	-.3264	.2928
	4.00	1.00	-.01236	.10441	1.000	-.2948	.2701
		2.00	-.06478	.11444	1.000	-.3744	.2448
		3.00	.01679	.11444	1.000	-.2928	.3264
Lectin7b	1.00	2.00	-.19230	.14087	1.000	-.5734	.1888
		3.00	.21770	.14087	.757	-.1634	.5988
		4.00	-.20278	.17087	1.000	-.6651	.2595
	2.00	1.00	.19230	.14087	1.000	-.1888	.5734
		3.00	.41000	.16038	.075	-.0239	.8439
		4.00	-.01048	.18729	1.000	-.5172	.4962
	3.00	1.00	-.21770	.14087	.757	-.5988	.1634
		2.00	-.41000	.16038	.075	-.8439	.0239
		4.00	-.42048	.18729	.165	-.9272	.0862
	4.00	1.00	.20278	.17087	1.000	-.2595	.6651
		2.00	.01048	.18729	1.000	-.4962	.5172
		3.00	.42048	.18729	.165	-.0862	.9272
Lectin8b	1.00	2.00	.05582	.04694	1.000	-.0712	.1828
		3.00	.07950	.04694	.565	-.0475	.2065
		4.00	.00644	.05693	1.000	-.1476	.1605
	2.00	1.00	-.05582	.04694	1.000	-.1828	.0712
		3.00	.02368	.05344	1.000	-.1209	.1683
		4.00	-.04938	.06240	1.000	-.2182	.1195
	3.00	1.00	-.07950	.04694	.565	-.2065	.0475
		2.00	-.02368	.05344	1.000	-.1683	.1209
		4.00	-.07306	.06240	1.000	-.2419	.0958
	4.00	1.00	-.00644	.05693	1.000	-.1605	.1476
		2.00	.04938	.06240	1.000	-.1195	.2182
		3.00	.07306	.06240	1.000	-.0958	.2419
Lectin9b	1.00	2.00	-.10423	.07908	1.000	-.3182	.1097
		3.00	-.03791	.07908	1.000	-.2519	.1760
		4.00	-.05049	.09593	1.000	-.3100	.2090
	2.00	1.00	.10423	.07908	1.000	-.1097	.3182
		3.00	.06632	.09004	1.000	-.1773	.3099
		4.00	.05373	.10514	1.000	-.2307	.3382
	3.00	1.00	.03791	.07908	1.000	-.1760	.2519
		2.00	-.06632	.09004	1.000	-.3099	.1773
		4.00	-.01258	.10514	1.000	-.2970	.2719
	4.00	1.00	.05049	.09593	1.000	-.2090	.3100
		2.00	-.05373	.10514	1.000	-.3382	.2307
		3.00	.01258	.10514	1.000	-.2719	.2970
Lectin10b	1.00	2.00	-.02913	.06753	1.000	-.2118	.1536
		3.00	.03298	.06753	1.000	-.1497	.2157
		4.00	-.05319	.08191	1.000	-.2748	.1684
	2.00	1.00	.02913	.06753	1.000	-.1536	.2118
		3.00	.06211	.07688	1.000	-.1459	.2701
		4.00	-.02407	.08978	1.000	-.2670	.2188
	3.00	1.00	-.03298	.06753	1.000	-.2157	.1497
		2.00	-.06211	.07688	1.000	-.2701	.1459
		4.00	-.08617	.08978	1.000	-.3291	.1567
	4.00	1.00	.05319	.08191	1.000	-.1684	.2748
		2.00	.02407	.08978	1.000	-.2188	.2670
		3.00	.08617	.08978	1.000	-.1567	.3291
Lectin11b	1.00	2.00	.01316	.03536	1.000	-.0825	.1088
		3.00	.02842	.03536	1.000	-.0673	.1241
		4.00	-.03818	.04290	1.000	-.1542	.0779
	2.00	1.00	-.01316	.03536	1.000	-.1088	.0825
		3.00	.01526	.04026	1.000	-.0937	.1242
		4.00	-.05134	.04702	1.000	-.1785	.0759
	3.00	1.00	-.02842	.03536	1.000	-.1241	.0673
		2.00	-.01526	.04026	1.000	-.1242	.0937
		4.00	-.06660	.04702	.963	-.1938	.0606
	4.00	1.00	.03818	.04290	1.000	-.0779	.1542

		2.00	.05134	.04702	1.000	-.0759	.1785
		3.00	.06660	.04702	.963	-.0606	.1938
Lectin12b	1.00	2.00	.03105	.04735	1.000	-.0970	.1592
		3.00	-.00684	.04735	1.000	-.1349	.1213
		4.00	.01091	.05743	1.000	-.1445	.1663
	2.00	1.00	-.03105	.04735	1.000	-.1592	.0970
		3.00	-.03789	.05391	1.000	-.1837	.1080
		4.00	-.02014	.06295	1.000	-.1905	.1502
	3.00	1.00	.00684	.04735	1.000	-.1213	.1349
		2.00	.03789	.05391	1.000	-.1080	.1837
		4.00	.01775	.06295	1.000	-.1526	.1881
	4.00	1.00	-.01091	.05743	1.000	-.1663	.1445
		2.00	.02014	.06295	1.000	-.1502	.1905
		3.00	-.01775	.06295	1.000	-.1881	.1526
Lectin13b	1.00	2.00	.01944	.07032	1.000	-.1708	.2097
		3.00	-.04845	.07032	1.000	-.2387	.1418
		4.00	.00083	.08530	1.000	-.2299	.2316
	2.00	1.00	-.01944	.07032	1.000	-.2097	.1708
		3.00	-.06789	.08006	1.000	-.2845	.1487
		4.00	-.01861	.09349	1.000	-.2716	.2343
	3.00	1.00	.04845	.07032	1.000	-.1418	.2387
		2.00	.06789	.08006	1.000	-.1487	.2845
		4.00	.04928	.09349	1.000	-.2037	.3022
	4.00	1.00	-.00083	.08530	1.000	-.2316	.2299
		2.00	.01861	.09349	1.000	-.2343	.2716
		3.00	-.04928	.09349	1.000	-.3022	.2037
Lectin14b	1.00	2.00	-.00878	.06096	1.000	-.1737	.1561
		3.00	.03964	.06096	1.000	-.1253	.2046
		4.00	-.08094	.07395	1.000	-.2810	.1191
	2.00	1.00	.00878	.06096	1.000	-.1561	.1737
		3.00	.04842	.06941	1.000	-.1394	.2362
		4.00	-.07215	.08105	1.000	-.2914	.1471
	3.00	1.00	-.03964	.06096	1.000	-.2046	.1253
		2.00	-.04842	.06941	1.000	-.2362	.1394
		4.00	-.12057	.08105	.845	-.3399	.0987
	4.00	1.00	.08094	.07395	1.000	-.1191	.2810
		2.00	.07215	.08105	1.000	-.1471	.2914
		3.00	.12057	.08105	.845	-.0987	.3399
Lectin15b	1.00	2.00	.00702	.07553	1.000	-.1973	.2114
		3.00	.03176	.07553	1.000	-.1726	.2361
		4.00	-.03044	.09162	1.000	-.2783	.2174
	2.00	1.00	-.00702	.07553	1.000	-.2114	.1973
		3.00	.02474	.08600	1.000	-.2079	.2574
		4.00	-.03746	.10042	1.000	-.3092	.2342
	3.00	1.00	-.03176	.07553	1.000	-.2361	.1726
		2.00	-.02474	.08600	1.000	-.2574	.2079
		4.00	-.06220	.10042	1.000	-.3339	.2095
	4.00	1.00	.03044	.09162	1.000	-.2174	.2783
		2.00	.03746	.10042	1.000	-.2342	.3092
		3.00	.06220	.10042	1.000	-.2095	.3339
Lectin16b	1.00	2.00	-.03460	.11180	1.000	-.3371	.2679
		3.00	.09329	.11180	1.000	-.2092	.3958
		4.00	.16626	.13561	1.000	-.2006	.5332
	2.00	1.00	.03460	.11180	1.000	-.2679	.3371
		3.00	.12789	.12729	1.000	-.2165	.4723
		4.00	.20086	.14864	1.000	-.2013	.6030
	3.00	1.00	-.09329	.11180	1.000	-.3958	.2092
		2.00	-.12789	.12729	1.000	-.4723	.2165
		4.00	.07297	.14864	1.000	-.3292	.4751
	4.00	1.00	-.16626	.13561	1.000	-.5332	.2006
		2.00	-.20086	.14864	1.000	-.6030	.2013
		3.00	-.07297	.14864	1.000	-.4751	.3292
Lectin17b	1.00	2.00	-.13759	.08715	.710	-.3734	.0982
		3.00	-.06970	.08715	1.000	-.3055	.1661
		4.00	-.09831	.10571	1.000	-.3843	.1877
	2.00	1.00	.13759	.08715	.710	-.0982	.3734
		3.00	.06789	.09923	1.000	-.2006	.3363
		4.00	.03928	.11587	1.000	-.2742	.3528
	3.00	1.00	.06970	.08715	1.000	-.1661	.3055
		2.00	-.06789	.09923	1.000	-.3363	.2006
		4.00	-.02861	.11587	1.000	-.3421	.2849
	4.00	1.00	.09831	.10571	1.000	-.1877	.3843
		2.00	-.03928	.11587	1.000	-.3528	.2742
		3.00	.02861	.11587	1.000	-.2849	.3421

Lectin18b	1.00	2.00	-.11198	.08133	1.000	-.3320	.1080
		3.00	.07065	.08133	1.000	-.1494	.2907
		4.00	-.14332	.09865	.901	-.4102	.1236
	2.00	1.00	.11198	.08133	1.000	-.1080	.3320
		3.00	.18263	.09260	.312	-.0679	.4331
		4.00	-.03134	.10813	1.000	-.3239	.2612
	3.00	1.00	-.07065	.08133	1.000	-.2907	.1494
		2.00	-.18263	.09260	.312	-.4331	.0679
		4.00	-.21397	.10813	.308	-.5065	.0786
	4.00	1.00	.14332	.09865	.901	-.1236	.4102
		2.00	.03134	.10813	1.000	-.2612	.3239
		3.00	.21397	.10813	.308	-.0786	.5065
Lectin19b	1.00	2.00	.01988	.03108	1.000	-.0642	.1040
		3.00	.01304	.03108	1.000	-.0710	.0971
		4.00	.01423	.03769	1.000	-.0877	.1162
	2.00	1.00	-.01988	.03108	1.000	-.1040	.0642
		3.00	-.00684	.03538	1.000	-.1026	.0889
		4.00	-.00565	.04132	1.000	-.1174	.1061
	3.00	1.00	-.01304	.03108	1.000	-.0971	.0710
		2.00	.00684	.03538	1.000	-.0889	.1026
		4.00	.00120	.04132	1.000	-.1106	.1130
	4.00	1.00	-.01423	.03769	1.000	-.1162	.0877
		2.00	.00565	.04132	1.000	-.1061	.1174
		3.00	-.00120	.04132	1.000	-.1130	.1106
Lectin20b	1.00	2.00	-.06180	.05943	1.000	-.2226	.0990
		3.00	.00609	.05943	1.000	-.1547	.1669
		4.00	-.07922	.07209	1.000	-.2743	.1158
	2.00	1.00	.06180	.05943	1.000	-.0990	.2226
		3.00	.06789	.06766	1.000	-.1152	.2510
		4.00	-.01742	.07901	1.000	-.2312	.1964
	3.00	1.00	-.00609	.05943	1.000	-.1669	.1547
		2.00	-.06789	.06766	1.000	-.2510	.1152
		4.00	-.08531	.07901	1.000	-.2991	.1285
	4.00	1.00	.07922	.07209	1.000	-.1158	.2743
		2.00	.01742	.07901	1.000	-.1964	.2312
		3.00	.08531	.07901	1.000	-.1285	.2991

6.3 Glycoprotein H

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Lectin1b	Mixed	4	.1700	.02449	.01225	.1310	.2090	.14	.19
	1.00	44	.2241	.13939	.02101	.1817	.2665	.09	.88
	2.00	36	.2108	.08709	.01452	.1814	.2403	.09	.42
	Total	84	.2158	.11591	.01265	.1907	.2410	.09	.88
Lectin2b	Mixed	4	.6725	.27281	.13640	.2384	1.1066	.51	1.08
	1.00	44	.5627	.22912	.03454	.4931	.6324	.20	1.17
	2.00	36	.5314	.12401	.02067	.4894	.5733	.33	.83
	Total	84	.5545	.19316	.02108	.5126	.5964	.20	1.17
Lectin3b	Mixed	4	.7600	.18601	.09301	.4640	1.0560	.56	1.01
	1.00	44	.6811	.26241	.03956	.6014	.7609	.34	1.48
	2.00	36	.7203	.25920	.04320	.6326	.8080	.32	1.47
	Total	84	.7017	.25650	.02799	.6460	.7573	.32	1.48
Lectin4b	Mixed	4	.8975	.34558	.17279	.3476	1.4474	.61	1.40
	1.00	44	.7761	.35726	.05386	.6675	.8848	.36	2.27
	2.00	36	.7072	.23658	.03943	.6272	.7873	.30	1.20
	Total	84	.7524	.31023	.03385	.6851	.8197	.30	2.27
Lectin5b	Mixed	4	.4450	.51131	.25565	-.3686	1.2586	.12	1.20
	1.00	44	.2075	.12221	.01842	.1703	.2447	.08	.74
	2.00	36	.2114	.13624	.02271	.1653	.2575	.07	.78
	Total	84	.2205	.16604	.01812	.1844	.2565	.07	1.20
Lectin6b	Mixed	4	1.1125	.31532	.15766	.6108	1.6142	.65	1.36
	1.00	44	.7950	.29985	.04520	.7038	.8862	.35	1.57
	2.00	36	.8675	.28446	.04741	.7713	.9637	.44	1.51
	Total	84	.8412	.29879	.03260	.7763	.9060	.35	1.57

Lectin7b	Mixed	4	1.1800	.86753	.43376	-.2004	2.5604	.35	2.08
	1.00	44	1.0698	.48147	.07258	.9234	1.2162	.18	2.41
	2.00	36	1.0503	.53985	.08997	.8676	1.2329	.17	2.50
	Total	84	1.0667	.52051	.05679	.9537	1.1796	.17	2.50
Lectin8b	Mixed	4	.3125	.06702	.03351	.2059	.4191	.26	.41
	1.00	44	.3982	.13258	.01999	.3579	.4385	.16	.75
	2.00	36	.4056	.20565	.03428	.3360	.4751	.12	1.14
	Total	84	.3973	.16577	.01809	.3613	.4332	.12	1.14
Lectin9b	Mixed	4	.6300	.16432	.08216	.3685	.8915	.50	.87
	1.00	44	.6693	.31003	.04674	.5751	.7636	.32	1.71
	2.00	36	.6822	.24135	.04023	.6006	.7639	.36	1.54
	Total	84	.6730	.27472	.02997	.6134	.7326	.32	1.71
Lectin10b	Mixed	4	.6875	.40343	.20172	.0455	1.3295	.34	1.26
	1.00	44	.6445	.26160	.03944	.5650	.7241	.15	1.12
	2.00	36	.5647	.16838	.02806	.5078	.6217	.27	.95
	Total	84	.6124	.23473	.02561	.5614	.6633	.15	1.26
Lectin11b	Mixed	4	.2600	.20083	.10042	-.0596	.5796	.10	.54
	1.00	44	.2457	.12496	.01884	.2077	.2837	.08	.59
	2.00	36	.2181	.11600	.01933	.1788	.2573	.09	.62
	Total	84	.2345	.12425	.01356	.2076	.2615	.08	.62
Lectin12b	Mixed	4	.1750	.13102	.06551	-.0335	.3835	.09	.37
	1.00	44	.2095	.21167	.03191	.1452	.2739	.08	1.39
	2.00	36	.1472	.07094	.01182	.1232	.1712	.07	.38
	Total	84	.1812	.16396	.01789	.1456	.2168	.07	1.39
Lectin13b	Mixed	4	.4850	.23979	.11990	.1034	.8666	.33	.84
	1.00	44	.4573	.31756	.04787	.3607	.5538	.17	2.35
	2.00	36	.3939	.09667	.01611	.3612	.4266	.24	.63
	Total	84	.4314	.24365	.02658	.3786	.4843	.17	2.35
Lectin14b	Mixed	4	.5550	.29490	.14745	.0857	1.0243	.30	.98
	1.00	44	.5616	.23604	.03558	.4898	.6334	.21	1.39
	2.00	36	.5036	.17147	.02858	.4456	.5616	.26	.96
	Total	84	.5364	.21266	.02320	.4903	.5826	.21	1.39
Lectin15b	Mixed	4	.7725	.35920	.17960	.2009	1.3441	.54	1.30
	1.00	44	.6930	.27282	.04113	.6100	.7759	.11	1.47
	2.00	36	.6378	.23188	.03865	.5593	.7162	.22	1.16
	Total	84	.6731	.25908	.02827	.6169	.7293	.11	1.47
Lectin16b	Mixed	4	.3900	.35412	.17706	-.1735	.9535	.08	.74
	1.00	44	.3691	.35984	.05425	.2597	.4785	.07	1.46
	2.00	36	.2950	.26446	.04408	.2055	.3845	.07	.81
	Total	84	.3383	.32024	.03494	.2688	.4078	.07	1.46
Lectin17b	Mixed	4	.7600	.30452	.15226	.2754	1.2446	.50	1.20
	1.00	44	.8455	.33471	.05046	.7437	.9472	.38	2.15
	2.00	36	.8333	.25518	.04253	.7470	.9197	.45	1.52
	Total	84	.8362	.29863	.03258	.7714	.9010	.38	2.15
Lectin18b	Mixed	4	.8275	.31160	.15580	.3317	1.3233	.59	1.27
	1.00	44	.7693	.26414	.03982	.6890	.8496	.22	1.44
	2.00	36	.7831	.32399	.05400	.6734	.8927	.31	1.91
	Total	84	.7780	.28998	.03164	.7150	.8409	.22	1.91
Lectin19b	Mixed	4	.3025	.20320	.10160	-.0208	.6258	.13	.57
	1.00	44	.2700	.10090	.01521	.2393	.3007	.11	.51
	2.00	36	.2550	.10670	.01778	.2189	.2911	.08	.57
	Total	84	.2651	.10813	.01180	.2417	.2886	.08	.57
Lectin20b	Mixed	4	.6325	.16317	.08159	.3729	.8921	.45	.78
	1.00	44	.5234	.22754	.03430	.4542	.5926	.14	1.44
	2.00	36	.5100	.19171	.03195	.4451	.5749	.15	1.14
	Total	84	.5229	.20961	.02287	.4774	.5683	.14	1.44

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lectin1b	**Between Groups**	**.012**	**2**	**.006**	**.452**	**.638**
	Within Groups	1.103	81	.014		
	Total	1.115	83			

Lectin2b	Between Groups	.078	2	.039	1.045	.356
	Within Groups	3.019	81	.037		
	Total	3.097	83			
Lectin3b	Between Groups	.045	2	.022	.334	.717
	Within Groups	5.416	81	.067		
	Total	5.461	83			
Lectin4b	Between Groups	.182	2	.091	.947	.392
	Within Groups	7.806	81	.096		
	Total	7.988	83			
Lectin5b	Between Groups	.212	2	.106	4.136	.019
	Within Groups	2.076	81	.026		
	Total	2.288	83			
Lectin6b	Between Groups	.413	2	.207	2.392	.098
	Within Groups	6.996	81	.086		
	Total	7.410	83			
Lectin7b	Between Groups	.061	2	.031	.111	.895
	Within Groups	22.426	81	.277		
	Total	22.487	83			
Lectin8b	Between Groups	.031	2	.016	.563	.572
	Within Groups	2.250	81	.028		
	Total	2.281	83			
Lectin9b	Between Groups	.011	2	.006	.072	.931
	Within Groups	6.253	81	.077		
	Total	6.264	83			
Lectin10b	Between Groups	.150	2	.075	1.372	.259
	Within Groups	4.423	81	.055		
	Total	4.573	83			
Lectin11b	Between Groups	.018	2	.009	.572	.567
	Within Groups	1.263	81	.016		
	Total	1.281	83			
Lectin12b	Between Groups	.077	2	.039	1.449	.241
	Within Groups	2.154	81	.027		
	Total	2.231	83			
Lectin13b	Between Groups	.092	2	.046	.767	.468
	Within Groups	4.836	81	.060		
	Total	4.927	83			
Lectin14b	Between Groups	.068	2	.034	.747	.477
	Within Groups	3.686	81	.046		
	Total	3.754	83			
Lectin15b	Between Groups	.102	2	.051	.754	.474
	Within Groups	5.469	81	.068		
	Total	5.571	83			
Lectin16b	Between Groups	.120	2	.060	.579	.563
	Within Groups	8.392	81	.104		
	Total	8.512	83			
Lectin17b	Between Groups	.027	2	.014	.150	.861
	Within Groups	7.374	81	.091		
	Total	7.402	83			
Lectin18b	Between Groups	.014	2	.007	.082	.922
	Within Groups	6.965	81	.086		
	Total	6.979	83			
Lectin19b	Between Groups	.010	2	.005	.435	.648
	Within Groups	.960	81	.012		
	Total	.970	83			
Lectin20b	Between Groups	.054	2	.027	.609	.546
	Within Groups	3.593	81	.044		
	Total	3.647	83			

Multiple Comparisons							
Post hoc test: Bonferroni							
Dependent Variable	(I) gH	(J) gH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Lectin1b	Mixed	1.00	-.05409	.06093	1.000	-.2031	.0949
		2.00	-.04083	.06150	1.000	-.1912	.1095
	1.00	Mixed	.05409	.06093	1.000	-.0949	.2031
		2.00	.01326	.02622	1.000	-.0508	.0774
	2.00	Mixed	.04083	.06150	1.000	-.1095	.1912
		1.00	-.01326	.02622	1.000	-.0774	.0508
Lectin2b	Mixed	1.00	.10977	.10082	.838	-.1367	.3562
		2.00	.14111	.10175	.508	-.1076	.3899
	1.00	Mixed	-.10977	.10082	.838	-.3562	.1367
		2.00	.03134	.04339	1.000	-.0747	.1374
	2.00	Mixed	-.14111	.10175	.508	-.3899	.1076
		1.00	-.03134	.04339	1.000	-.1374	.0747
Lectin3b	Mixed	1.00	.07886	.13504	1.000	-.2513	.4090
		2.00	.03972	.13629	1.000	-.2935	.3729
	1.00	Mixed	-.07886	.13504	1.000	-.4090	.2513
		2.00	-.03914	.05811	1.000	-.1812	.1029
	2.00	Mixed	-.03972	.13629	1.000	-.3729	.2935
		1.00	.03914	.05811	1.000	-.1029	.1812
Lectin4b	Mixed	1.00	.12136	.16212	1.000	-.2750	.5177
		2.00	.19028	.16361	.745	-.2097	.5903
	1.00	Mixed	-.12136	.16212	1.000	-.5177	.2750
		2.00	.06891	.06976	.979	-.1016	.2395
	2.00	Mixed	-.19028	.16361	.745	-.5903	.2097
		1.00	-.06891	.06976	.979	-.2395	.1016
Lectin5b	Mixed	1.00	.23750*	.08361	.017	.0331	.4419
		2.00	.23361*	.08438	.021	.0273	.4399
	1.00	Mixed	-.23750*	.08361	.017	-.4419	-.0331
		2.00	-.00389	.03598	1.000	-.0918	.0841
	2.00	Mixed	-.23361*	.08438	.021	-.4399	-.0273
		1.00	.00389	.03598	1.000	-.0841	.0918
Lectin6b	Mixed	1.00	.31750	.15348	.125	-.0577	.6927
		2.00	.24500	.15490	.353	-.1337	.6237
	1.00	Mixed	-.31750	.15348	.125	-.6927	.0577
		2.00	-.07250	.06605	.827	-.2340	.0890
	2.00	Mixed	-.24500	.15490	.353	-.6237	.1337
		1.00	.07250	.06605	.827	-.0890	.2340
Lectin7b	Mixed	1.00	.11023	.27479	1.000	-.5616	.7820
		2.00	.12972	.27732	1.000	-.5482	.8077
	1.00	Mixed	-.11023	.27479	1.000	-.7820	.5616
		2.00	.01949	.11825	1.000	-.2696	.3086
	2.00	Mixed	-.12972	.27732	1.000	-.8077	.5482
		1.00	-.01949	.11825	1.000	-.3086	.2696
Lectin8b	Mixed	1.00	-.08568	.08703	.983	-.2984	.1271
		2.00	-.09306	.08783	.878	-.3078	.1217
	1.00	Mixed	.08568	.08703	.983	-.1271	.2984
		2.00	-.00737	.03745	1.000	-.0989	.0842
	2.00	Mixed	.09306	.08783	.878	-.1217	.3078
		1.00	.00737	.03745	1.000	-.0842	.0989
Lectin9b	Mixed	1.00	-.03932	.14510	1.000	-.3940	.3154
		2.00	-.05222	.14644	1.000	-.4102	.3058
	1.00	Mixed	.03932	.14510	1.000	-.3154	.3940
		2.00	-.01290	.06244	1.000	-.1656	.1397
	2.00	Mixed	.05222	.14644	1.000	-.3058	.4102
		1.00	.01290	.06244	1.000	-.1397	.1656
Lectin10b	Mixed	1.00	.04295	.12204	1.000	-.2554	.3413
		2.00	.12278	.12316	.965	-.1783	.4239
	1.00	Mixed	-.04295	.12204	1.000	-.3413	.2554
		2.00	.07982	.05252	.397	-.0486	.2082
	2.00	Mixed	-.12278	.12316	.965	-.4239	.1783
		1.00	-.07982	.05252	.397	-.2082	.0486
Lectin11b	Mixed	1.00	.01432	.06522	1.000	-.1451	.1738
		2.00	.04194	.06582	1.000	-.1190	.2029
	1.00	Mixed	-.01432	.06522	1.000	-.1738	.1451
		2.00	.02763	.02807	.984	-.0410	.0962
	2.00	Mixed	-.04194	.06582	1.000	-.2029	.1190

		1.00	-.02763	.02807	.984	-.0962	.0410
Lectin12b	Mixed	1.00	-.03455	.08517	1.000	-.2428	.1737
		2.00	.02778	.08595	1.000	-.1823	.2379
	1.00	Mixed	.03455	.08517	1.000	-.1737	.2428
		2.00	.06232	.03665	.279	-.0273	.1519
	2.00	Mixed	-.02778	.08595	1.000	-.2379	.1823
		1.00	-.06232	.03665	.279	-.1519	.0273
Lectin13b	Mixed	1.00	.02773	.12760	1.000	-.2842	.3397
		2.00	.09111	.12878	1.000	-.2237	.4059
	1.00	Mixed	-.02773	.12760	1.000	-.3397	.2842
		2.00	.06338	.05491	.755	-.0709	.1976
	2.00	Mixed	-.09111	.12878	1.000	-.4059	.2237
		1.00	-.06338	.05491	.755	-.1976	.0709
Lectin14b	Mixed	1.00	-.00659	.11140	1.000	-.2789	.2657
		2.00	.05139	.11243	1.000	-.2235	.3262
	1.00	Mixed	.00659	.11140	1.000	-.2657	.2789
		2.00	.05798	.04794	.690	-.0592	.1752
	2.00	Mixed	-.05139	.11243	1.000	-.3262	.2235
		1.00	-.05798	.04794	.690	-.1752	.0592
Lectin15b	Mixed	1.00	.07955	.13570	1.000	-.2522	.4113
		2.00	.13472	.13695	.985	-.2001	.4695
	1.00	Mixed	-.07955	.13570	1.000	-.4113	.2522
		2.00	.05518	.05840	1.000	-.0876	.1979
	2.00	Mixed	-.13472	.13695	.985	-.4695	.2001
		1.00	-.05518	.05840	1.000	-.1979	.0876
Lectin16b	Mixed	1.00	.02091	.16809	1.000	-.3900	.4318
		2.00	.09500	.16964	1.000	-.3197	.5097
	1.00	Mixed	-.02091	.16809	1.000	-.4318	.3900
		2.00	.07409	.07234	.926	-.1027	.2509
	2.00	Mixed	-.09500	.16964	1.000	-.5097	.3197
		1.00	-.07409	.07234	.926	-.2509	.1027
Lectin17b	Mixed	1.00	-.08545	.15758	1.000	-.4707	.2998
		2.00	-.07333	.15903	1.000	-.4621	.3154
	1.00	Mixed	.08545	.15758	1.000	-.2998	.4707
		2.00	.01212	.06781	1.000	-.1537	.1779
	2.00	Mixed	.07333	.15903	1.000	-.3154	.4621
		1.00	-.01212	.06781	1.000	-.1779	.1537
Lectin18b	Mixed	1.00	.05818	.15314	1.000	-.3162	.4326
		2.00	.04444	.15455	1.000	-.3334	.4223
	1.00	Mixed	-.05818	.15314	1.000	-.4326	.3162
		2.00	-.01374	.06590	1.000	-.1748	.1474
	2.00	Mixed	-.04444	.15455	1.000	-.4223	.3334
		1.00	.01374	.06590	1.000	-.1474	.1748
Lectin19b	Mixed	1.00	.03250	.05686	1.000	-.1065	.1715
		2.00	.04750	.05738	1.000	-.0928	.1878
	1.00	Mixed	-.03250	.05686	1.000	-.1715	.1065
		2.00	.01500	.02447	1.000	-.0448	.0748
	2.00	Mixed	-.04750	.05738	1.000	-.1878	.0928
		1.00	-.01500	.02447	1.000	-.0748	.0448
Lectin20b	Mixed	1.00	.10909	.10998	.973	-.1598	.3780
		2.00	.12250	.11100	.819	-.1489	.3939
	1.00	Mixed	-.10909	.10998	.973	-.3780	.1598
		2.00	.01341	.04733	1.000	-.1023	.1291
	2.00	Mixed	-.12250	.11100	.819	-.3939	.1489
		1.00	-.01341	.04733	1.000	-.1291	.1023

6.4 Glycoprotein L

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Lectin1b	Mixed	14	.2543	.19821	.05297	.1398	.3687	.09	.88
	1.00	6	.1950	.07396	.03019	.1174	.2726	.14	.33
	2.00	18	.2194	.10597	.02498	.1667	.2721	.11	.44
	3.00	8	.2262	.06093	.02154	.1753	.2772	.15	.34
	4.00	37	.2070	.10124	.01664	.1733	.2408	.09	.45
	Total	83	.2187	.11845	.01300	.1928	.2445	.09	.88
Lectin2	Mixed	14	.5464	.13843	.03700	.4665	.6264	.33	.80

b	1.00	6	.4700	.09960	.04066	.3655	.5745	.36	.64
	2.00	18	.5911	.24217	.05708	.4707	.7115	.29	1.08
	3.00	8	.6575	.25905	.09159	.4409	.8741	.34	1.17
	4.00	37	.5286	.17794	.02925	.4693	.5880	.20	1.14
	Total	83	.5534	.19389	.02128	.5110	.5957	.20	1.17
Lectin3b	Mixed	14	.7543	.28164	.07527	.5917	.9169	.45	1.48
	1.00	6	.5817	.18324	.07481	.3894	.7740	.35	.82
	2.00	18	.6722	.25965	.06120	.5431	.8013	.32	1.23
	3.00	8	.6775	.19499	.06894	.5145	.8405	.43	1.07
	4.00	37	.6895	.23365	.03841	.6116	.7674	.34	1.37
	Total	83	.6877	.23970	.02631	.6354	.7401	.32	1.48
Lectin4b	Mixed	14	.8264	.45099	.12053	.5660	1.0868	.50	2.27
	1.00	6	.6267	.18886	.07710	.4285	.8249	.40	.94
	2.00	18	.8089	.32033	.07550	.6496	.9682	.37	1.48
	3.00	8	.8262	.33239	.11752	.5484	1.1041	.46	1.31
	4.00	37	.7138	.25223	.04147	.6297	.7979	.30	1.24
	Total	83	.7580	.31148	.03419	.6899	.8260	.30	2.27
Lectin5b	Mixed	14	.2671	.21931	.05861	.1405	.3938	.09	.78
	1.00	6	.1900	.10621	.04336	.0785	.3015	.12	.40
	2.00	18	.1944	.07430	.01751	.1575	.2314	.10	.32
	3.00	8	.2200	.09457	.03343	.1409	.2991	.13	.42
	4.00	37	.2135	.19344	.03180	.1490	.2780	.07	1.20
	Total	83	.2173	.16507	.01812	.1813	.2534	.07	1.20
Lectin6b	Mixed	14	.8843	.31917	.08530	.7000	1.0686	.44	1.57
	1.00	6	.6600	.21679	.08851	.4325	.8875	.35	.91
	2.00	18	.7894	.29566	.06969	.6424	.9365	.44	1.39
	3.00	8	.7800	.26398	.09333	.5593	1.0007	.48	1.12
	4.00	37	.8914	.31442	.05169	.7865	.9962	.43	1.57
	Total	83	.8406	.30216	.03317	.7746	.9066	.35	1.57
Lectin7b	Mixed	14	1.0907	.49169	.13141	.8068	1.3746	.33	2.08
	1.00	6	.9233	.52064	.21255	.3770	1.4697	.18	1.50
	2.00	18	1.0628	.47984	.11310	.8242	1.3014	.30	2.07
	3.00	8	.9588	.46557	.16460	.5695	1.3480	.21	1.62
	4.00	37	1.1427	.57377	.09433	.9514	1.3340	.17	2.50
	Total	83	1.0830	.52029	.05711	.9694	1.1966	.17	2.50
Lectin8b	Mixed	14	.4871	.27170	.07262	.3303	.6440	.18	1.14
	1.00	6	.3933	.09070	.03703	.2981	.4885	.27	.54
	2.00	18	.3400	.12742	.03003	.2766	.4034	.15	.58
	3.00	8	.4488	.16234	.05740	.3130	.5845	.24	.75
	4.00	37	.3895	.13199	.02170	.3455	.4335	.12	.70
	Total	83	.4012	.16685	.01831	.3648	.4376	.12	1.14
Lectin9b	Mixed	14	.6593	.31604	.08447	.4768	.8418	.42	1.71
	1.00	6	.5867	.19582	.07994	.3812	.7922	.36	.92
	2.00	18	.7094	.29505	.06954	.5627	.8562	.32	1.35
	3.00	8	.7250	.29573	.10456	.4778	.9722	.42	1.27
	4.00	37	.6549	.26668	.04384	.5659	.7438	.34	1.54
	Total	83	.6693	.27600	.03030	.6090	.7295	.32	1.71
Lectin10b	Mixed	14	.6164	.23575	.06301	.4803	.7525	.15	1.11
	1.00	6	.5250	.17627	.07196	.3400	.7100	.31	.78
	2.00	18	.6289	.22927	.05404	.5149	.7429	.34	1.26
	3.00	8	.7000	.28224	.09979	.4640	.9360	.29	1.02
	4.00	37	.6084	.23923	.03933	.5286	.6881	.26	1.12

	Total	83	.6170	.23427	.02572	.5658	.6681	.15	1.26
Lectin11b	Mixed	14	.2507	.15005	.04010	.1641	.3374	.10	.62
	1.00	6	.1967	.03933	.01606	.1554	.2379	.15	.25
	2.00	18	.2511	.15107	.03561	.1760	.3262	.11	.58
	3.00	8	.2700	.09577	.03386	.1899	.3501	.14	.42
	4.00	37	.2251	.11541	.01897	.1867	.2636	.08	.59
	Total	83	.2373	.12406	.01362	.2103	.2644	.08	.62
Lectin12b	Mixed	14	.2593	.33525	.08960	.0657	.4529	.09	1.39
	1.00	6	.1217	.03061	.01249	.0895	.1538	.08	.17
	2.00	18	.1794	.09000	.02121	.1347	.2242	.10	.37
	3.00	8	.1863	.06368	.02251	.1330	.2395	.07	.25
	4.00	37	.1659	.11536	.01897	.1275	.2044	.07	.71
	Total	83	.1834	.16476	.01809	.1474	.2194	.07	1.39
Lectin13b	Mixed	14	.5464	.52352	.13992	.2442	.8487	.32	2.35
	1.00	6	.3433	.04719	.01926	.2938	.3929	.28	.42
	2.00	18	.4250	.14565	.03433	.3526	.4974	.24	.84
	3.00	8	.4313	.12541	.04434	.3264	.5361	.30	.59
	4.00	37	.4108	.13118	.02157	.3671	.4545	.17	.68
	Total	83	.4339	.24475	.02686	.3804	.4873	.17	2.35
Lectin14b	Mixed	14	.5743	.26503	.07083	.4213	.7273	.33	1.39
	1.00	6	.4450	.11380	.04646	.3256	.5644	.27	.57
	2.00	18	.5367	.22319	.05261	.4257	.6477	.27	1.02
	3.00	8	.5000	.16423	.05806	.3627	.6373	.26	.75
	4.00	37	.5557	.21243	.03492	.4848	.6265	.21	1.10
	Total	83	.5413	.21329	.02341	.4948	.5879	.21	1.39
Lectin15b	Mixed	14	.7136	.32872	.08785	.5238	.9034	.11	1.47
	1.00	6	.5300	.14100	.05756	.3820	.6780	.33	.68
	2.00	18	.6728	.24600	.05798	.5504	.7951	.43	1.30
	3.00	8	.6463	.22633	.08002	.4570	.8355	.32	.92
	4.00	37	.7146	.25063	.04120	.6310	.7982	.37	1.31
	Total	83	.6854	.25556	.02805	.6296	.7412	.11	1.47
Lectin16b	Mixed	14	.5929	.59630	.15937	.2486	.9372	.08	2.41
	1.00	6	.0983	.02858	.01167	.0683	.1283	.07	.15
	2.00	18	.1683	.15629	.03684	.0906	.2461	.07	.74
	3.00	8	.4413	.36459	.12890	.1364	.7461	.08	1.02
	4.00	37	.4100	.36034	.05924	.2899	.5301	.07	1.46
	Total	83	.3689	.39225	.04306	.2833	.4546	.07	2.41
Lectin17b	Mixed	14	.9064	.45329	.12115	.6447	1.1681	.48	2.15
	1.00	6	.7250	.24337	.09936	.4696	.9804	.38	1.02
	2.00	18	.9228	.29919	.07052	.7740	1.0716	.54	1.52
	3.00	8	.8475	.27820	.09836	.6149	1.0801	.52	1.31
	4.00	37	.7897	.25370	.04171	.7051	.8743	.40	1.31

	Total	83	.8392	.30656	.03365	.7722	.9061	.38	2.15
Lectin18b	Mixed	14	.7521	.24055	.06429	.6133	.8910	.22	1.19
	1.00	6	.6483	.26393	.10775	.3714	.9253	.42	1.13
	2.00	18	.8122	.30562	.07204	.6602	.9642	.42	1.44
	3.00	8	.7488	.27000	.09546	.5230	.9745	.43	1.20
	4.00	37	.7995	.31672	.05207	.6939	.9051	.31	1.91
	Total	83	.7784	.29145	.03199	.7148	.8421	.22	1.91
Lectin19b	Mixed	14	.2843	.10610	.02836	.2230	.3455	.15	.51
	1.00	6	.2100	.06066	.02477	.1463	.2737	.12	.29
	2.00	18	.2928	.11192	.02638	.2371	.3484	.15	.57
	3.00	8	.2463	.08943	.03162	.1715	.3210	.12	.39
	4.00	37	.2608	.11526	.01895	.2224	.2992	.08	.57
	Total	83	.2666	.10777	.01183	.2431	.2902	.08	.57
Lectin20b	Mixed	14	.5721	.30803	.08232	.3943	.7500	.14	1.44
	1.00	6	.5117	.20203	.08248	.2996	.7237	.25	.72
	2.00	18	.5344	.23640	.05572	.4169	.6520	.25	1.14
	3.00	8	.5225	.16663	.05891	.3832	.6618	.35	.88
	4.00	37	.4943	.16540	.02719	.4392	.5495	.15	1.00
	Total	83	.5201	.21050	.02311	.4742	.5661	.14	1.44

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lectin1b	Between Groups	.027	4	.007	.462	.764
	Within Groups	1.124	78	.014		
	Total	1.151	82			
Lectin2b	Between Groups	.177	4	.044	1.191	.322
	Within Groups	2.905	78	.037		
	Total	3.083	82			
Lectin3b	Between Groups	.135	4	.034	.574	.682
	Within Groups	4.577	78	.059		
	Total	4.711	82			
Lectin4b	Between Groups	.325	4	.081	.831	.509
	Within Groups	7.630	78	.098		
	Total	7.956	82			
Lectin5b	Between Groups	.049	4	.012	.439	.780
	Within Groups	2.185	78	.028		
	Total	2.234	82			
Lectin6b	Between Groups	.394	4	.099	1.084	.370
	Within Groups	7.092	78	.091		
	Total	7.486	82			
Lectin7b	Between Groups	.417	4	.104	.373	.827
	Within Groups	21.781	78	.279		
	Total	22.198	82			
Lectin8b	Between Groups	.194	4	.049	1.815	.134
	Within Groups	2.088	78	.027		
	Total	2.283	82			
Lectin9b	Between Groups	.104	4	.026	.330	.857
	Within Groups	6.143	78	.079		
	Total	6.247	82			
Lectin10b	Between Groups	.111	4	.028	.494	.740
	Within Groups	4.389	78	.056		

	Total	4.501	82			
Lectin11b	Between Groups	.030	4	.007	.473	.755
	Within Groups	1.232	78	.016		
	Total	1.262	82			
Lectin12b	Between Groups	.115	4	.029	1.063	.380
	Within Groups	2.111	78	.027		
	Total	2.226	82			
Lectin13b	Between Groups	.248	4	.062	1.036	.394
	Within Groups	4.664	78	.060		
	Total	4.912	82			
Lectin14b	Between Groups	.093	4	.023	.496	.739
	Within Groups	3.638	78	.047		
	Total	3.731	82			
Lectin15b	Between Groups	.203	4	.051	.767	.550
	Within Groups	5.153	78	.066		
	Total	5.355	82			
Lectin16b	Between Groups	1.970	4	.492	3.608	.009
	Within Groups	10.647	78	.136		
	Total	12.617	82			
Lectin17b	Between Groups	.358	4	.090	.951	.439
	Within Groups	7.348	78	.094		
	Total	7.706	82			
Lectin18b	Between Groups	.155	4	.039	.444	.776
	Within Groups	6.810	78	.087		
	Total	6.965	82			
Lectin19b	Between Groups	.040	4	.010	.866	.488
	Within Groups	.912	78	.012		
	Total	.952	82			
Lectin20b	Between Groups	.067	4	.017	.365	.833
	Within Groups	3.567	78	.046		
	Total	3.633	82			

Multiple Comparisons

Post hoc test: Bonferroni

Dependent Variable	(I) gL	(J) gL	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Lectin1b	Mixed	1.00	.05929	.05857	1.000	-.1099	.2285
		2.00	.03484	.04278	1.000	-.0887	.1584
		3.00	.02804	.05320	1.000	-.1257	.1817
		4.00	.04726	.03767	1.000	-.0616	.1561
	1.00	Mixed	-.05929	.05857	1.000	-.2285	.1099
		2.00	-.02444	.05659	1.000	-.1879	.1390
		3.00	-.03125	.06483	1.000	-.2185	.1560
		4.00	-.01203	.05283	1.000	-.1647	.1406
	2.00	Mixed	-.03484	.04278	1.000	-.1584	.0887
		1.00	.02444	.05659	1.000	-.1390	.1879
		3.00	-.00681	.05101	1.000	-.1542	.1406
		4.00	.01242	.03450	1.000	-.0872	.1121
	3.00	Mixed	-.02804	.05320	1.000	-.1817	.1257
		1.00	.03125	.06483	1.000	-.1560	.2185
		2.00	.00681	.05101	1.000	-.1406	.1542
		4.00	.01922	.04680	1.000	-.1160	.1544
	4.00	Mixed	-.04726	.03767	1.000	-.1561	.0616
		1.00	.01203	.05283	1.000	-.1406	.1647
		2.00	-.01242	.03450	1.000	-.1121	.0872
		3.00	-.01922	.04680	1.000	-.1544	.1160
Lectin2b	Mixed	1.00	.07643	.09417	1.000	-.1956	.3485
		2.00	-.04468	.06877	1.000	-.2434	.1540
		3.00	-.11107	.08554	1.000	-.3582	.1360
		4.00	.01778	.06056	1.000	-.1572	.1927
	1.00	Mixed	-.07643	.09417	1.000	-.3485	.1956
		2.00	-.12111	.09098	1.000	-.3840	.1417
		3.00	-.18750	.10423	.759	-.4886	.1136
		4.00	-.05865	.08494	1.000	-.3040	.1867
	2.00	Mixed	.04468	.06877	1.000	-.1540	.2434

		1.00	.12111	.09098	1.000	-.1417	.3840
		3.00	-.06639	.08201	1.000	-.3033	.1705
		4.00	.06246	.05546	1.000	-.0978	.2227
	3.00	Mixed	.11107	.08554	1.000	-.1360	.3582
		1.00	.18750	.10423	.759	-.1136	.4886
		2.00	.06639	.08201	1.000	-.1705	.3033
		4.00	.12885	.07525	.908	-.0886	.3463
	4.00	Mixed	-.01778	.06056	1.000	-.1927	.1572
		1.00	.05865	.08494	1.000	-.1867	.3040
		2.00	-.06246	.05546	1.000	-.2227	.0978
		3.00	-.12885	.07525	.908	-.3463	.0886
Lectin3b	Mixed	1.00	.17262	.11820	1.000	-.1689	.5141
		2.00	.08206	.08632	1.000	-.1673	.3314
		3.00	.07679	.10736	1.000	-.2334	.3869
		4.00	.06483	.07601	1.000	-.1548	.2844
	1.00	Mixed	-.17262	.11820	1.000	-.5141	.1689
		2.00	-.09056	.11419	1.000	-.4205	.2393
		3.00	-.09583	.13082	1.000	-.4738	.2821
		4.00	-.10779	.10661	1.000	-.4158	.2002
	2.00	Mixed	-.08206	.08632	1.000	-.3314	.1673
		1.00	.09056	.11419	1.000	-.2393	.4205
		3.00	-.00528	.10293	1.000	-.3026	.2921
		4.00	-.01724	.06961	1.000	-.2183	.1839
	3.00	Mixed	-.07679	.10736	1.000	-.3869	.2334
		1.00	.09583	.13082	1.000	-.2821	.4738
		2.00	.00528	.10293	1.000	-.2921	.3026
		4.00	-.01196	.09445	1.000	-.2848	.2609
	4.00	Mixed	-.06483	.07601	1.000	-.2844	.1548
		1.00	.10779	.10661	1.000	-.2002	.4158
		2.00	.01724	.06961	1.000	-.1839	.2183
		3.00	.01196	.09445	1.000	-.2609	.2848
Lectin4b	Mixed	1.00	.19976	.15262	1.000	-.2412	.6407
		2.00	.01754	.11146	1.000	-.3045	.3395
		3.00	.00018	.13862	1.000	-.4003	.4007
		4.00	.11264	.09814	1.000	-.1709	.3962
	1.00	Mixed	-.19976	.15262	1.000	-.6407	.2412
		2.00	-.18222	.14744	1.000	-.6082	.2438
		3.00	-.19958	.16892	1.000	-.6876	.2884
		4.00	-.08712	.13765	1.000	-.4848	.3106
	2.00	Mixed	-.01754	.11146	1.000	-.3395	.3045
		1.00	.18222	.14744	1.000	-.2438	.6082
		3.00	-.01736	.13290	1.000	-.4013	.3666
		4.00	.09511	.08988	1.000	-.1646	.3548
	3.00	Mixed	-.00018	.13862	1.000	-.4007	.4003
		1.00	.19958	.16892	1.000	-.2884	.6876
		2.00	.01736	.13290	1.000	-.3666	.4013
		4.00	.11247	.12195	1.000	-.2399	.4648
	4.00	Mixed	-.11264	.09814	1.000	-.3962	.1709
		1.00	.08712	.13765	1.000	-.3106	.4848
		2.00	-.09511	.08988	1.000	-.3548	.1646
		3.00	-.11247	.12195	1.000	-.4648	.2399
Lectin5b	Mixed	1.00	.07714	.08167	1.000	-.1588	.3131
		2.00	.07270	.05964	1.000	-.0996	.2450
		3.00	.04714	.07418	1.000	-.1672	.2615
		4.00	.05363	.05252	1.000	-.0981	.2054
	1.00	Mixed	-.07714	.08167	1.000	-.3131	.1588
		2.00	-.00444	.07890	1.000	-.2324	.2235
		3.00	-.03000	.09039	1.000	-.2912	.2312
		4.00	-.02351	.07366	1.000	-.2363	.1893
	2.00	Mixed	-.07270	.05964	1.000	-.2450	.0996
		1.00	.00444	.07890	1.000	-.2235	.2324
		3.00	-.02556	.07112	1.000	-.2310	.1799
		4.00	-.01907	.04810	1.000	-.1580	.1199
	3.00	Mixed	-.04714	.07418	1.000	-.2615	.1672
		1.00	.03000	.09039	1.000	-.2312	.2912
		2.00	.02556	.07112	1.000	-.1799	.2310
		4.00	.00649	.06526	1.000	-.1821	.1950
	4.00	Mixed	-.05363	.05252	1.000	-.2054	.0981
		1.00	.02351	.07366	1.000	-.1893	.2363
		2.00	.01907	.04810	1.000	-.1199	.1580
		3.00	-.00649	.06526	1.000	-.1950	.1821
Lectin6b	Mixed	1.00	.22429	.14714	1.000	-.2008	.6494
		2.00	.09484	.10745	1.000	-.2156	.4053
		3.00	.10429	.13364	1.000	-.2818	.4904
		4.00	-.00707	.09462	1.000	-.2804	.2663
	1.00	Mixed	-.22429	.14714	1.000	-.6494	.2008

		2.00	-.12944	.14215	1.000	-.5401	.2812
		3.00	-.12000	.16285	1.000	-.5905	.3505
		4.00	-.23135	.13271	.852	-.6148	.1521
	2.00	Mixed	-.09484	.10745	1.000	-.4053	.2156
		1.00	.12944	.14215	1.000	-.2812	.5401
		3.00	.00944	.12813	1.000	-.3607	.3796
		4.00	-.10191	.08665	1.000	-.3523	.1484
	3.00	Mixed	-.10429	.13364	1.000	-.4904	.2818
		1.00	.12000	.16285	1.000	-.3505	.5905
		2.00	-.00944	.12813	1.000	-.3796	.3607
		4.00	-.11135	.11757	1.000	-.4510	.2283
	4.00	Mixed	.00707	.09462	1.000	-.2663	.2804
		1.00	.23135	.13271	.852	-.1521	.6148
		2.00	.10191	.08665	1.000	-.1484	.3523
		3.00	.11135	.11757	1.000	-.2283	.4510
Lectin7b	Mixed	1.00	.16738	.25785	1.000	-.5776	.9123
		2.00	.02794	.18831	1.000	-.5161	.5720
		3.00	.13196	.23421	1.000	-.5447	.8086
		4.00	-.05199	.16581	1.000	-.5310	.4271
	1.00	Mixed	-.16738	.25785	1.000	-.9123	.5776
		2.00	-.13944	.24911	1.000	-.8591	.5803
		3.00	-.03542	.28539	1.000	-.8599	.7891
		4.00	-.21937	.23257	1.000	-.8913	.4525
	2.00	Mixed	-.02794	.18831	1.000	-.5720	.5161
		1.00	.13944	.24911	1.000	-.5803	.8591
		3.00	.10403	.22454	1.000	-.5447	.7528
		4.00	-.07992	.15186	1.000	-.5187	.3588
	3.00	Mixed	-.13196	.23421	1.000	-.8086	.5447
		1.00	.03542	.28539	1.000	-.7891	.8599
		2.00	-.10403	.22454	1.000	-.7528	.5447
		4.00	-.18395	.20604	1.000	-.7792	.4113
	4.00	Mixed	.05199	.16581	1.000	-.4271	.5310
		1.00	.21937	.23257	1.000	-.4525	.8913
		2.00	.07992	.15186	1.000	-.3588	.5187
		3.00	.18395	.20604	1.000	-.4113	.7792
Lectin8b	Mixed	1.00	.09381	.07984	1.000	-.1369	.3245
		2.00	.14714	.05831	.137	-.0213	.3156
		3.00	.03839	.07252	1.000	-.1711	.2479
		4.00	.09768	.05134	.608	-.0507	.2460
	1.00	Mixed	-.09381	.07984	1.000	-.3245	.1369
		2.00	.05333	.07714	1.000	-.1695	.2762
		3.00	-.05542	.08837	1.000	-.3107	.1999
		4.00	.00387	.07202	1.000	-.2042	.2119
	2.00	Mixed	-.14714	.05831	.137	-.3156	.0213
		1.00	-.05333	.07714	1.000	-.2762	.1695
		3.00	-.10875	.06953	1.000	-.3096	.0921
		4.00	-.04946	.04702	1.000	-.1853	.0864
	3.00	Mixed	-.03839	.07252	1.000	-.2479	.1711
		1.00	.05542	.08837	1.000	-.1999	.3107
		2.00	.10875	.06953	1.000	-.0921	.3096
		4.00	.05929	.06380	1.000	-.1250	.2436
	4.00	Mixed	-.09768	.05134	.608	-.2460	.0507
		1.00	-.00387	.07202	1.000	-.2119	.2042
		2.00	.04946	.04702	1.000	-.0864	.1853
		3.00	-.05929	.06380	1.000	-.2436	.1250
Lectin9b	Mixed	1.00	.07262	.13693	1.000	-.3230	.4682
		2.00	-.05016	.10000	1.000	-.3391	.2388
		3.00	-.06571	.12437	1.000	-.4250	.2936
		4.00	.00442	.08805	1.000	-.2500	.2588
	1.00	Mixed	-.07262	.13693	1.000	-.4682	.3230
		2.00	-.12278	.13229	1.000	-.5050	.2594
		3.00	-.13833	.15156	1.000	-.5762	.2995
		4.00	-.06820	.12351	1.000	-.4250	.2886
	2.00	Mixed	.05016	.10000	1.000	-.2388	.3391
		1.00	.12278	.13229	1.000	-.2594	.5050
		3.00	-.01556	.11924	1.000	-.3601	.3289
		4.00	.05458	.08064	1.000	-.1784	.2876
	3.00	Mixed	.06571	.12437	1.000	-.2936	.4250
		1.00	.13833	.15156	1.000	-.2995	.5762
		2.00	.01556	.11924	1.000	-.3289	.3601
		4.00	.07014	.10942	1.000	-.2460	.3863
	4.00	Mixed	-.00442	.08805	1.000	-.2588	.2500
		1.00	.06820	.12351	1.000	-.2886	.4250
		2.00	-.05458	.08064	1.000	-.2876	.1784
		3.00	-.07014	.10942	1.000	-.3863	.2460
Lectin10b	Mixed	1.00	.09143	.11575	1.000	-.2430	.4258

		2.00	-.01246	.08453	1.000	-.2567	.2318
		3.00	-.08357	.10514	1.000	-.3873	.2202
		4.00	.00805	.07443	1.000	-.2070	.2231
	1.00	Mixed	-.09143	.11575	1.000	-.4258	.2430
		2.00	-.10389	.11183	1.000	-.4270	.2192
		3.00	-.17500	.12811	1.000	-.5451	.1951
		4.00	-.08338	.10440	1.000	-.3850	.2182
	2.00	Mixed	.01246	.08453	1.000	-.2318	.2567
		1.00	.10389	.11183	1.000	-.2192	.4270
		3.00	-.07111	.10080	1.000	-.3623	.2201
		4.00	.02051	.06817	1.000	-.1764	.2175
	3.00	Mixed	.08357	.10514	1.000	-.2202	.3873
		1.00	.17500	.12811	1.000	-.1951	.5451
		2.00	.07111	.10080	1.000	-.2201	.3623
		4.00	.09162	.09249	1.000	-.1756	.3588
	4.00	Mixed	-.00805	.07443	1.000	-.2231	.2070
		1.00	.08338	.10440	1.000	-.2182	.3850
		2.00	-.02051	.06817	1.000	-.2175	.1764
		3.00	-.09162	.09249	1.000	-.3588	.1756
Lectin11b	Mixed	1.00	.05405	.06133	1.000	-.1231	.2312
		2.00	-.00040	.04479	1.000	-.1298	.1290
		3.00	-.01929	.05570	1.000	-.1802	.1416
		4.00	.02558	.03944	1.000	-.0884	.1395
	1.00	Mixed	-.05405	.06133	1.000	-.2312	.1231
		2.00	-.05444	.05925	1.000	-.2256	.1167
		3.00	-.07333	.06788	1.000	-.2694	.1228
		4.00	-.02847	.05531	1.000	-.1883	.1313
	2.00	Mixed	.00040	.04479	1.000	-.1290	.1298
		1.00	.05444	.05925	1.000	-.1167	.2256
		3.00	-.01889	.05341	1.000	-.1732	.1354
		4.00	.02598	.03612	1.000	-.0784	.1303
	3.00	Mixed	.01929	.05570	1.000	-.1416	.1802
		1.00	.07333	.06788	1.000	-.1228	.2694
		2.00	.01889	.05341	1.000	-.1354	.1732
		4.00	.04486	.04901	1.000	-.0967	.1864
	4.00	Mixed	-.02558	.03944	1.000	-.1395	.0884
		1.00	.02847	.05531	1.000	-.1313	.1883
		2.00	-.02598	.03612	1.000	-.1303	.0784
		3.00	-.04486	.04901	1.000	-.1864	.0967
Lectin12b	Mixed	1.00	.13762	.08027	.904	-.0943	.3695
		2.00	.07984	.05862	1.000	-.0895	.2492
		3.00	.07304	.07291	1.000	-.1376	.2837
		4.00	.09334	.05162	.744	-.0558	.2425
	1.00	Mixed	-.13762	.08027	.904	-.3695	.0943
		2.00	-.05778	.07755	1.000	-.2818	.1663
		3.00	-.06458	.08885	1.000	-.3213	.1921
		4.00	-.04428	.07240	1.000	-.2535	.1649
	2.00	Mixed	-.07984	.05862	1.000	-.2492	.0895
		1.00	.05778	.07755	1.000	-.1663	.2818
		3.00	-.00681	.06990	1.000	-.2088	.1952
		4.00	.01350	.04728	1.000	-.1231	.1501
	3.00	Mixed	-.07304	.07291	1.000	-.2837	.1376
		1.00	.06458	.08885	1.000	-.1921	.3213
		2.00	.00681	.06990	1.000	-.1952	.2088
		4.00	.02030	.06414	1.000	-.1650	.2056
	4.00	Mixed	-.09334	.05162	.744	-.2425	.0558
		1.00	.04428	.07240	1.000	-.1649	.2535
		2.00	-.01350	.04728	1.000	-.1501	.1231
		3.00	-.02030	.06414	1.000	-.2056	.1650
Lectin13b	Mixed	1.00	.20310	.11932	.927	-.1416	.5478
		2.00	.12143	.08714	1.000	-.1303	.3732
		3.00	.11518	.10838	1.000	-.1979	.4283
		4.00	.13562	.07673	.811	-.0861	.3573
	1.00	Mixed	-.20310	.11932	.927	-.5478	.1416
		2.00	-.08167	.11528	1.000	-.4147	.2514
		3.00	-.08792	.13207	1.000	-.4695	.2936
		4.00	-.06748	.10762	1.000	-.3784	.2435
	2.00	Mixed	-.12143	.08714	1.000	-.3732	.1303
		1.00	.08167	.11528	1.000	-.2514	.4147
		3.00	-.00625	.10391	1.000	-.3064	.2939
		4.00	.01419	.07027	1.000	-.1888	.2172
	3.00	Mixed	-.11518	.10838	1.000	-.4283	.1979
		1.00	.08792	.13207	1.000	-.2936	.4695
		2.00	.00625	.10391	1.000	-.2939	.3064
		4.00	.02044	.09535	1.000	-.2550	.2959
	4.00	Mixed	-.13562	.07673	.811	-.3573	.0861

		1.00	.06748	.10762	1.000	-.2435	.3784
		2.00	-.01419	.07027	1.000	-.2172	.1888
		3.00	-.02044	.09535	1.000	-.2959	.2550
Lectin14b	Mixed	1.00	.12929	.10538	1.000	-.1752	.4337
		2.00	.03762	.07696	1.000	-.1847	.2600
		3.00	.07429	.09572	1.000	-.2022	.3508
		4.00	.01861	.06776	1.000	-.1772	.2144
	1.00	Mixed	-.12929	.10538	1.000	-.4337	.1752
		2.00	-.09167	.10181	1.000	-.3858	.2025
		3.00	-.05500	.11663	1.000	-.3920	.2820
		4.00	-.11068	.09505	1.000	-.3853	.1639
	2.00	Mixed	-.03762	.07696	1.000	-.2600	.1847
		1.00	.09167	.10181	1.000	-.2025	.3858
		3.00	.03667	.09177	1.000	-.2285	.3018
		4.00	-.01901	.06206	1.000	-.1983	.1603
	3.00	Mixed	-.07429	.09572	1.000	-.3508	.2022
		1.00	.05500	.11663	1.000	-.2820	.3920
		2.00	-.03667	.09177	1.000	-.3018	.2285
		4.00	-.05568	.08421	1.000	-.2990	.1876
	4.00	Mixed	-.01861	.06776	1.000	-.2144	.1772
		1.00	.11068	.09505	1.000	-.1639	.3853
		2.00	.01901	.06206	1.000	-.1603	.1983
		3.00	.05568	.08421	1.000	-.1876	.2990
Lectin15b	Mixed	1.00	.18357	.12541	1.000	-.1788	.5459
		2.00	.04079	.09159	1.000	-.2238	.3054
		3.00	.06732	.11391	1.000	-.2618	.3964
		4.00	-.00102	.08065	1.000	-.2340	.2320
	1.00	Mixed	-.18357	.12541	1.000	-.5459	.1788
		2.00	-.14278	.12116	1.000	-.4928	.2073
		3.00	-.11625	.13881	1.000	-.5173	.2848
		4.00	-.18459	.11312	1.000	-.5114	.1422
	2.00	Mixed	-.04079	.09159	1.000	-.3054	.2238
		1.00	.14278	.12116	1.000	-.2073	.4928
		3.00	.02653	.10921	1.000	-.2890	.3421
		4.00	-.04182	.07386	1.000	-.2552	.1716
	3.00	Mixed	-.06732	.11391	1.000	-.3964	.2618
		1.00	.11625	.13881	1.000	-.2848	.5173
		2.00	-.02653	.10921	1.000	-.3421	.2890
		4.00	-.06834	.10022	1.000	-.3579	.2212
	4.00	Mixed	.00102	.08065	1.000	-.2320	.2340
		1.00	.18459	.11312	1.000	-.1422	.5114
		2.00	.04182	.07386	1.000	-.1716	.2552
		3.00	.06834	.10022	1.000	-.2212	.3579
Lectin16b	Mixed	1.00	.49452	.18028	.075	-.0263	1.0154
		2.00	.42452*	.13165	.018	.0442	.8049
		3.00	.15161	.16374	1.000	-.3215	.6247
		4.00	.18286	.11593	1.000	-.1521	.5178
	1.00	Mixed	-.49452	.18028	.075	-1.0154	.0263
		2.00	-.07000	.17416	1.000	-.5732	.4332
		3.00	-.34292	.19953	.896	-.9194	.2335
		4.00	-.31167	.16260	.589	-.7814	.1581
	2.00	Mixed	-.42452*	.13165	.018	-.8049	-.0442
		1.00	.07000	.17416	1.000	-.4332	.5732
		3.00	-.27292	.15699	.861	-.7265	.1806
		4.00	-.24167	.10617	.256	-.5484	.0651
	3.00	Mixed	-.15161	.16374	1.000	-.6247	.3215
		1.00	.34292	.19953	.896	-.2335	.9194
		2.00	.27292	.15699	.861	-.1806	.7265
		4.00	.03125	.14405	1.000	-.3849	.4474
	4.00	Mixed	-.18286	.11593	1.000	-.5178	.1521
		1.00	.31167	.16260	.589	-.1581	.7814
		2.00	.24167	.10617	.256	-.0651	.5484
		3.00	-.03125	.14405	1.000	-.4474	.3849
Lectin17b	Mixed	1.00	.18143	.14976	1.000	-.2513	.6141
		2.00	-.01635	.10937	1.000	-.3323	.2996
		3.00	.05893	.13603	1.000	-.3341	.4519
		4.00	.11670	.09631	1.000	-.1615	.3949
	1.00	Mixed	-.18143	.14976	1.000	-.6141	.2513
		2.00	-.19778	.14469	1.000	-.6158	.2202
		3.00	-.12250	.16576	1.000	-.6014	.3564
		4.00	-.06473	.13508	1.000	-.4550	.3255
	2.00	Mixed	.01635	.10937	1.000	-.2996	.3323
		1.00	.19778	.14469	1.000	-.2202	.6158
		3.00	.07528	.13042	1.000	-.3015	.4521
		4.00	.13305	.08820	1.000	-.1218	.3879

	3.00	Mixed	-.05893	.13603	1.000	-.4519	.3341
		1.00	.12250	.16576	1.000	-.3564	.6014
		2.00	-.07528	.13042	1.000	-.4521	.3015
		4.00	.05777	.11967	1.000	-.2880	.4035
	4.00	Mixed	-.11670	.09631	1.000	-.3949	.1615
		1.00	.06473	.13508	1.000	-.3255	.4550
		2.00	-.13305	.08820	1.000	-.3879	.1218
		3.00	-.05777	.11967	1.000	-.4035	.2880
Lectin18b	Mixed	1.00	.10381	.14418	1.000	-.3127	.5204
		2.00	-.06008	.10529	1.000	-.3643	.2441
		3.00	.00339	.13096	1.000	-.3749	.3817
		4.00	-.04732	.09271	1.000	-.3152	.2205
	1.00	Mixed	-.10381	.14418	1.000	-.5204	.3127
		2.00	-.16389	.13929	1.000	-.5663	.2385
		3.00	-.10042	.15958	1.000	-.5614	.3606
		4.00	-.15113	.13004	1.000	-.5268	.2246
	2.00	Mixed	.06008	.10529	1.000	-.2441	.3643
		1.00	.16389	.13929	1.000	-.2385	.5663
		3.00	.06347	.12555	1.000	-.2993	.4262
		4.00	.01276	.08491	1.000	-.2326	.2581
	3.00	Mixed	-.00339	.13096	1.000	-.3817	.3749
		1.00	.10042	.15958	1.000	-.3606	.5614
		2.00	-.06347	.12555	1.000	-.4262	.2993
		4.00	-.05071	.11521	1.000	-.3836	.2821
	4.00	Mixed	.04732	.09271	1.000	-.2205	.3152
		1.00	.15113	.13004	1.000	-.2246	.5268
		2.00	-.01276	.08491	1.000	-.2581	.2326
		3.00	.05071	.11521	1.000	-.2821	.3836
Lectin19b	Mixed	1.00	.07429	.05276	1.000	-.0781	.2267
		2.00	-.00849	.03853	1.000	-.1198	.1028
		3.00	.03804	.04792	1.000	-.1004	.1765
		4.00	.02347	.03393	1.000	-.0745	.1215
	1.00	Mixed	-.07429	.05276	1.000	-.2267	.0781
		2.00	-.08278	.05097	1.000	-.2300	.0645
		3.00	-.03625	.05840	1.000	-.2050	.1325
		4.00	-.05081	.04759	1.000	-.1883	.0867
	2.00	Mixed	.00849	.03853	1.000	-.1028	.1198
		1.00	.08278	.05097	1.000	-.0645	.2300
		3.00	.04653	.04595	1.000	-.0862	.1793
		4.00	.03197	.03107	1.000	-.0578	.1217
	3.00	Mixed	-.03804	.04792	1.000	-.1765	.1004
		1.00	.03625	.05840	1.000	-.1325	.2050
		2.00	-.04653	.04595	1.000	-.1793	.0862
		4.00	-.01456	.04216	1.000	-.1364	.1072
	4.00	Mixed	-.02347	.03393	1.000	-.1215	.0745
		1.00	.05081	.04759	1.000	-.0867	.1883
		2.00	-.03197	.03107	1.000	-.1217	.0578
		3.00	.01456	.04216	1.000	-.1072	.1364
Lectin20b	Mixed	1.00	.06048	.10434	1.000	-.2410	.3619
		2.00	.03770	.07620	1.000	-.1825	.2579
		3.00	.04964	.09478	1.000	-.2242	.3235
		4.00	.07782	.06710	1.000	-.1160	.2717
	1.00	Mixed	-.06048	.10434	1.000	-.3619	.2410
		2.00	-.02278	.10081	1.000	-.3140	.2685
		3.00	-.01083	.11549	1.000	-.3445	.3228
		4.00	.01734	.09411	1.000	-.2546	.2892
	2.00	Mixed	-.03770	.07620	1.000	-.2579	.1825
		1.00	.02278	.10081	1.000	-.2685	.3140
		3.00	.01194	.09087	1.000	-.2506	.2745
		4.00	.04012	.06145	1.000	-.1374	.2177
	3.00	Mixed	-.04964	.09478	1.000	-.3235	.2242
		1.00	.01083	.11549	1.000	-.3228	.3445
		2.00	-.01194	.09087	1.000	-.2745	.2506
		4.00	.02818	.08338	1.000	-.2127	.2691
	4.00	Mixed	-.07782	.06710	1.000	-.2717	.1160
		1.00	-.01734	.09411	1.000	-.2892	.2546
		2.00	-.04012	.06145	1.000	-.2177	.1374
		3.00	-.02818	.08338	1.000	-.2691	.2127

6.5 Glycoprotein M

Descriptives									
						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Lectin1b	1.00	16	.2200	.08959	.02240	.1723	.2677	.13	.42
	2.00	4	.2125	.09946	.04973	.0542	.3708	.13	.35
	3.00	53	.2268	.13135	.01804	.1906	.2630	.09	.88
	Total	73	.2245	.12067	.01412	.1964	.2527	.09	.88
Lectin2b	1.00	16	.5306	.13626	.03406	.4580	.6032	.28	.79
	2.00	4	.5175	.21469	.10734	.1759	.8591	.33	.75
	3.00	53	.5636	.20717	.02846	.5065	.6207	.20	1.17
	Total	73	.5538	.19248	.02253	.5089	.5987	.20	1.17
Lectin3b	1.00	16	.6606	.19379	.04845	.5574	.7639	.35	1.06
	2.00	4	.5900	.24590	.12295	.1987	.9813	.32	.89
	3.00	53	.7045	.26273	.03609	.6321	.7769	.34	1.48
	Total	73	.6886	.24718	.02893	.6310	.7463	.32	1.48
Lectin4b	1.00	16	.6556	.20176	.05044	.5481	.7631	.40	1.16
	2.00	4	.6625	.35046	.17523	.1048	1.2202	.41	1.18
	3.00	53	.7975	.33991	.04669	.7039	.8912	.30	2.27
	Total	73	.7590	.31785	.03720	.6849	.8332	.30	2.27
Lectin5b	1.00	16	.2000	.09940	.02485	.1470	.2530	.09	.42
	2.00	4	.1800	.07789	.03894	.0561	.3039	.12	.29
	3.00	53	.2383	.19286	.02649	.1851	.2915	.07	1.20
	Total	73	.2267	.17191	.02012	.1866	.2668	.07	1.20
Lectin6b	1.00	16	.7838	.28378	.07095	.6325	.9350	.48	1.39
	2.00	4	.8000	.48888	.24444	.0221	1.5779	.44	1.51
	3.00	53	.8589	.29486	.04050	.7776	.9401	.35	1.57
	Total	73	.8392	.30096	.03523	.7690	.9094	.35	1.57
Lectin7b	1.00	16	.9150	.44288	.11072	.6790	1.1510	.17	1.58
	2.00	4	.9750	.62979	.31489	-.0271	1.9771	.55	1.91
	3.00	53	1.1209	.52880	.07264	.9752	1.2667	.18	2.50
	Total	73	1.0678	.51681	.06049	.9472	1.1884	.17	2.50
Lectin8b	1.00	16	.4494	.22398	.05599	.3300	.5687	.21	1.14
	2.00	4	.3700	.14491	.07246	.1394	.6006	.24	.51
	3.00	53	.4070	.15493	.02128	.3643	.4497	.12	.95
	Total	73	.4142	.17054	.01996	.3745	.4540	.12	1.14
Lectin9b	1.00	16	.6556	.22663	.05666	.5349	.7764	.36	1.07
	2.00	4	.5625	.27765	.13883	.1207	1.0043	.36	.97
	3.00	53	.6960	.30894	.04244	.6109	.7812	.32	1.71
	Total	73	.6799	.28971	.03391	.6123	.7475	.32	1.71
Lectin10b	1.00	16	.5488	.18439	.04610	.4505	.6470	.27	.95
	2.00	4	.5300	.18815	.09407	.2306	.8294	.38	.78
	3.00	53	.6402	.24189	.03323	.5735	.7069	.15	1.12
	Total	73	.6141	.22947	.02686	.5606	.6676	.15	1.12
Lectin11b	1.00	16	.2363	.11313	.02828	.1760	.2965	.11	.48
	2.00	4	.2175	.08098	.04049	.0886	.3464	.14	.31
	3.00	53	.2432	.13094	.01799	.2071	.2793	.09	.62
	Total	73	.2403	.12394	.01451	.2114	.2692	.09	.62
Lectin12b	1.00	16	.1544	.08246	.02061	.1104	.1983	.07	.38
	2.00	4	.1625	.04573	.02287	.0897	.2353	.11	.22
	3.00	53	.1987	.19684	.02704	.1444	.2529	.07	1.39
	Total	73	.1870	.17279	.02022	.1467	.2273	.07	1.39
Lectin13b	1.00	16	.3675	.09609	.02402	.3163	.4187	.23	.59
	2.00	4	.3650	.07594	.03797	.2442	.4858	.30	.47
	3.00	53	.4638	.28796	.03955	.3844	.5431	.24	2.35
	Total	73	.4373	.25286	.02960	.3783	.4963	.23	2.35
Lectin14b	1.00	16	.4594	.15906	.03976	.3746	.5441	.26	.72
	2.00	4	.4875	.16070	.08035	.2318	.7432	.36	.71
	3.00	53	.5791	.22638	.03110	.5167	.6415	.26	1.39
	Total	73	.5478	.21452	.02511	.4978	.5979	.26	1.39
Lectin15b	1.00	16	.6275	.19975	.04994	.5211	.7339	.32	1.04
	2.00	4	.6000	.18166	.09083	.3109	.8891	.47	.86

	3.00	53	.7136	.27085	.03720	.6389	.7882	.11	1.47
	Total	73	.6885	.25376	.02970	.6293	.7477	.11	1.47
Lectin16b	1.00	16	.3081	.36830	.09207	.1119	.5044	.07	1.46
	2.00	4	.3800	.33635	.16818	-.1552	.9152	.09	.75
	3.00	53	.3328	.31864	.04377	.2450	.4207	.07	1.40
	Total	73	.3300	.32642	.03820	.2538	.4062	.07	1.46
Lectin17b	1.00	16	.7831	.26902	.06725	.6398	.9265	.38	1.20
	2.00	4	.6500	.14283	.07141	.4227	.8773	.54	.86
	3.00	53	.8798	.31880	.04379	.7919	.9677	.40	2.15
	Total	73	.8460	.30526	.03573	.7748	.9173	.38	2.15
Lectin18b	1.00	16	.6956	.21945	.05486	.5787	.8126	.33	1.01
	2.00	4	.6100	.19799	.09899	.2950	.9250	.43	.81
	3.00	53	.8079	.32169	.04419	.7193	.8966	.22	1.91
	Total	73	.7725	.30018	.03513	.7024	.8425	.22	1.91
Lectin19b	1.00	16	.2388	.08943	.02236	.1911	.2864	.11	.40
	2.00	4	.2825	.19190	.09595	-.0229	.5879	.18	.57
	3.00	53	.2745	.10246	.01407	.2463	.3028	.08	.51
	Total	73	.2671	.10495	.01228	.2426	.2916	.08	.57
Lectin20b	1.00	16	.4975	.16715	.04179	.4084	.5866	.25	.81
	2.00	4	.3900	.10165	.05083	.2282	.5518	.25	.47
	3.00	53	.5340	.23365	.03209	.4696	.5984	.14	1.44
	Total	73	.5181	.21650	.02534	.4676	.5686	.14	1.44

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lectin1b	Between Groups	.001	2	.001	.039	.961
	Within Groups	1.047	70	.015		
	Total	1.048	72			
Lectin2b	Between Groups	.019	2	.009	.250	.779
	Within Groups	2.649	70	.038		
	Total	2.668	72			
Lectin3b	Between Groups	.065	2	.032	.524	.595
	Within Groups	4.334	70	.062		
	Total	4.399	72			
Lectin4b	Between Groups	.287	2	.143	1.438	.244
	Within Groups	6.987	70	.100		
	Total	7.274	72			
Lectin5b	Between Groups	.027	2	.014	.454	.637
	Within Groups	2.101	70	.030		
	Total	2.128	72			
Lectin6b	Between Groups	.076	2	.038	.412	.664
	Within Groups	6.446	70	.092		
	Total	6.522	72			
Lectin7b	Between Groups	.558	2	.279	1.045	.357
	Within Groups	18.673	70	.267		
	Total	19.230	72			
Lectin8b	Between Groups	.030	2	.015	.515	.600
	Within Groups	2.064	70	.029		
	Total	2.094	72			
Lectin9b	Between Groups	.078	2	.039	.460	.633
	Within Groups	5.965	70	.085		
	Total	6.043	72			
Lectin10b	Between Groups	.133	2	.066	1.269	.287
	Within Groups	3.659	70	.052		
	Total	3.791	72			
Lectin11b	Between Groups	.003	2	.001	.089	.915
	Within Groups	1.103	70	.016		
	Total	1.106	72			
Lectin12b	Between Groups	.027	2	.013	.440	.646
	Within Groups	2.123	70	.030		
	Total	2.150	72			

Lectin13b	Between Groups	.136	2	.068	1.065	.350
	Within Groups	4.468	70	.064		
	Total	4.604	72			
Lectin14b	Between Groups	.191	2	.096	2.146	.125
	Within Groups	3.122	70	.045		
	Total	3.313	72			
Lectin15b	Between Groups	.124	2	.062	.963	.387
	Within Groups	4.512	70	.064		
	Total	4.637	72			
Lectin16b	Between Groups	.018	2	.009	.083	.921
	Within Groups	7.654	70	.109		
	Total	7.672	72			
Lectin17b	Between Groups	.278	2	.139	1.510	.228
	Within Groups	6.432	70	.092		
	Total	6.709	72			
Lectin18b	Between Groups	.267	2	.133	1.500	.230
	Within Groups	6.221	70	.089		
	Total	6.488	72			
Lectin19b	Between Groups	.017	2	.008	.754	.474
	Within Groups	.776	70	.011		
	Total	.793	72			
Lectin20b	Between Groups	.086	2	.043	.913	.406
	Within Groups	3.289	70	.047		
	Total	3.375	72			

Multiple Comparisons

Post hoc test: Bonferroni

Dependent Variable	(I) gM.r	(J) gM.r	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Lectin1b	1.00	2.00	.00750	.06837	1.000	-.1602	.1752
		3.00	-.00679	.03489	1.000	-.0924	.0788
	2.00	1.00	-.00750	.06837	1.000	-.1752	.1602
		3.00	-.01429	.06342	1.000	-.1699	.1413
	3.00	1.00	.00679	.03489	1.000	-.0788	.0924
		2.00	.01429	.06342	1.000	-.1413	.1699
Lectin2b	1.00	2.00	.01313	.10874	1.000	-.2536	.2798
		3.00	-.03296	.05549	1.000	-.1691	.1031
	2.00	1.00	-.01313	.10874	1.000	-.2798	.2536
		3.00	-.04608	.10086	1.000	-.2935	.2013
	3.00	1.00	.03296	.05549	1.000	-.1031	.1691
		2.00	.04608	.10086	1.000	-.2013	.2935
Lectin3b	1.00	2.00	.07062	.13910	1.000	-.2706	.4118
		3.00	-.04390	.07098	1.000	-.2180	.1302
	2.00	1.00	-.07062	.13910	1.000	-.4118	.2706
		3.00	-.11453	.12903	1.000	-.4310	.2020
	3.00	1.00	.04390	.07098	1.000	-.1302	.2180
		2.00	.11453	.12903	1.000	-.2020	.4310
Lectin4b	1.00	2.00	-.00687	.17661	1.000	-.4401	.4263
		3.00	-.14192	.09012	.359	-.3630	.0791
	2.00	1.00	.00687	.17661	1.000	-.4263	.4401
		3.00	-.13505	.16382	1.000	-.5369	.2668
	3.00	1.00	.14192	.09012	.359	-.0791	.3630
		2.00	.13505	.16382	1.000	-.2668	.5369
Lectin5b	1.00	2.00	.02000	.09684	1.000	-.2175	.2575
		3.00	-.03830	.04941	1.000	-.1595	.0829
	2.00	1.00	-.02000	.09684	1.000	-.2575	.2175
		3.00	-.05830	.08982	1.000	-.2786	.1620
	3.00	1.00	.03830	.04941	1.000	-.0829	.1595
		2.00	.05830	.08982	1.000	-.1620	.2786
Lectin6b	1.00	2.00	-.01625	.16964	1.000	-.4323	.3998
		3.00	-.07512	.08656	1.000	-.2874	.1372
	2.00	1.00	.01625	.16964	1.000	-.3998	.4323

		3.00	-.05887	.15735	1.000	-.4448	.3271
	3.00	1.00	.07512	.08656	1.000	-.1372	.2874
		2.00	.05887	.15735	1.000	-.3271	.4448
Lectin7b	1.00	2.00	-.06000	.28872	1.000	-.7682	.6482
		3.00	-.20594	.14733	.500	-.5673	.1554
	2.00	1.00	.06000	.28872	1.000	-.6482	.7682
		3.00	-.14594	.26781	1.000	-.8028	.5110
	3.00	1.00	.20594	.14733	.500	-.1554	.5673
		2.00	.14594	.26781	1.000	-.5110	.8028
Lectin8b	1.00	2.00	.07937	.09598	1.000	-.1561	.3148
		3.00	.04239	.04898	1.000	-.0777	.1625
	2.00	1.00	-.07937	.09598	1.000	-.3148	.1561
		3.00	-.03698	.08903	1.000	-.2554	.1814
	3.00	1.00	-.04239	.04898	1.000	-.1625	.0777
		2.00	.03698	.08903	1.000	-.1814	.2554
Lectin9b	1.00	2.00	.09313	.16318	1.000	-.3071	.4934
		3.00	-.04041	.08327	1.000	-.2447	.1638
	2.00	1.00	-.09313	.16318	1.000	-.4934	.3071
		3.00	-.13354	.15136	1.000	-.5048	.2377
	3.00	1.00	.04041	.08327	1.000	-.1638	.2447
		2.00	.13354	.15136	1.000	-.2377	.5048
Lectin10b	1.00	2.00	.01875	.12780	1.000	-.2947	.3322
		3.00	-.09144	.06521	.496	-.2514	.0685
	2.00	1.00	-.01875	.12780	1.000	-.3322	.2947
		3.00	-.11019	.11854	1.000	-.4010	.1806
	3.00	1.00	.09144	.06521	.496	-.0685	.2514
		2.00	.11019	.11854	1.000	-.1806	.4010
Lectin11b	1.00	2.00	.01875	.07018	1.000	-.1534	.1909
		3.00	-.00696	.03581	1.000	-.0948	.0809
	2.00	1.00	-.01875	.07018	1.000	-.1909	.1534
		3.00	-.02571	.06510	1.000	-.1854	.1340
	3.00	1.00	.00696	.03581	1.000	-.0809	.0948
		2.00	.02571	.06510	1.000	-.1340	.1854
Lectin12b	1.00	2.00	-.00812	.09736	1.000	-.2469	.2307
		3.00	-.04430	.04968	1.000	-.1662	.0775
	2.00	1.00	.00812	.09736	1.000	-.2307	.2469
		3.00	-.03618	.09030	1.000	-.2577	.1853
	3.00	1.00	.04430	.04968	1.000	-.0775	.1662
		2.00	.03618	.09030	1.000	-.1853	.2577
Lectin13b	1.00	2.00	.00250	.14123	1.000	-.3439	.3489
		3.00	-.09627	.07206	.558	-.2730	.0805
	2.00	1.00	-.00250	.14123	1.000	-.3489	.3439
		3.00	-.09877	.13100	1.000	-.4201	.2225
	3.00	1.00	.09627	.07206	.558	-.0805	.2730
		2.00	.09877	.13100	1.000	-.2225	.4201
Lectin14b	1.00	2.00	-.02813	.11805	1.000	-.3177	.2614
		3.00	-.11968	.06024	.153	-.2674	.0281
	2.00	1.00	.02813	.11805	1.000	-.2614	.3177
		3.00	-.09156	.10950	1.000	-.3602	.1770
	3.00	1.00	.11968	.06024	.153	-.0281	.2674
		2.00	.09156	.10950	1.000	-.1770	.3602
Lectin15b	1.00	2.00	.02750	.14193	1.000	-.3206	.3756
		3.00	-.08608	.07242	.716	-.2637	.0916
	2.00	1.00	-.02750	.14193	1.000	-.3756	.3206
		3.00	-.11358	.13165	1.000	-.4365	.2093
	3.00	1.00	.08608	.07242	.716	-.0916	.2637
		2.00	.11358	.13165	1.000	-.2093	.4365
Lectin16b	1.00	2.00	-.07188	.18484	1.000	-.5253	.3815
		3.00	-.02471	.09432	1.000	-.2561	.2067
	2.00	1.00	.07188	.18484	1.000	-.3815	.5253
		3.00	.04717	.17146	1.000	-.3734	.4677
	3.00	1.00	.02471	.09432	1.000	-.2067	.2561
		2.00	-.04717	.17146	1.000	-.4677	.3734
Lectin17b	1.00	2.00	.13313	.16945	1.000	-.2825	.5488
		3.00	-.09669	.08647	.802	-.3088	.1154
	2.00	1.00	-.13313	.16945	1.000	-.5488	.2825
		3.00	-.22981	.15718	.445	-.6153	.1557

	3.00	1.00	.09669	.08647	.802	-.1154	.3088
		2.00	.22981	.15718	.445	-.1557	.6153
Lectin18b	1.00	2.00	.08562	.16665	1.000	-.3232	.4944
		3.00	-.11230	.08504	.573	-.3209	.0963
	2.00	1.00	-.08562	.16665	1.000	-.4944	.3232
		3.00	-.19792	.15458	.614	-.5771	.1812
	3.00	1.00	.11230	.08504	.573	-.0963	.3209
		2.00	.19792	.15458	.614	-.1812	.5771
Lectin19b	1.00	2.00	-.04375	.05887	1.000	-.1882	.1007
		3.00	-.03578	.03004	.713	-.1095	.0379
	2.00	1.00	.04375	.05887	1.000	-.1007	.1882
		3.00	.00797	.05461	1.000	-.1260	.1419
	3.00	1.00	.03578	.03004	.713	-.0379	.1095
		2.00	-.00797	.05461	1.000	-.1419	.1260
Lectin20b	1.00	2.00	.10750	.12117	1.000	-.1897	.4047
		3.00	-.03646	.06183	1.000	-.1881	.1152
	2.00	1.00	-.10750	.12117	1.000	-.4047	.1897
		3.00	-.14396	.11240	.613	-.4197	.1317
	3.00	1.00	.03646	.06183	1.000	-.1152	.1881
		2.00	.14396	.11240	.613	-.1317	.4197

6.6 Glycoprotein N

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Lectin1b	1	6	.2600	.04817	.01966	.2095	.3105	.19	.32
	3a	52	.2200	.13408	.01859	.1827	.2573	.09	.88
	4a	6	.2000	.08343	.03406	.1124	.2876	.13	.36
	4c	4	.2700	.12675	.06338	.0683	.4717	.16	.42
	Total	68	.2247	.12410	.01505	.1947	.2547	.09	.88
Lectin2b	1	6	.5700	.17504	.07146	.3863	.7537	.33	.84
	3a	52	.5431	.19168	.02658	.4897	.5964	.20	1.08
	4a	6	.5217	.10265	.04191	.4139	.6294	.39	.68
	4c	4	.5300	.11804	.05902	.3422	.7178	.36	.63
	Total	68	.5428	.17827	.02162	.4996	.5859	.20	1.08
Lectin3b	1	6	.6983	.18946	.07735	.4995	.8972	.46	.96
	3a	52	.6810	.27634	.03832	.6040	.7579	.32	1.48
	4a	6	.6533	.20829	.08504	.4347	.8719	.34	.93
	4c	4	.7200	.19983	.09992	.4020	1.0380	.47	.94
	Total	68	.6824	.25693	.03116	.6202	.7445	.32	1.48
Lectin4b	1	6	.8617	.24095	.09837	.6088	1.1145	.57	1.15
	3a	52	.7567	.35741	.04956	.6572	.8562	.30	2.27
	4a	6	.6433	.16293	.06652	.4723	.8143	.36	.79
	4c	4	.5975	.09106	.04553	.4526	.7424	.50	.68
	Total	68	.7466	.32783	.03975	.6673	.8260	.30	2.27
Lectin5b	1	6	.2200	.08462	.03454	.1312	.3088	.14	.37
	3a	52	.1933	.11160	.01548	.1622	.2243	.07	.74
	4a	6	.4650	.43071	.17584	.0130	.9170	.14	1.20
	4c	4	.2300	.10893	.05447	.0567	.4033	.10	.32
	Total	68	.2218	.17413	.02112	.1796	.2639	.07	1.20
Lectin6b	1	6	.7617	.20817	.08499	.5432	.9801	.52	1.02
	3a	52	.8562	.31980	.04435	.7671	.9452	.35	1.57
	4a	6	.7850	.31533	.12873	.4541	1.1159	.43	1.22
	4c	4	.7700	.25206	.12603	.3689	1.1711	.52	1.11
	Total	68	.8365	.30438	.03691	.7628	.9101	.35	1.57
Lectin7b	1	6	1.2350	.29304	.11963	.9275	1.5425	.82	1.53
	3a	52	1.0960	.57505	.07974	.9359	1.2561	.17	2.50
	4a	6	.8333	.54394	.22206	.2625	1.4042	.33	1.47
	4c	4	1.1025	.40525	.20262	.4577	1.7473	.72	1.51
	Total	68	1.0854	.54346	.06590	.9539	1.2170	.17	2.50
Lectin8b	1	6	.3917	.06853	.02798	.3197	.4636	.28	.49
	3a	52	.3829	.15991	.02217	.3384	.4274	.12	1.14
	4a	6	.3733	.13277	.05420	.2340	.5127	.18	.53
	4c	4	.4950	.16663	.08332	.2298	.7602	.27	.64
	Total	68	.3894	.15197	.01843	.3526	.4262	.12	1.14
Lectin9	1	6	.6933	.28479	.11627	.3945	.9922	.41	1.20

b	3a	52	.6933	.31691	.04395	.6050	.7815	.32	1.71
	4a	6	.5267	.12242	.04998	.3982	.6551	.34	.72
	4c	4	.6750	.10878	.05439	.5019	.8481	.56	.82
	Total	68	.6775	.29394	.03565	.6064	.7486	.32	1.71
Lectin10b	1	6	.6100	.26803	.10942	.3287	.8913	.15	.90
	3a	52	.6262	.22956	.03183	.5622	.6901	.27	1.26
	4a	6	.4717	.16654	.06799	.2969	.6464	.34	.80
	4c	4	.6375	.22794	.11397	.2748	1.0002	.41	.94
	Total	68	.6118	.22766	.02761	.5567	.6669	.15	1.26
Lectin11b	1	6	.2150	.07259	.02964	.1388	.2912	.14	.34
	3a	52	.2379	.12372	.01716	.2034	.2723	.09	.58
	4a	6	.2183	.08727	.03563	.1267	.3099	.13	.37
	4c	4	.3200	.20314	.10157	-.0032	.6432	.19	.62
	Total	68	.2390	.12224	.01482	.2094	.2686	.09	.62
Lectin12b	1	6	.1933	.07501	.03062	.1146	.2721	.11	.31
	3a	52	.1852	.18676	.02590	.1332	.2372	.07	1.39
	4a	6	.1617	.06401	.02613	.0945	.2288	.11	.27
	4c	4	.1900	.10231	.05115	.0272	.3528	.10	.33
	Total	68	.1841	.16674	.02022	.1438	.2245	.07	1.39
Lectin13b	1	6	.4333	.07789	.03180	.3516	.5151	.37	.58
	3a	52	.4465	.29608	.04106	.3641	.5290	.23	2.35
	4a	6	.3800	.07899	.03225	.2971	.4629	.26	.48
	4c	4	.4200	.11518	.05759	.2367	.6033	.27	.52
	Total	68	.4379	.26195	.03177	.3745	.5013	.23	2.35
Lectin14b	1	6	.6150	.19149	.07818	.4140	.8160	.38	.96
	3a	52	.5490	.22942	.03181	.4852	.6129	.26	1.39
	4a	6	.4633	.16561	.06761	.2895	.6371	.28	.72
	4c	4	.4625	.14751	.07375	.2278	.6972	.33	.60
	Total	68	.5422	.21741	.02637	.4896	.5948	.26	1.39
Lectin15b	1	6	.7233	.35229	.14382	.3536	1.0930	.11	1.16
	3a	52	.6792	.25776	.03575	.6075	.7510	.33	1.47
	4a	6	.6000	.17697	.07225	.4143	.7857	.37	.88
	4c	4	.6525	.14500	.07250	.4218	.8832	.48	.80
	Total	68	.6746	.25273	.03065	.6134	.7357	.11	1.47
Lectin16b	1	6	.5283	.50261	.20519	.0009	1.0558	.11	1.40
	3a	52	.3417	.41287	.05725	.2268	.4567	.07	2.41
	4a	6	.1267	.09543	.03896	.0265	.2268	.08	.32
	4c	4	.4425	.22515	.11257	.0842	.8008	.21	.72
	Total	68	.3451	.39926	.04842	.2485	.4418	.07	2.41
Lectin17b	1	6	.8167	.23192	.09468	.5733	1.0601	.52	1.03
	3a	52	.8769	.34520	.04787	.7808	.9730	.38	2.15
	4a	6	.6983	.18236	.07445	.5070	.8897	.40	.92
	4c	4	.7025	.29736	.14868	.2293	1.1757	.41	1.04
	Total	68	.8456	.32429	.03933	.7671	.9241	.38	2.15
Lectin18b	1	6	.8850	.55497	.22656	.3026	1.4674	.22	1.91
	3a	52	.7863	.29393	.04076	.7045	.8682	.31	1.51
	4a	6	.6317	.19426	.07931	.4278	.8355	.32	.93
	4c	4	.6950	.21917	.10958	.3463	1.0437	.45	.93
	Total	68	.7760	.31164	.03779	.7006	.8515	.22	1.91
Lectin19b	1	6	.3183	.08448	.03449	.2297	.4070	.20	.44
	3a	52	.2713	.11305	.01568	.2399	.3028	.08	.57
	4a	6	.2250	.07092	.02895	.1506	.2994	.13	.30
	4c	4	.3125	.10563	.05282	.1444	.4806	.16	.40
	Total	68	.2738	.10780	.01307	.2477	.2999	.08	.57
Lectin20b	1	6	.4617	.20331	.08300	.2483	.6750	.14	.74
	3a	52	.5294	.23926	.03318	.4628	.5960	.15	1.44
	4a	6	.4250	.13383	.05464	.2846	.5654	.22	.60
	4c	4	.5025	.16256	.08128	.2438	.7612	.31	.70
	Total	68	.5126	.22433	.02720	.4583	.5669	.14	1.44

ANOVA						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Lectin1b	Between Groups	.020	3	.007	.432	.731
	Within Groups	1.011	64	.016		
	Total	1.032	67			
Lectin2b	Between Groups	.008	3	.003	.078	.972
	Within Groups	2.121	64	.033		
	Total	2.129	67			
Lectin3b	Between Groups	.012	3	.004	.060	.981
	Within Groups	4.411	64	.069		
	Total	4.423	67			
Lectin4b	Between Groups	.238	3	.079	.728	.539
	Within Groups	6.963	64	.109		
	Total	7.201	67			
Lectin5b	Between Groups	.397	3	.132	5.189	.003
	Within Groups	1.634	64	.026		
	Total	2.032	67			
Lectin6b	Between Groups	.087	3	.029	.304	.822
	Within Groups	6.120	64	.096		
	Total	6.208	67			
Lectin7b	Between Groups	.522	3	.174	.579	.631
	Within Groups	19.266	64	.301		
	Total	19.788	67			
Lectin8b	Between Groups	.048	3	.016	.689	.562
	Within Groups	1.499	64	.023		
	Total	1.547	67			
Lectin9b	Between Groups	.151	3	.050	.571	.636
	Within Groups	5.638	64	.088		
	Total	5.789	67			
Lectin10b	Between Groups	.131	3	.044	.838	.478
	Within Groups	3.341	64	.052		
	Total	3.473	67			
Lectin11b	Between Groups	.032	3	.011	.712	.549
	Within Groups	.969	64	.015		
	Total	1.001	67			
Lectin12b	Between Groups	.004	3	.001	.043	.988
	Within Groups	1.859	64	.029		
	Total	1.863	67			
Lectin13b	Between Groups	.025	3	.008	.119	.949
	Within Groups	4.572	64	.071		
	Total	4.598	67			
Lectin14b	Between Groups	.097	3	.032	.674	.571
	Within Groups	3.070	64	.048		
	Total	3.167	67			
Lectin15b	Between Groups	.051	3	.017	.256	.857
	Within Groups	4.229	64	.066		
	Total	4.279	67			
Lectin16b	Between Groups	.526	3	.175	1.106	.353
	Within Groups	10.154	64	.159		
	Total	10.680	67			
Lectin17b	Between Groups	.268	3	.089	.844	.475
	Within Groups	6.778	64	.106		
	Total	7.046	67			
Lectin18b	Between Groups	.228	3	.076	.775	.512
	Within Groups	6.279	64	.098		
	Total	6.507	67			
Lectin19b	Between Groups	.032	3	.011	.929	.432
	Within Groups	.746	64	.012		
	Total	.779	67			

Lectin20b	Between Groups	.077	3	.026	.497	.686
	Within Groups	3.295	64	.051		
	Total	3.372	67			

Multiple Comparisons

Post hoc test: Bonferroni

Dependent Variable	(I) gN.r	(J) gN.r	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Lectin1b	1	3a	.04000	.05420	1.000	-.1076	.1876
		4a	.06000	.07258	1.000	-.1376	.2576
		4c	-.01000	.08115	1.000	-.2309	.2109
	3a	1	-.04000	.05420	1.000	-.1876	.1076
		4a	.02000	.05420	1.000	-.1276	.1676
		4c	-.05000	.06523	1.000	-.2276	.1276
	4a	1	-.06000	.07258	1.000	-.2576	.1376
		3a	-.02000	.05420	1.000	-.1676	.1276
		4c	-.07000	.08115	1.000	-.2909	.1509
	4c	1	.01000	.08115	1.000	-.2109	.2309
		3a	.05000	.06523	1.000	-.1276	.2276
		4a	.07000	.08115	1.000	-.1509	.2909
Lectin2b	1	3a	.02692	.07850	1.000	-.1868	.2407
		4a	.04833	.10511	1.000	-.2379	.3345
		4c	.04000	.11752	1.000	-.2800	.3600
	3a	1	-.02692	.07850	1.000	-.2407	.1868
		4a	.02141	.07850	1.000	-.1923	.2351
		4c	.01308	.09447	1.000	-.2441	.2703
	4a	1	-.04833	.10511	1.000	-.3345	.2379
		3a	-.02141	.07850	1.000	-.2351	.1923
		4c	-.00833	.11752	1.000	-.3283	.3116
	4c	1	-.04000	.11752	1.000	-.3600	.2800
		3a	-.01308	.09447	1.000	-.2703	.2441
		4a	.00833	.11752	1.000	-.3116	.3283
Lectin3b	1	3a	.01737	.11319	1.000	-.2908	.3256
		4a	.04500	.15157	1.000	-.3677	.4577
		4c	-.02167	.16946	1.000	-.4831	.4397
	3a	1	-.01737	.11319	1.000	-.3256	.2908
		4a	.02763	.11319	1.000	-.2806	.3358
		4c	-.03904	.13621	1.000	-.4099	.3318
	4a	1	-.04500	.15157	1.000	-.4577	.3677
		3a	-.02763	.11319	1.000	-.3358	.2806
		4c	-.06667	.16946	1.000	-.5281	.3947
	4c	1	.02167	.16946	1.000	-.4397	.4831
		3a	.03904	.13621	1.000	-.3318	.4099
		4a	.06667	.16946	1.000	-.3947	.5281
Lectin4b	1	3a	.10494	.14221	1.000	-.2823	.4921
		4a	.21833	.19043	1.000	-.3002	.7368
		4c	.26417	.21291	1.000	-.3155	.8439
	3a	1	-.10494	.14221	1.000	-.4921	.2823
		4a	.11340	.14221	1.000	-.2738	.5006
		4c	.15923	.17115	1.000	-.3068	.6252
	4a	1	-.21833	.19043	1.000	-.7368	.3002
		3a	-.11340	.14221	1.000	-.5006	.2738
		4c	.04583	.21291	1.000	-.5339	.6255
	4c	1	-.26417	.21291	1.000	-.8439	.3155
		3a	-.15923	.17115	1.000	-.6252	.3068
		4a	-.04583	.21291	1.000	-.6255	.5339
Lectin5b	1	3a	.02673	.06889	1.000	-.1609	.2143
		4a	-.24500	.09225	.060	-.4962	.0062
		4c	-.01000	.10314	1.000	-.2908	.2708
	3a	1	-.02673	.06889	1.000	-.2143	.1609
		4a	-.27173*	.06889	.001	-.4593	-.0841
		4c	-.03673	.08291	1.000	-.2625	.1890
	4a	1	.24500	.09225	.060	-.0062	.4962
		3a	.27173*	.06889	.001	.0841	.4593
		4c	.23500	.10314	.156	-.0458	.5158
	4c	1	.01000	.10314	1.000	-.2708	.2908
		3a	.03673	.08291	1.000	-.1890	.2625
		4a	-.23500	.10314	.156	-.5158	.0458
Lectin6b	1	3a	-.09449	.13333	1.000	-.4575	.2685
		4a	-.02333	.17854	1.000	-.5095	.4628
		4c	-.00833	.19961	1.000	-.5518	.5352
	3a	1	.09449	.13333	1.000	-.2685	.4575

		4a	.07115	.13333	1.000	-.2919	.4342
		4c	.08615	.16046	1.000	-.3507	.5230
	4a	1	.02333	.17854	1.000	-.4628	.5095
		3a	-.07115	.13333	1.000	-.4342	.2919
		4c	.01500	.19961	1.000	-.5285	.5585
	4c	1	.00833	.19961	1.000	-.5352	.5518
		3a	-.08615	.16046	1.000	-.5230	.3507
		4a	-.01500	.19961	1.000	-.5585	.5285
Lectin7b	1	3a	.13904	.23656	1.000	-.5051	.7831
		4a	.40167	.31677	1.000	-.4608	1.2642
		4c	.13250	.35416	1.000	-.8318	1.0968
	3a	1	-.13904	.23656	1.000	-.7831	.5051
		4a	.26263	.23656	1.000	-.3815	.9067
		4c	-.00654	.28469	1.000	-.7817	.7686
	4a	1	-.40167	.31677	1.000	-1.2642	.4608
		3a	-.26263	.23656	1.000	-.9067	.3815
		4c	-.26917	.35416	1.000	-1.2335	.6951
	4c	1	-.13250	.35416	1.000	-1.0968	.8318
		3a	.00654	.28469	1.000	-.7686	.7817
		4a	.26917	.35416	1.000	-.6951	1.2335
Lectin8b	1	3a	.00878	.06598	1.000	-.1709	.1884
		4a	.01833	.08836	1.000	-.2222	.2589
		4c	-.10333	.09879	1.000	-.3723	.1656
	3a	1	-.00878	.06598	1.000	-.1884	.1709
		4a	.00955	.06598	1.000	-.1701	.1892
		4c	-.11212	.07941	.977	-.3283	.1041
	4a	1	-.01833	.08836	1.000	-.2589	.2222
		3a	-.00955	.06598	1.000	-.1892	.1701
		4c	-.12167	.09879	1.000	-.3906	.1473
	4c	1	.10333	.09879	1.000	-.1656	.3723
		3a	.11212	.07941	.977	-.1041	.3283
		4a	.12167	.09879	1.000	-.1473	.3906
Lectin9b	1	3a	.00006	.12797	1.000	-.3484	.3485
		4a	.16667	.17136	1.000	-.2999	.6332
		4c	.01833	.19159	1.000	-.5033	.5400
	3a	1	-.00006	.12797	1.000	-.3485	.3484
		4a	.16660	.12797	1.000	-.1818	.5150
		4c	.01827	.15400	1.000	-.4010	.4376
	4a	1	-.16667	.17136	1.000	-.6332	.2999
		3a	-.16660	.12797	1.000	-.5150	.1818
		4c	-.14833	.19159	1.000	-.6700	.3733
	4c	1	-.01833	.19159	1.000	-.5400	.5033
		3a	-.01827	.15400	1.000	-.4376	.4010
		4a	.14833	.19159	1.000	-.3733	.6700
Lectin10b	1	3a	-.01615	.09852	1.000	-.2844	.2521
		4a	.13833	.13192	1.000	-.2209	.4975
		4c	-.02750	.14749	1.000	-.4291	.3741
	3a	1	.01615	.09852	1.000	-.2521	.2844
		4a	.15449	.09852	.731	-.1137	.4227
		4c	-.01135	.11856	1.000	-.3342	.3115
	4a	1	-.13833	.13192	1.000	-.4975	.2209
		3a	-.15449	.09852	.731	-.4227	.1137
		4c	-.16583	.14749	1.000	-.5674	.2357
	4c	1	.02750	.14749	1.000	-.3741	.4291
		3a	.01135	.11856	1.000	-.3115	.3342
		4a	.16583	.14749	1.000	-.2357	.5674
Lectin11b	1	3a	-.02288	.05305	1.000	-.1673	.1216
		4a	-.00333	.07104	1.000	-.1968	.1901
		4c	-.10500	.07942	1.000	-.3212	.1112
	3a	1	.02288	.05305	1.000	-.1216	.1673
		4a	.01955	.05305	1.000	-.1249	.1640
		4c	-.08212	.06384	1.000	-.2559	.0917
	4a	1	.00333	.07104	1.000	-.1901	.1968
		3a	-.01955	.05305	1.000	-.1640	.1249
		4c	-.10167	.07942	1.000	-.3179	.1146
	4c	1	.10500	.07942	1.000	-.1112	.3212
		3a	.08212	.06384	1.000	-.0917	.2559
		4a	.10167	.07942	1.000	-.1146	.3179
Lectin12b	1	3a	.00814	.07348	1.000	-.1919	.2082
		4a	.03167	.09840	1.000	-.2362	.2996
		4c	.00333	.11001	1.000	-.2962	.3029
	3a	1	-.00814	.07348	1.000	-.2082	.1919

		4a	.02353	.07348	1.000	-.1765	.2236
		4c	-.00481	.08843	1.000	-.2456	.2360
	4a	1	-.03167	.09840	1.000	-.2996	.2362
		3a	-.02353	.07348	1.000	-.2236	.1765
		4c	-.02833	.11001	1.000	-.3279	.2712
	4c	1	-.00333	.11001	1.000	-.3029	.2962
		3a	.00481	.08843	1.000	-.2360	.2456
		4a	.02833	.11001	1.000	-.2712	.3279
Lectin13b	1	3a	-.01321	.11524	1.000	-.3270	.3006
		4a	.05333	.15431	1.000	-.3668	.4735
		4c	.01333	.17253	1.000	-.4564	.4831
	3a	1	.01321	.11524	1.000	-.3006	.3270
		4a	.06654	.11524	1.000	-.2472	.3803
		4c	.02654	.13869	1.000	-.3511	.4041
	4a	1	-.05333	.15431	1.000	-.4735	.3668
		3a	-.06654	.11524	1.000	-.3803	.2472
		4c	-.04000	.17253	1.000	-.5098	.4298
	4c	1	-.01333	.17253	1.000	-.4831	.4564
		3a	-.02654	.13869	1.000	-.4041	.3511
		4a	.04000	.17253	1.000	-.4298	.5098
Lectin14b	1	3a	.06596	.09443	1.000	-.1912	.3231
		4a	.15167	.12645	1.000	-.1926	.4960
		4c	.15250	.14138	1.000	-.2324	.5374
	3a	1	-.06596	.09443	1.000	-.3231	.1912
		4a	.08571	.09443	1.000	-.1714	.3428
		4c	.08654	.11364	1.000	-.2229	.3960
	4a	1	-.15167	.12645	1.000	-.4960	.1926
		3a	-.08571	.09443	1.000	-.3428	.1714
		4c	.00083	.14138	1.000	-.3841	.3858
	4c	1	-.15250	.14138	1.000	-.5374	.2324
		3a	-.08654	.11364	1.000	-.3960	.2229
		4a	-.00083	.14138	1.000	-.3858	.3841
Lectin15b	1	3a	.04410	.11083	1.000	-.2577	.3459
		4a	.12333	.14841	1.000	-.2807	.5274
		4c	.07083	.16592	1.000	-.3809	.5226
	3a	1	-.04410	.11083	1.000	-.3459	.2577
		4a	.07923	.11083	1.000	-.2225	.3810
		4c	.02673	.13338	1.000	-.3364	.3899
	4a	1	-.12333	.14841	1.000	-.5274	.2807
		3a	-.07923	.11083	1.000	-.3810	.2225
		4c	-.05250	.16592	1.000	-.5043	.3993
	4c	1	-.07083	.16592	1.000	-.5226	.3809
		3a	-.02673	.13338	1.000	-.3899	.3364
		4a	.05250	.16592	1.000	-.3993	.5043
Lectin16b	1	3a	.18660	.17174	1.000	-.2810	.6542
		4a	.40167	.22997	.513	-.2245	1.0278
		4c	.08583	.25711	1.000	-.6142	.7859
	3a	1	-.18660	.17174	1.000	-.6542	.2810
		4a	.21506	.17174	1.000	-.2525	.6827
		4c	-.10077	.20668	1.000	-.6635	.4620
	4a	1	-.40167	.22997	.513	-1.0278	.2245
		3a	-.21506	.17174	1.000	-.6827	.2525
		4c	-.31583	.25711	1.000	-1.0159	.3842
	4c	1	-.08583	.25711	1.000	-.7859	.6142
		3a	.10077	.20668	1.000	-.4620	.6635
		4a	.31583	.25711	1.000	-.3842	1.0159
Lectin17b	1	3a	-.06026	.14031	1.000	-.4423	.3218
		4a	.11833	.18789	1.000	-.3932	.6299
		4c	.11417	.21006	1.000	-.4578	.6861
	3a	1	.06026	.14031	1.000	-.3218	.4423
		4a	.17859	.14031	1.000	-.2034	.5606
		4c	.17442	.16886	1.000	-.2853	.6342
	4a	1	-.11833	.18789	1.000	-.6299	.3932
		3a	-.17859	.14031	1.000	-.5606	.2034
		4c	-.00417	.21006	1.000	-.5761	.5678
	4c	1	-.11417	.21006	1.000	-.6861	.4578
		3a	-.17442	.16886	1.000	-.6342	.2853
		4a	.00417	.21006	1.000	-.5678	.5761
Lectin18b	1	3a	.09865	.13505	1.000	-.2690	.4664
		4a	.25333	.18084	.996	-.2390	.7457
		4c	.19000	.20218	1.000	-.3605	.7405

	3a	1	-.09865	.13505	1.000	-.4664	.2690
		4a	.15468	.13505	1.000	-.2130	.5224
		4c	.09135	.16252	1.000	-.3512	.5338
	4a	1	-.25333	.18084	.996	-.7457	.2390
		3a	-.15468	.13505	1.000	-.5224	.2130
		4c	-.06333	.20218	1.000	-.6138	.4872
	4c	1	-.19000	.20218	1.000	-.7405	.3605
		3a	-.09135	.16252	1.000	-.5338	.3512
		4a	.06333	.20218	1.000	-.4872	.6138
Lectin19b	1	3a	.04699	.04655	1.000	-.0798	.1737
		4a	.09333	.06234	.836	-.0764	.2631
		4c	.00583	.06970	1.000	-.1839	.1956
	3a	1	-.04699	.04655	1.000	-.1737	.0798
		4a	.04635	.04655	1.000	-.0804	.1731
		4c	-.04115	.05602	1.000	-.1937	.1114
	4a	1	-.09333	.06234	.836	-.2631	.0764
		3a	-.04635	.04655	1.000	-.1731	.0804
		4c	-.08750	.06970	1.000	-.2773	.1023
	4c	1	-.00583	.06970	1.000	-.1956	.1839
		3a	.04115	.05602	1.000	-.1114	.1937
		4a	.08750	.06970	1.000	-.1023	.2773
Lectin20b	1	3a	-.06776	.09783	1.000	-.3341	.1986
		4a	.03667	.13100	1.000	-.3200	.3934
		4c	-.04083	.14646	1.000	-.4396	.3580
	3a	1	.06776	.09783	1.000	-.1986	.3341
		4a	.10442	.09783	1.000	-.1619	.3708
		4c	.02692	.11773	1.000	-.2936	.3475
	4a	1	-.03667	.13100	1.000	-.3934	.3200
		3a	-.10442	.09783	1.000	-.3708	.1619
		4c	-.07750	.14646	1.000	-.4763	.3213
	4c	1	.04083	.14646	1.000	-.3580	.4396
		3a	-.02692	.11773	1.000	-.3475	.2936
		4a	.07750	.14646	1.000	-.3213	.4763

6.7 Glycoprotein O

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Lectin1b	1a	17	.2400	.08993	.02181	.1938	.2862	.14	.44
	1c	42	.2331	.14304	.02207	.1885	.2777	.09	.88
	4	6	.1783	.07885	.03219	.0956	.2611	.10	.33
	Total	65	.2298	.12609	.01564	.1986	.2611	.09	.88
Lectin2b	1a	17	.5482	.18719	.04540	.4520	.6445	.33	1.01
	1c	42	.5619	.20918	.03228	.4967	.6271	.20	1.14
	4	6	.4850	.10913	.04455	.3705	.5995	.36	.67
	Total	65	.5512	.19548	.02425	.5028	.5997	.20	1.14
Lectin3b	1a	17	.6600	.16688	.04048	.5742	.7458	.35	.93
	1c	42	.7005	.29487	.04550	.6086	.7924	.32	1.48
	4	6	.6967	.15449	.06307	.5345	.8588	.52	.94
	Total	65	.6895	.25465	.03159	.6264	.7526	.32	1.48
Lectin4b	1a	17	.7124	.22706	.05507	.5956	.8291	.37	1.18
	1c	42	.8100	.37948	.05855	.6917	.9283	.30	2.27
	4	6	.6667	.15016	.06130	.5091	.8242	.50	.90
	Total	65	.7712	.33142	.04111	.6891	.8534	.30	2.27
Lectin5b	1a	17	.3212	.27027	.06555	.1822	.4601	.13	1.20
	1c	42	.2045	.12675	.01956	.1650	.2440	.07	.74
	4	6	.1917	.09347	.03816	.0936	.2898	.08	.32
	Total	65	.2338	.17887	.02219	.1895	.2782	.07	1.20
Lectin6b	1a	17	.7924	.23931	.05804	.6693	.9154	.44	1.22
	1c	42	.9012	.35384	.05460	.7909	1.0115	.35	1.57
	4	6	.7767	.15782	.06443	.6110	.9423	.54	.94
	Total	65	.8612	.31536	.03912	.7831	.9394	.35	1.57
Lectin7b	1a	17	1.0241	.52975	.12848	.7517	1.2965	.17	1.81
	1c	42	1.1726	.55587	.08577	.9994	1.3458	.18	2.50
	4	6	1.0350	.31150	.12717	.7081	1.3619	.70	1.52

	Total	65	1.1211	.52974	.06571	.9898	1.2523	.17	2.50
Lectin8b	1a	17	.3941	.12400	.03007	.3304	.4579	.18	.64
	1c	42	.3807	.13243	.02043	.3394	.4220	.12	.70
	4	6	.6400	.33544	.13694	.2880	.9920	.28	1.14
	Total	65	.4082	.17163	.02129	.3656	.4507	.12	1.14
Lectin9b	1a	17	.6282	.22600	.05481	.5120	.7444	.36	1.25
	1c	42	.7388	.33846	.05223	.6333	.8443	.32	1.71
	4	6	.5600	.09839	.04017	.4567	.6633	.45	.68
	Total	65	.6934	.30177	.03743	.6186	.7682	.32	1.71
Lectin10b	1a	17	.5682	.24775	.06009	.4409	.6956	.15	.96
	1c	42	.6617	.24049	.03711	.5867	.7366	.28	1.26
	4	6	.5100	.13251	.05410	.3709	.6491	.31	.68
	Total	65	.6232	.23820	.02955	.5642	.6823	.15	1.26
Lectin11b	1a	17	.2235	.08624	.02092	.1792	.2679	.13	.46
	1c	42	.2552	.13897	.02144	.2119	.2985	.09	.59
	4	6	.2367	.18960	.07740	.0377	.4356	.12	.62
	Total	65	.2452	.13129	.01628	.2127	.2778	.09	.62
Lectin12b	1a	17	.1753	.06746	.01636	.1406	.2100	.09	.31
	1c	42	.2064	.22078	.03407	.1376	.2752	.07	1.39
	4	6	.1450	.09225	.03766	.0482	.2418	.08	.33
	Total	65	.1926	.18289	.02268	.1473	.2379	.07	1.39
Lectin13b	1a	17	.4159	.09663	.02344	.3662	.4656	.31	.65
	1c	42	.4724	.32778	.05058	.3702	.5745	.24	2.35
	4	6	.3833	.09626	.03930	.2823	.4844	.25	.52
	Total	65	.4494	.27007	.03350	.3825	.5163	.24	2.35
Lectin14b	1a	17	.5341	.18080	.04385	.4412	.6271	.27	1.02
	1c	42	.5783	.25462	.03929	.4990	.6577	.26	1.39
	4	6	.5300	.15033	.06137	.3722	.6878	.33	.66
	Total	65	.5623	.22792	.02827	.5058	.6188	.26	1.39
Lectin15b	1a	17	.6482	.22935	.05563	.5303	.7662	.11	1.06
	1c	42	.7236	.29305	.04522	.6323	.8149	.33	1.47
	4	6	.7733	.25137	.10262	.5095	1.0371	.49	1.04
	Total	65	.7085	.27315	.03388	.6408	.7761	.11	1.47
Lectin16b	1a	17	.3165	.35826	.08689	.1323	.5007	.07	1.40
	1c	42	.2600	.24588	.03794	.1834	.3366	.07	.79
	4	6	.3967	.29609	.12088	.0859	.7074	.08	.69
	Total	65	.2874	.28196	.03497	.2175	.3573	.07	1.40
Lectin17b	1a	17	.7894	.23610	.05726	.6680	.9108	.45	1.17
	1c	42	.8952	.33340	.05145	.7913	.9991	.38	2.15
	4	6	.7783	.26739	.10916	.4977	1.0589	.50	1.13
	Total	65	.8568	.30575	.03792	.7810	.9325	.38	2.15
Lectin18b	1a	17	.6882	.21640	.05248	.5770	.7995	.22	1.02
	1c	42	.8352	.34760	.05364	.7269	.9436	.31	1.91
	4	6	.6767	.16379	.06687	.5048	.8486	.45	.93
	Total	65	.7822	.31055	.03852	.7052	.8591	.22	1.91
Lectin19b	1a	17	.2812	.10283	.02494	.2283	.3340	.13	.44
	1c	42	.2845	.12011	.01853	.2471	.3220	.08	.57
	4	6	.2350	.07662	.03128	.1546	.3154	.17	.36
	Total	65	.2791	.11201	.01389	.2513	.3068	.08	.57
Lectin20b	1a	17	.4894	.14571	.03534	.4145	.5643	.14	.70
	1c	42	.5195	.24611	.03798	.4428	.5962	.15	1.44
	4	6	.5533	.18435	.07526	.3599	.7468	.35	.84
	Total	65	.5148	.21700	.02692	.4610	.5685	.14	1.44

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lectin1b	Between Groups	.018	2	.009	.562	.573
	Within Groups	.999	62	.016		
	Total	1.017	64			
Lectin2b	Between Groups	.031	2	.016	.401	.671
	Within Groups	2.414	62	.039		
	Total	2.446	64			

Lectin3b	Between Groups	.020	2	.010	.151	.860
	Within Groups	4.130	62	.067		
	Total	4.150	64			
Lectin4b	Between Groups	.188	2	.094	.850	.432
	Within Groups	6.842	62	.110		
	Total	7.030	64			
Lectin5b	Between Groups	.176	2	.088	2.923	.061
	Within Groups	1.871	62	.030		
	Total	2.048	64			
Lectin6b	Between Groups	.191	2	.095	.957	.390
	Within Groups	6.174	62	.100		
	Total	6.365	64			
Lectin7b	Between Groups	.316	2	.158	.555	.577
	Within Groups	17.644	62	.285		
	Total	17.960	64			
Lectin8b	Between Groups	.357	2	.179	7.254	.001
	Within Groups	1.528	62	.025		
	Total	1.885	64			
Lectin9b	Between Groups	.266	2	.133	1.480	.236
	Within Groups	5.562	62	.090		
	Total	5.828	64			
Lectin10b	Between Groups	.190	2	.095	1.715	.188
	Within Groups	3.441	62	.056		
	Total	3.631	64			
Lectin11b	Between Groups	.013	2	.006	.360	.699
	Within Groups	1.091	62	.018		
	Total	1.103	64			
Lectin12b	Between Groups	.027	2	.013	.392	.677
	Within Groups	2.114	62	.034		
	Total	2.141	64			
Lectin13b	Between Groups	.067	2	.034	.455	.637
	Within Groups	4.601	62	.074		
	Total	4.668	64			
Lectin14b	Between Groups	.031	2	.015	.288	.751
	Within Groups	3.294	62	.053		
	Total	3.325	64			
Lectin15b	Between Groups	.097	2	.048	.639	.531
	Within Groups	4.679	62	.075		
	Total	4.775	64			
Lectin16b	Between Groups	.118	2	.059	.733	.485
	Within Groups	4.971	62	.080		
	Total	5.088	64			
Lectin17b	Between Groups	.176	2	.088	.941	.396
	Within Groups	5.807	62	.094		
	Total	5.983	64			
Lectin18b	Between Groups	.335	2	.168	1.779	.177
	Within Groups	5.837	62	.094		
	Total	6.172	64			
Lectin19b	Between Groups	.013	2	.006	.509	.603
	Within Groups	.790	62	.013		
	Total	.803	64			
Lectin20b	Between Groups	.021	2	.010	.215	.807
	Within Groups	2.993	62	.048		
	Total	3.014	64			

Multiple Comparisons

Post hoc test: Bonferroni

Dependent Variable	(I) gO.r	(J) gO.r	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Lectin1b	1a	1c	.00690	.03650	1.000	-.0829	.0967
		4	.06167	.06029	.931	-.0867	.2100

	1c	1a	-.00690	.03650	1.000	-.0967	.0829
		4	.05476	.05541	.981	-.0816	.1911
	4	1a	-.06167	.06029	.931	-.2100	.0867
		1c	-.05476	.05541	.981	-.1911	.0816
Lectin2b	1a	1c	-.01367	.05672	1.000	-.1533	.1259
		4	.06324	.09370	1.000	-.1673	.2938
	1c	1a	.01367	.05672	1.000	-.1259	.1533
		4	.07690	.08612	1.000	-.1350	.2888
	4	1a	-.06324	.09370	1.000	-.2938	.1673
		1c	-.07690	.08612	1.000	-.2888	.1350
Lectin3b	1a	1c	-.04048	.07419	1.000	-.2230	.1421
		4	-.03667	.12256	1.000	-.3382	.2649
	1c	1a	.04048	.07419	1.000	-.1421	.2230
		4	.00381	.11264	1.000	-.2734	.2810
	4	1a	.03667	.12256	1.000	-.2649	.3382
		1c	-.00381	.11264	1.000	-.2810	.2734
Lectin4b	1a	1c	-.09765	.09549	.931	-.3326	.1373
		4	.04569	.15774	1.000	-.3425	.4338
	1c	1a	.09765	.09549	.931	-.1373	.3326
		4	.14333	.14498	.980	-.2134	.5001
	4	1a	-.04569	.15774	1.000	-.4338	.3425
		1c	-.14333	.14498	.980	-.5001	.2134
Lectin5b	1a	1c	.11665	.04994	.068	-.0062	.2395
		4	.12951	.08249	.365	-.0735	.3325
	1c	1a	-.11665	.04994	.068	-.2395	.0062
		4	.01286	.07582	1.000	-.1737	.1994
	4	1a	-.12951	.08249	.365	-.3325	.0735
		1c	-.01286	.07582	1.000	-.1994	.1737
Lectin6b	1a	1c	-.10884	.09071	.704	-.3321	.1144
		4	.01569	.14985	1.000	-.3530	.3844
	1c	1a	.10884	.09071	.704	-.1144	.3321
		4	.12452	.13773	1.000	-.2144	.4634
	4	1a	-.01569	.14985	1.000	-.3844	.3530
		1c	-.12452	.13773	1.000	-.4634	.2144
Lectin7b	1a	1c	-.14850	.15335	1.000	-.5258	.2288
		4	-.01088	.25332	1.000	-.6342	.6125
	1c	1a	.14850	.15335	1.000	-.2288	.5258
		4	.13762	.23282	1.000	-.4353	.7105
	4	1a	.01088	.25332	1.000	-.6125	.6342
		1c	-.13762	.23282	1.000	-.7105	.4353
Lectin8b	1a	1c	.01340	.04512	1.000	-.0976	.1244
		4	-.24588*	.07454	.005	-.4293	-.0625
	1c	1a	-.01340	.04512	1.000	-.1244	.0976
		4	-.25929*	.06851	.001	-.4279	-.0907
	4	1a	.24588*	.07454	.005	.0625	.4293
		1c	.25929*	.06851	.001	.0907	.4279
Lectin9b	1a	1c	-.11057	.08610	.612	-.3224	.1013
		4	.06824	.14223	1.000	-.2818	.4182
	1c	1a	.11057	.08610	.612	-.1013	.3224
		4	.17881	.13073	.529	-.1429	.5005
	4	1a	-.06824	.14223	1.000	-.4182	.2818
		1c	-.17881	.13073	.529	-.5005	.1429
Lectin10b	1a	1c	-.09343	.06772	.518	-.2601	.0732
		4	.05824	.11187	1.000	-.2170	.3335
	1c	1a	.09343	.06772	.518	-.0732	.2601
		4	.15167	.10282	.436	-.1013	.4047
	4	1a	-.05824	.11187	1.000	-.3335	.2170
		1c	-.15167	.10282	.436	-.4047	.1013
Lectin11b	1a	1c	-.03171	.03812	1.000	-.1255	.0621
		4	-.01314	.06298	1.000	-.1681	.1418
	1c	1a	.03171	.03812	1.000	-.0621	.1255
		4	.01857	.05788	1.000	-.1239	.1610
	4	1a	.01314	.06298	1.000	-.1418	.1681
		1c	-.01857	.05788	1.000	-.1610	.1239
Lectin12b	1a	1c	-.03113	.05308	1.000	-.1617	.0995
		4	.03029	.08768	1.000	-.1855	.2461
	1c	1a	.03113	.05308	1.000	-.0995	.1617
		4	.06143	.08059	1.000	-.1369	.2597
	4	1a	-.03029	.08768	1.000	-.2461	.1855
		1c	-.06143	.08059	1.000	-.2597	.1369
Lectin13b	1a	1c	-.05650	.07831	1.000	-.2492	.1362
		4	.03255	.12935	1.000	-.2857	.3508
	1c	1a	.05650	.07831	1.000	-.1362	.2492
		4	.08905	.11889	1.000	-.2035	.3816
	4	1a	-.03255	.12935	1.000	-.3508	.2857
		1c	-.08905	.11889	1.000	-.3816	.2035

Lectin14b	1a	1c	-.04422	.06626	1.000	-.2073	.1188
		4	.00412	.10946	1.000	-.2652	.2735
	1c	1a	.04422	.06626	1.000	-.1188	.2073
		4	.04833	.10060	1.000	-.1992	.2959
	4	1a	-.00412	.10946	1.000	-.2735	.2652
		1c	-.04833	.10060	1.000	-.2959	.1992
Lectin15b	1a	1c	-.07534	.07897	1.000	-.2696	.1190
		4	-.12510	.13044	1.000	-.4461	.1959
	1c	1a	.07534	.07897	1.000	-.1190	.2696
		4	-.04976	.11989	1.000	-.3448	.2452
	4	1a	.12510	.13044	1.000	-.1959	.4461
		1c	.04976	.11989	1.000	-.2452	.3448
Lectin16b	1a	1c	.05647	.08139	1.000	-.1438	.2568
		4	-.08020	.13446	1.000	-.4110	.2507
	1c	1a	-.05647	.08139	1.000	-.2568	.1438
		4	-.13667	.12358	.819	-.4407	.1674
	4	1a	.08020	.13446	1.000	-.2507	.4110
		1c	.13667	.12358	.819	-.1674	.4407
Lectin17b	1a	1c	-.10583	.08797	.701	-.3223	.1106
		4	.01108	.14532	1.000	-.3465	.3687
	1c	1a	.10583	.08797	.701	-.1106	.3223
		4	.11690	.13357	1.000	-.2118	.4456
	4	1a	-.01108	.14532	1.000	-.3687	.3465
		1c	-.11690	.13357	1.000	-.4456	.2118
Lectin18b	1a	1c	-.14700	.08820	.302	-.3640	.0700
		4	.01157	.14570	1.000	-.3470	.3701
	1c	1a	.14700	.08820	.302	-.0700	.3640
		4	.15857	.13391	.723	-.1709	.4881
	4	1a	-.01157	.14570	1.000	-.3701	.3470
		1c	-.15857	.13391	.723	-.4881	.1709
Lectin19b	1a	1c	-.00335	.03245	1.000	-.0832	.0765
		4	.04618	.05360	1.000	-.0857	.1781
	1c	1a	.00335	.03245	1.000	-.0765	.0832
		4	.04952	.04926	.956	-.0717	.1707
	4	1a	-.04618	.05360	1.000	-.1781	.0857
		1c	-.04952	.04926	.956	-.1707	.0717
Lectin20b	1a	1c	-.03011	.06316	1.000	-.1855	.1253
		4	-.06392	.10433	1.000	-.3207	.1928
	1c	1a	.03011	.06316	1.000	-.1253	.1855
		4	-.03381	.09589	1.000	-.2698	.2021
	4	1a	.06392	.10433	1.000	-.1928	.3207
		1c	.03381	.09589	1.000	-.2021	.2698

Appendix 7: Tables of One-way ANOVA results for the relation between HCMV infection category and the glycosylation of the glycoproteins.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Lectin1b	congenital infection	12	.2017	.09861	.02847	.1390	.2643	.11	.42
	Not known infection	5	.2340	.07893	.03530	.1360	.3320	.15	.33
	Primary from Immunocompetent	7	.1886	.07290	.02755	.1212	.2560	.09	.29
	Primary from immunocompromized	26	.2238	.09475	.01858	.1856	.2621	.09	.45
	Recurrent Infections	35	.2306	.14828	.02506	.1796	.2815	.09	.88
	Total	85	.2212	.11710	.01270	.1959	.2464	.09	.88
Lectin2b	congenital infection	12	.5342	.16059	.04636	.4321	.6362	.20	.71
	Not known infection	5	.4640	.08142	.03641	.3629	.5651	.34	.55
	Primary from Immunocompetent	7	.4786	.09459	.03575	.3911	.5661	.35	.58
	Primary from immunocompromized	26	.5827	.25029	.04909	.4816	.6838	.34	1.17
	Recurrent Infections	35	.5614	.17043	.02881	.5029	.6200	.29	1.08
	Total	85	.5515	.18978	.02058	.5106	.5925	.20	1.17
Lectin3b	congenital infection	12	.5950	.10379	.02996	.5291	.6609	.40	.75
	Not known infection	5	.6280	.14096	.06304	.4530	.8030	.48	.82
	Primary from Immunocompetent	7	.7443	.23042	.08709	.5312	.9574	.39	1.01
	Primary from immunocompromized	26	.7077	.21068	.04132	.6226	.7928	.34	1.23
	Recurrent Infections	35	.7391	.32099	.05426	.6289	.8494	.32	1.48
	Total	85	.7031	.25250	.02739	.6486	.7575	.32	1.48
Lectin4b	congenital infection	12	.6842	.17650	.05095	.5720	.7963	.41	1.05
	Not known infection	5	.7960	.36842	.16476	.3386	1.2534	.50	1.38
	Primary from Immunocompetent	7	.7414	.28257	.10680	.4801	1.0028	.30	1.15
	Primary from immunocompromized	26	.7738	.29754	.05835	.6537	.8940	.36	1.48
	Recurrent Infections	35	.7777	.36269	.06131	.6531	.9023	.41	2.27
	Total	85	.7614	.31138	.03377	.6942	.8286	.30	2.27
Lectin5b	congenital infection	12	.1758	.09491	.02740	.1155	.2361	.08	.37
	Not known infection	5	.2880	.16285	.07283	.0858	.4902	.16	.52
	Primary from Immunocompetent	7	.2757	.40898	.15458	-.1025	.6540	.07	1.20
	Primary from immunocompromized	26	.2046	.07850	.01539	.1729	.2363	.10	.42

	Recurrent Infections	35	.2314	.16223	.02742	.1757	.2872	.08	.78
	Total	85	.2224	.16677	.01809	.1864	.2583	.07	1.20
Lectin6b	congenital infection	12	.7975	.22872	.06602	.6522	.9428	.50	1.29
	Not known infection	5	.7900	.34037	.15222	.3674	1.2126	.35	1.15
	Primary from Immunocompetent	7	.9929	.25824	.09761	.7540	1.2317	.68	1.36
	Primary from immunocompromized	26	.8173	.30364	.05955	.6947	.9400	.43	1.57
	Recurrent Infections	35	.8640	.31190	.05272	.7569	.9711	.43	1.57
	Total	85	.8466	.29437	.03193	.7831	.9101	.35	1.57
Lectin7b	congenital infection	12	.9908	.32318	.09329	.7855	1.1962	.53	1.55
	Not known infection	5	1.0040	.55797	.24953	.3112	1.6968	.18	1.44
	Primary from Immunocompetent	7	1.1500	.69632	.26319	.5060	1.7940	.35	2.08
	Primary from immunocompromized	26	1.1631	.54500	.10688	.9429	1.3832	.17	2.50
	Recurrent Infections	35	1.0660	.51491	.08704	.8891	1.2429	.33	2.41
	Total	85	1.0884	.51273	.05561	.9778	1.1989	.17	2.50
Lectin8b	congenital infection	12	.3583	.09456	.02730	.2982	.4184	.24	.54
	Not known infection	5	.4720	.13936	.06232	.2990	.6450	.27	.65
	Primary from Immunocompetent	7	.3386	.13533	.05115	.2134	.4637	.12	.56
	Primary from immunocompromized	26	.4400	.18564	.03641	.3650	.5150	.15	.95
	Recurrent Infections	35	.3891	.17406	.02942	.3294	.4489	.18	1.14
	Total	85	.4011	.16534	.01793	.3654	.4367	.12	1.14
Lectin9b	congenital infection	12	.5283	.11527	.03328	.4551	.6016	.35	.74
	Not known infection	5	.6100	.19339	.08649	.3699	.8501	.42	.92
	Primary from Immunocompetent	7	.7314	.25680	.09706	.4939	.9689	.48	1.20
	Primary from immunocompromized	26	.6538	.24736	.04851	.5539	.7538	.34	1.35
	Recurrent Infections	35	.7169	.33036	.05584	.6034	.8303	.32	1.71
	Total	85	.6659	.27385	.02970	.6068	.7249	.32	1.71
Lectin10b	congenital infection	12	.6642	.17916	.05172	.5503	.7780	.36	.92
	Not known infection	5	.5300	.22226	.09940	.2540	.8060	.31	.84
	Primary from Immunocompetent	7	.5314	.25790	.09748	.2929	.7699	.27	.95
	Primary from immunocompromized	26	.6446	.25439	.04989	.5419	.7474	.26	1.02
	Recurrent Infections	35	.6283	.23814	.04025	.5465	.7101	.15	1.26
	Total	85	.6246	.23480	.02547	.5739	.6752	.15	1.26
Lectin11b	congenital infection	12	.2250	.09140	.02639	.1669	.2831	.10	.43

	Not known infection	5	.2100	.05339	.02387	.1437	.2763	.15	.27
	Primary from Immunocompetent	7	.1686	.11481	.04339	.0624	.2748	.09	.42
	Primary from immunocompromized	26	.2719	.14541	.02852	.2132	.3307	.08	.62
	Recurrent Infections	35	.2323	.11941	.02018	.1913	.2733	.11	.54
	Total	85	.2368	.12279	.01332	.2103	.2633	.08	.62
Lectin12b	congenital infection	12	.1633	.06315	.01823	.1232	.2035	.09	.26
	Not known infection	5	.1460	.04506	.02015	.0901	.2019	.08	.20
	Primary from Immunocompetent	7	.1171	.04572	.01728	.0749	.1594	.07	.21
	Primary from immunocompromized	26	.1865	.12753	.02501	.1350	.2380	.09	.71
	Recurrent Infections	35	.2066	.22295	.03768	.1300	.2832	.08	1.39
	Total	85	.1834	.16267	.01764	.1483	.2185	.07	1.39
Lectin13b	congenital infection	12	.4267	.07703	.02224	.3777	.4756	.29	.54
	Not known infection	5	.4000	.08888	.03975	.2896	.5104	.32	.53
	Primary from Immunocompetent	7	.3586	.12034	.04548	.2473	.4699	.23	.59
	Primary from immunocompromized	26	.4038	.12384	.02429	.3538	.4539	.17	.67
	Recurrent Infections	35	.4826	.35077	.05929	.3621	.6031	.24	2.35
	Total	85	.4355	.24159	.02620	.3834	.4876	.17	2.35
Lectin14b	congenital infection	12	.5408	.12369	.03571	.4622	.6194	.28	.73
	Not known infection	5	.4680	.14957	.06689	.2823	.6537	.27	.66
	Primary from Immunocompetent	7	.4986	.24162	.09132	.2751	.7220	.26	.96
	Primary from immunocompromized	26	.5331	.18992	.03725	.4564	.6098	.21	1.02
	Recurrent Infections	35	.5780	.24632	.04164	.4934	.6626	.27	1.39
	Total	85	.5460	.20872	.02264	.5010	.5910	.21	1.39
Lectin15b	congenital infection	12	.6775	.19127	.05521	.5560	.7990	.41	1.02
	Not known infection	5	.5760	.17757	.07941	.3555	.7965	.33	.78
	Primary from Immunocompetent	7	.6971	.23078	.08722	.4837	.9106	.48	1.16
	Primary from immunocompromized	26	.6715	.20474	.04015	.5888	.7542	.37	1.06
	Recurrent Infections	35	.7043	.32297	.05459	.5933	.8152	.11	1.47
	Total	85	.6824	.25636	.02781	.6271	.7376	.11	1.47

Lectin16b	congenital infection	12	.7175	.66102	.19082	.2975	1.1375	.08	2.41
	Not known infection	5	.6420	1.20670	.53965	-.8563	2.1403	.08	2.80
	Primary from Immunocompetent	7	.2400	.22353	.08449	.0333	.4467	.07	.65
	Primary from immunocompromized	26	.3069	.34635	.06792	.1670	.4468	.07	1.46
	Recurrent Infections	35	.3626	.27819	.04702	.2670	.4581	.07	.81
	Total	85	.4020	.46951	.05093	.3007	.5033	.07	2.80
Lectin17b	congenital infection	12	.8275	.29564	.08534	.6397	1.0153	.46	1.49
	Not known infection	5	.8380	.19189	.08581	.5997	1.0763	.54	1.02
	Primary from Immunocompetent	7	.7443	.15263	.05769	.6031	.8854	.53	1.01
	Primary from immunocompromized	26	.8288	.29269	.05740	.7106	.9471	.40	1.36
	Recurrent Infections	35	.8889	.34770	.05877	.7694	1.0083	.48	2.15
	Total	85	.8469	.30177	.03273	.7819	.9120	.40	2.15
Lectin18b	congenital infection	12	.7750	.13318	.03845	.6904	.8596	.59	1.04
	Not known infection	5	.7380	.30752	.13753	.3562	1.1198	.43	1.13
	Primary from Immunocompetent	7	.8586	.51454	.19448	.3827	1.3344	.31	1.91
	Primary from immunocompromised	26	.7492	.24764	.04857	.6492	.8493	.32	1.44
	Recurrent Infections	35	.8126	.29771	.05032	.7103	.9148	.22	1.51
	Total	85	.7873	.28490	.03090	.7258	.8487	.22	1.91
Lectin19b	congenital infection	12	.2492	.08816	.02545	.1932	.3052	.12	.43
	Not known infection	5	.2160	.07092	.03172	.1279	.3041	.12	.31
	Primary from Immunocompetent	7	.2143	.12660	.04785	.0972	.3314	.08	.39
	Primary from immunocompromised	26	.2612	.09301	.01824	.2236	.2987	.11	.46
	Recurrent Infections	35	.2989	.11641	.01968	.2589	.3388	.13	.57
	Total	85	.2685	.10642	.01154	.2455	.2914	.08	.57
Lectin20b	congenital infection	12	.4483	.06043	.01744	.4099	.4867	.32	.54
	Not known infection	5	.5260	.15962	.07139	.3278	.7242	.37	.72
	Primary from Immunocompetent	7	.5557	.22127	.08363	.3511	.7604	.15	.78
	Primary from immunocompromized	26	.5208	.14832	.02909	.4609	.5807	.22	.84

	Recurrent Infections	35	.5426	.26912	.04549	.4501	.6350	.14	1.44
	Total	85	.5227	.20518	.02225	.4784	.5670	.14	1.44

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lectin1b	Between Groups	.016	4	.004	.284	.888
	Within Groups	1.136	80	.014		
	Total	1.152	84			
Lectin2b	Between Groups	.108	4	.027	.739	.568
	Within Groups	2.918	80	.036		
	Total	3.026	84			
Lectin3b	Between Groups	.226	4	.057	.882	.478
	Within Groups	5.129	80	.064		
	Total	5.356	84			
Lectin4b	Between Groups	.094	4	.023	.233	.919
	Within Groups	8.051	80	.101		
	Total	8.144	84			
Lectin5b	Between Groups	.079	4	.020	.696	.597
	Within Groups	2.258	80	.028		
	Total	2.336	84			
Lectin6b	Between Groups	.228	4	.057	.646	.632
	Within Groups	7.052	80	.088		
	Total	7.279	84			
Lectin7b	Between Groups	.339	4	.085	.312	.869
	Within Groups	21.744	80	.272		
	Total	22.083	84			
Lectin8b	Between Groups	.119	4	.030	1.091	.367
	Within Groups	2.178	80	.027		
	Total	2.296	84			
Lectin9b	Between Groups	.367	4	.092	1.239	.301
	Within Groups	5.932	80	.074		
	Total	6.299	84			
Lectin10b	Between Groups	.135	4	.034	.601	.663
	Within Groups	4.496	80	.056		
	Total	4.631	84			
Lectin11b	Between Groups	.071	4	.018	1.181	.325
	Within Groups	1.196	80	.015		
	Total	1.266	84			
Lectin12b	Between Groups	.062	4	.015	.570	.685
	Within Groups	2.161	80	.027		
	Total	2.223	84			
Lectin13b	Between Groups	.152	4	.038	.641	.635
	Within Groups	4.750	80	.059		
	Total	4.903	84			
Lectin14b	Between Groups	.087	4	.022	.485	.747
	Within Groups	3.573	80	.045		
	Total	3.659	84			
Lectin15b	Between Groups	.078	4	.020	.288	.885
	Within Groups	5.442	80	.068		
	Total	5.521	84			
Lectin16b	Between Groups	1.956	4	.489	2.362	.060
	Within Groups	16.561	80	.207		
	Total	18.517	84			
Lectin17b	Between Groups	.149	4	.037	.397	.811
	Within Groups	7.501	80	.094		
	Total	7.649	84			
Lectin18b	Between Groups	.110	4	.027	.327	.859
	Within Groups	6.709	80	.084		
	Total	6.818	84			

Lectin19b	Between Groups	.072	4	.018	1.650	.170
	Within Groups	.879	80	.011		
	Total	.951	84			
Lectin20b	Between Groups	.088	4	.022	.510	.728
	Within Groups	3.448	80	.043		
	Total	3.536	84			

Multiple Comparison

Post hoc test: Bonferroni

Dependent Variable	(I) Infection type with not known.r	(J) (I) Infection type with not known.r	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Lectin1b	congenital infection	Not known infection	-.03233	.06342	1.000	-.2154	.1508
		Primary from Immunocompetent	.01310	.05667	1.000	-.1505	.1767
		Primary from immunocompromized	-.02218	.04158	1.000	-.1422	.0979
		Recurrent Infections	-.02890	.03986	1.000	-.1440	.0862
	Not known infection	congenital infection	.03233	.06342	1.000	-.1508	.2154
		Primary from Immunocompetent	.04543	.06977	1.000	-.1560	.2468
		Primary from immunocompromized	.01015	.05818	1.000	-.1578	.1781
		Recurrent Infections	.00343	.05697	1.000	-.1610	.1679
	Primary from Immunocompetent	congenital infection	-.01310	.05667	1.000	-.1767	.1505
		Not known infection	-.04543	.06977	1.000	-.2468	.1560
		Primary from immunocompromized	-.03527	.05074	1.000	-.1818	.1112
		Recurrent Infections	-.04200	.04933	1.000	-.1844	.1004
	Primary from immunocompromized	congenital infection	.02218	.04158	1.000	-.0979	.1422
		Not known infection	-.01015	.05818	1.000	-.1781	.1578
		Primary from Immunocompetent	.03527	.05074	1.000	-.1112	.1818
		Recurrent Infections	-.00673	.03085	1.000	-.0958	.0823
	Recurrent Infections	congenital infection	.02890	.03986	1.000	-.0862	.1440
		Not known infection	-.00343	.05697	1.000	-.1679	.1610
		Primary from Immunocompetent	.04200	.04933	1.000	-.1004	.1844
		Primary from immunocompromized	.00673	.03085	1.000	-.0823	.0958
Lectin2b	congenital infection	Not known infection	.07017	.10165	1.000	-.2233	.3636
		Primary from Immunocompetent	.05560	.09083	1.000	-.2066	.3178
		Primary from immunocompromized	-.04853	.06665	1.000	-.2409	.1439
		Recurrent Infections	-.02726	.06388	1.000	-.2117	.1572
	Not known infection	congenital infection	-.07017	.10165	1.000	-.3636	.2233
		Primary from Immunocompetent	-.01457	.11182	1.000	-.3374	.3083

		Primary from immunocompromized	-.11869	.09326	1.000	-.3879	.1505
		Recurrent Infections	-.09743	.09130	1.000	-.3610	.1662
	Primary from Immunocompetent	congenital infection	-.05560	.09083	1.000	-.3178	.2066
		Not known infection	.01457	.11182	1.000	-.3083	.3374
		Primary from immunocompromized	-.10412	.08132	1.000	-.3389	.1306
		Recurrent Infections	-.08286	.07907	1.000	-.3111	.1454
	Primary from immunocompromized	congenital infection	.04853	.06665	1.000	-.1439	.2409
		Not known infection	.11869	.09326	1.000	-.1505	.3879
		Primary from Immunocompetent	.10412	.08132	1.000	-.1306	.3389
		Recurrent Infections	.02126	.04944	1.000	-.1215	.1640
	Recurrent Infections	congenital infection	.02726	.06388	1.000	-.1572	.2117
		Not known infection	.09743	.09130	1.000	-.1662	.3610
		Primary from Immunocompetent	.08286	.07907	1.000	-.1454	.3111
		Primary from immunocompromized	-.02126	.04944	1.000	-.1640	.1215
Lectin3b	congenital infection	Not known infection	-.03300	.13478	1.000	-.4221	.3561
		Primary from Immunocompetent	-.14929	.12043	1.000	-.4970	.1984
		Primary from immunocompromized	-.11269	.08837	1.000	-.3678	.1424
		Recurrent Infections	-.14414	.08470	.927	-.3887	.1004
	Not known infection	congenital infection	.03300	.13478	1.000	-.3561	.4221
		Primary from Immunocompetent	-.11629	.14827	1.000	-.5443	.3118
		Primary from immunocompromized	-.07969	.12365	1.000	-.4367	.2773
		Recurrent Infections	-.11114	.12106	1.000	-.4606	.2383
	Primary from Immunocompetent	congenital infection	.14929	.12043	1.000	-.1984	.4970
		Not known infection	.11629	.14827	1.000	-.3118	.5443
		Primary from immunocompromized	.03659	.10782	1.000	-.2747	.3479
		Recurrent Infections	.00514	.10484	1.000	-.2975	.3078
	Primary from immunocompromized	congenital infection	.11269	.08837	1.000	-.1424	.3678
		Not known infection	.07969	.12365	1.000	-.2773	.4367
		Primary from Immunocompetent	-.03659	.10782	1.000	-.3479	.2747
		Recurrent Infections	-.03145	.06556	1.000	-.2207	.1578
	Recurrent Infections	congenital infection	.14414	.08470	.927	-.1004	.3887
		Not known infection	.11114	.12106	1.000	-.2383	.4606
		Primary from Immunocompetent	-.00514	.10484	1.000	-.3078	.2975
		Primary from immunocompromized	.03145	.06556	1.000	-.1578	.2207

Lectin4b	congenital infection	Not known infection	-.11183	.16886	1.000	-.5993	.3756
		Primary from Immunocompetent	-.05726	.15087	1.000	-.4928	.3783
		Primary from immunocompromized	-.08968	.11071	1.000	-.4093	.2299
		Recurrent Infections	-.09355	.10612	1.000	-.3999	.2128
	Not known infection	congenital infection	.11183	.16886	1.000	-.3756	.5993
		Primary from Immunocompetent	.05457	.18575	1.000	-.4817	.5908
		Primary from immunocompromized	.02215	.15491	1.000	-.4251	.4694
		Recurrent Infections	.01829	.15166	1.000	-.4196	.4561
	Primary from Immunocompetent	congenital infection	.05726	.15087	1.000	-.3783	.4928
		Not known infection	-.05457	.18575	1.000	-.5908	.4817
		Primary from immunocompromized	-.03242	.13508	1.000	-.4224	.3576
		Recurrent Infections	-.03629	.13134	1.000	-.4155	.3429
	Primary from immunocompromized	congenital infection	.08968	.11071	1.000	-.2299	.4093
		Not known infection	-.02215	.15491	1.000	-.4694	.4251
		Primary from Immunocompetent	.03242	.13508	1.000	-.3576	.4224
		Recurrent Infections	-.00387	.08213	1.000	-.2410	.2332
	Recurrent Infections	congenital infection	.09355	.10612	1.000	-.2128	.3999
		Not known infection	-.01829	.15166	1.000	-.4561	.4196
		Primary from Immunocompetent	.03629	.13134	1.000	-.3429	.4155
		Primary from immunocompromized	.00387	.08213	1.000	-.2332	.2410
Lectin5b	congenital infection	Not known infection	-.11217	.08942	1.000	-.3703	.1460
		Primary from Immunocompetent	-.09988	.07989	1.000	-.3305	.1308
		Primary from immunocompromized	-.02878	.05863	1.000	-.1980	.1405
		Recurrent Infections	-.05560	.05620	1.000	-.2178	.1066
	Not known infection	congenital infection	.11217	.08942	1.000	-.1460	.3703
		Primary from Immunocompetent	.01229	.09836	1.000	-.2717	.2963
		Primary from immunocompromized	.08338	.08203	1.000	-.1534	.3202
		Recurrent Infections	.05657	.08031	1.000	-.1753	.2884
	Primary from Immunocompetent	congenital infection	.09988	.07989	1.000	-.1308	.3305
		Not known infection	-.01229	.09836	1.000	-.2963	.2717
		Primary from immunocompromized	.07110	.07153	1.000	-.1354	.2776
		Recurrent Infections	.04429	.06955	1.000	-.1565	.2451
	Primary from immunocompromized	congenital infection	.02878	.05863	1.000	-.1405	.1980
		Not known infection	-.08338	.08203	1.000	-.3202	.1534

		Primary from Immunocompetent	-.07110	.07153	1.000	-.2776	.1354
		Recurrent Infections	-.02681	.04349	1.000	-.1524	.0988
	Recurrent Infections	congenital infection	.05560	.05620	1.000	-.1066	.2178
		Not known infection	-.05657	.08031	1.000	-.2884	.1753
		Primary from Immunocompetent	-.04429	.06955	1.000	-.2451	.1565
		Primary from immunocompromized	.02681	.04349	1.000	-.0988	.1524
Lectin6b	congenital infection	Not known infection	.00750	.15803	1.000	-.4487	.4637
		Primary from Immunocompetent	-.19536	.14120	1.000	-.6030	.2123
		Primary from immunocompromized	-.01981	.10361	1.000	-.3189	.2793
		Recurrent Infections	-.06650	.09932	1.000	-.3532	.2202
	Not known infection	congenital infection	-.00750	.15803	1.000	-.4637	.4487
		Primary from Immunocompetent	-.20286	.17384	1.000	-.7047	.2990
		Primary from immunocompromized	-.02731	.14498	1.000	-.4459	.3912
		Recurrent Infections	-.07400	.14194	1.000	-.4838	.3358
	Primary from Immunocompetent	congenital infection	.19536	.14120	1.000	-.2123	.6030
		Not known infection	.20286	.17384	1.000	-.2990	.7047
		Primary from immunocompromized	.17555	.12642	1.000	-.1894	.5405
		Recurrent Infections	.12886	.12292	1.000	-.2260	.4837
	Primary from immunocompromized	congenital infection	.01981	.10361	1.000	-.2793	.3189
		Not known infection	.02731	.14498	1.000	-.3912	.4459
		Primary from Immunocompetent	-.17555	.12642	1.000	-.5405	.1894
		Recurrent Infections	-.04669	.07687	1.000	-.2686	.1752
	Recurrent Infections	congenital infection	.06650	.09932	1.000	-.2202	.3532
		Not known infection	.07400	.14194	1.000	-.3358	.4838
		Primary from Immunocompetent	-.12886	.12292	1.000	-.4837	.2260
		Primary from immunocompromized	.04669	.07687	1.000	-.1752	.2686
Lectin7b	congenital infection	Not known infection	-.01317	.27751	1.000	-.8143	.7880
		Primary from Immunocompetent	-.15917	.24795	1.000	-.8750	.5567
		Primary from immunocompromized	-.17224	.18194	1.000	-.6975	.3530
		Recurrent Infections	-.07517	.17440	1.000	-.5787	.4283
	Not known infection	congenital infection	.01317	.27751	1.000	-.7880	.8143
		Primary from Immunocompetent	-.14600	.30527	1.000	-1.0273	.7353
		Primary from immunocompromized	-.15908	.25458	1.000	-.8941	.5759

		Recurrent Infections	-.06200	.24925	1.000	-.7816	.6576
	Primary from Immunocompetent	congenital infection	.15917	.24795	1.000	-.5567	.8750
		Not known infection	.14600	.30527	1.000	-.7353	1.0273
		Primary from immunocompromized	-.01308	.22200	1.000	-.6540	.6278
		Recurrent Infections	.08400	.21586	1.000	-.5392	.7072
	Primary from immunocompromized	congenital infection	.17224	.18194	1.000	-.3530	.6975
		Not known infection	.15908	.25458	1.000	-.5759	.8941
		Primary from Immunocompetent	.01308	.22200	1.000	-.6278	.6540
		Recurrent Infections	.09708	.13498	1.000	-.2926	.4868
	Recurrent Infections	congenital infection	.07517	.17440	1.000	-.4283	.5787
		Not known infection	.06200	.24925	1.000	-.6576	.7816
		Primary from Immunocompetent	-.08400	.21586	1.000	-.7072	.5392
		Primary from immunocompromized	-.09708	.13498	1.000	-.4868	.2926
Lectin8b	congenital infection	Not known infection	-.11367	.08782	1.000	-.3672	.1399
		Primary from Immunocompetent	.01976	.07847	1.000	-.2068	.2463
		Primary from immunocompromized	-.08167	.05758	1.000	-.2479	.0846
		Recurrent Infections	-.03081	.05519	1.000	-.1901	.1285
	Not known infection	congenital infection	.11367	.08782	1.000	-.1399	.3672
		Primary from Immunocompetent	.13343	.09661	1.000	-.1455	.4123
		Primary from immunocompromized	.03200	.08057	1.000	-.2006	.2646
		Recurrent Infections	.08286	.07888	1.000	-.1449	.3106
	Primary from Immunocompetent	congenital infection	-.01976	.07847	1.000	-.2463	.2068
		Not known infection	-.13343	.09661	1.000	-.4123	.1455
		Primary from immunocompromized	-.10143	.07025	1.000	-.3042	.1014
		Recurrent Infections	-.05057	.06831	1.000	-.2478	.1466
	Primary from immunocompromized	congenital infection	.08167	.05758	1.000	-.0846	.2479
		Not known infection	-.03200	.08057	1.000	-.2646	.2006
		Primary from Immunocompetent	.10143	.07025	1.000	-.1014	.3042
		Recurrent Infections	.05086	.04272	1.000	-.0725	.1742
	Recurrent Infections	congenital infection	.03081	.05519	1.000	-.1285	.1901
		Not known infection	-.08286	.07888	1.000	-.3106	.1449
		Primary from Immunocompetent	.05057	.06831	1.000	-.1466	.2478
		Primary from immunocompromized	-.05086	.04272	1.000	-.1742	.0725
Lectin9b	congenital infection	Not known infection	-.08167	.14494	1.000	-.5001	.3368

		Primary from Immunocompetent	-.20310	.12950	1.000	-.5770	.1708
		Primary from immunocompromized	-.12551	.09503	1.000	-.3999	.1488
		Recurrent Infections	-.18852	.09109	.417	-.4515	.0745
	Not known infection	congenital infection	.08167	.14494	1.000	-.3368	.5001
		Primary from Immunocompetent	-.12143	.15944	1.000	-.5817	.3389
		Primary from immunocompromized	-.04385	.13297	1.000	-.4277	.3400
		Recurrent Infections	-.10686	.13018	1.000	-.4827	.2690
	Primary from Immunocompetent	congenital infection	.20310	.12950	1.000	-.1708	.5770
		Not known infection	.12143	.15944	1.000	-.3389	.5817
		Primary from immunocompromized	.07758	.11595	1.000	-.2572	.4123
		Recurrent Infections	.01457	.11274	1.000	-.3109	.3401
	Primary from immunocompromized	congenital infection	.12551	.09503	1.000	-.1488	.3999
		Not known infection	.04385	.13297	1.000	-.3400	.4277
		Primary from Immunocompetent	-.07758	.11595	1.000	-.4123	.2572
		Recurrent Infections	-.06301	.07050	1.000	-.2665	.1405
	Recurrent Infections	congenital infection	.18852	.09109	.417	-.0745	.4515
		Not known infection	.10686	.13018	1.000	-.2690	.4827
		Primary from Immunocompetent	-.01457	.11274	1.000	-.3401	.3109
		Primary from immunocompromized	.06301	.07050	1.000	-.1405	.2665
Lectin10b	congenital infection	Not known infection	.13417	.12618	1.000	-.2301	.4985
		Primary from Immunocompetent	.13274	.11274	1.000	-.1927	.4582
		Primary from immunocompromized	.01955	.08273	1.000	-.2193	.2584
		Recurrent Infections	.03588	.07930	1.000	-.1931	.2648
	Not known infection	congenital infection	-.13417	.12618	1.000	-.4985	.2301
		Primary from Immunocompetent	-.00143	.13881	1.000	-.4022	.3993
		Primary from immunocompromized	-.11462	.11576	1.000	-.4488	.2196
		Recurrent Infections	-.09829	.11334	1.000	-.4255	.2289
	Primary from Immunocompetent	congenital infection	-.13274	.11274	1.000	-.4582	.1927
		Not known infection	.00143	.13881	1.000	-.3993	.4022
		Primary from immunocompromized	-.11319	.10094	1.000	-.4046	.1782
		Recurrent Infections	-.09686	.09815	1.000	-.3802	.1865
	Primary from immunocompromized	congenital infection	-.01955	.08273	1.000	-.2584	.2193
		Not known infection	.11462	.11576	1.000	-.2196	.4488

		Primary from Immunocompetent	.11319	.10094	1.000	-.1782	.4046
		Recurrent Infections	.01633	.06138	1.000	-.1609	.1935
	Recurrent Infections	congenital infection	-.03588	.07930	1.000	-.2648	.1931
		Not known infection	.09829	.11334	1.000	-.2289	.4255
		Primary from Immunocompetent	.09686	.09815	1.000	-.1865	.3802
		Primary from immunocompromized	-.01633	.06138	1.000	-.1935	.1609
Lectin11b	congenital infection	Not known infection	.01500	.06508	1.000	-.1729	.2029
		Primary from Immunocompetent	.05643	.05815	1.000	-.1114	.2243
		Primary from immunocompromized	-.04692	.04267	1.000	-.1701	.0763
		Recurrent Infections	-.00729	.04090	1.000	-.1254	.1108
	Not known infection	congenital infection	-.01500	.06508	1.000	-.2029	.1729
		Primary from Immunocompetent	.04143	.07159	1.000	-.1652	.2481
		Primary from immunocompromized	-.06192	.05970	1.000	-.2343	.1104
		Recurrent Infections	-.02229	.05845	1.000	-.1910	.1465
	Primary from Immunocompetent	congenital infection	-.05643	.05815	1.000	-.2243	.1114
		Not known infection	-.04143	.07159	1.000	-.2481	.1652
		Primary from immunocompromized	-.10335	.05206	.505	-.2536	.0469
		Recurrent Infections	-.06371	.05062	1.000	-.2099	.0824
	Primary from immunocompromized	congenital infection	.04692	.04267	1.000	-.0763	.1701
		Not known infection	.06192	.05970	1.000	-.1104	.2343
		Primary from Immunocompetent	.10335	.05206	.505	-.0469	.2536
		Recurrent Infections	.03964	.03165	1.000	-.0517	.1310
	Recurrent Infections	congenital infection	.00729	.04090	1.000	-.1108	.1254
		Not known infection	.02229	.05845	1.000	-.1465	.1910
		Primary from Immunocompetent	.06371	.05062	1.000	-.0824	.2099
		Primary from immunocompromized	-.03964	.03165	1.000	-.1310	.0517
Lectin12b	congenital infection	Not known infection	.01733	.08749	1.000	-.2352	.2699
		Primary from Immunocompetent	.04619	.07817	1.000	-.1795	.2719
		Primary from immunocompromized	-.02321	.05736	1.000	-.1888	.1424
		Recurrent Infections	-.04324	.05498	1.000	-.2020	.1155
	Not known infection	congenital infection	-.01733	.08749	1.000	-.2699	.2352
		Primary from Immunocompetent	.02886	.09624	1.000	-.2490	.3067
		Primary from immunocompromized	-.04054	.08026	1.000	-.2722	.1912

		Recurrent Infections	-.06057	.07858	1.000	-.2874	.1663
	Primary from Immunocompetent	congenital infection	-.04619	.07817	1.000	-.2719	.1795
		Not known infection	-.02886	.09624	1.000	-.3067	.2490
		Primary from immunocompromized	-.06940	.06999	1.000	-.2714	.1327
		Recurrent Infections	-.08943	.06805	1.000	-.2859	.1070
	Primary from immunocompromized	congenital infection	.02321	.05736	1.000	-.1424	.1888
		Not known infection	.04054	.08026	1.000	-.1912	.2722
		Primary from Immunocompetent	.06940	.06999	1.000	-.1327	.2714
		Recurrent Infections	-.02003	.04255	1.000	-.1429	.1028
	Recurrent Infections	congenital infection	.04324	.05498	1.000	-.1155	.2020
		Not known infection	.06057	.07858	1.000	-.1663	.2874
		Primary from Immunocompetent	.08943	.06805	1.000	-.1070	.2859
		Primary from immunocompromized	.02003	.04255	1.000	-.1028	.1429
Lectin13b	congenital infection	Not known infection	.02667	.12971	1.000	-.3478	.4011
		Primary from Immunocompetent	.06810	.11589	1.000	-.2665	.4027
		Primary from immunocompromized	.02282	.08504	1.000	-.2227	.2683
		Recurrent Infections	-.05590	.08152	1.000	-.2912	.1794
	Not known infection	congenital infection	-.02667	.12971	1.000	-.4011	.3478
		Primary from Immunocompetent	.04143	.14269	1.000	-.3705	.4534
		Primary from immunocompromized	-.00385	.11900	1.000	-.3474	.3397
		Recurrent Infections	-.08257	.11650	1.000	-.4189	.2538
	Primary from Immunocompetent	congenital infection	-.06810	.11589	1.000	-.4027	.2665
		Not known infection	-.04143	.14269	1.000	-.4534	.3705
		Primary from immunocompromized	-.04527	.10376	1.000	-.3448	.2543
		Recurrent Infections	-.12400	.10089	1.000	-.4153	.1673
	Primary from immunocompromized	congenital infection	-.02282	.08504	1.000	-.2683	.2227
		Not known infection	.00385	.11900	1.000	-.3397	.3474
		Primary from Immunocompetent	.04527	.10376	1.000	-.2543	.3448
		Recurrent Infections	-.07873	.06309	1.000	-.2609	.1034
	Recurrent Infections	congenital infection	.05590	.08152	1.000	-.1794	.2912
		Not known infection	.08257	.11650	1.000	-.2538	.4189
		Primary from Immunocompetent	.12400	.10089	1.000	-.1673	.4153
		Primary from immunocompromized	.07873	.06309	1.000	-.1034	.2609
Lectin14b	congenital infection	Not known infection	.07283	.11249	1.000	-.2519	.3976

		Primary from Immunocompetent	.04226	.10051	1.000	-.2479	.3324
		Primary from immunocompromized	.00776	.07375	1.000	-.2052	.2207
		Recurrent Infections	-.03717	.07069	1.000	-.2413	.1669
	Not known infection	congenital infection	-.07283	.11249	1.000	-.3976	.2519
		Primary from Immunocompetent	-.03057	.12374	1.000	-.3878	.3267
		Primary from immunocompromized	-.06508	.10320	1.000	-.3630	.2328
		Recurrent Infections	-.11000	.10103	1.000	-.4017	.1817
	Primary from Immunocompetent	congenital infection	-.04226	.10051	1.000	-.3324	.2479
		Not known infection	.03057	.12374	1.000	-.3267	.3878
		Primary from immunocompromized	-.03451	.08999	1.000	-.2943	.2253
		Recurrent Infections	-.07943	.08750	1.000	-.3320	.1732
	Primary from immunocompromized	congenital infection	-.00776	.07375	1.000	-.2207	.2052
		Not known infection	.06508	.10320	1.000	-.2328	.3630
		Primary from Immunocompetent	.03451	.08999	1.000	-.2253	.2943
		Recurrent Infections	-.04492	.05471	1.000	-.2029	.1130
	Recurrent Infections	congenital infection	.03717	.07069	1.000	-.1669	.2413
		Not known infection	.11000	.10103	1.000	-.1817	.4017
		Primary from Immunocompetent	.07943	.08750	1.000	-.1732	.3320
		Primary from immunocompromized	.04492	.05471	1.000	-.1130	.2029
Lectin15b	congenital infection	Not known infection	.10150	.13884	1.000	-.2993	.5023
		Primary from Immunocompetent	-.01964	.12405	1.000	-.3778	.3385
		Primary from immunocompromized	.00596	.09103	1.000	-.2568	.2688
		Recurrent Infections	-.02679	.08725	1.000	-.2787	.2251
	Not known infection	congenital infection	-.10150	.13884	1.000	-.5023	.2993
		Primary from Immunocompetent	-.12114	.15273	1.000	-.5621	.3198
		Primary from immunocompromized	-.09554	.12737	1.000	-.4632	.2722
		Recurrent Infections	-.12829	.12470	1.000	-.4883	.2317
	Primary from Immunocompetent	congenital infection	.01964	.12405	1.000	-.3385	.3778
		Not known infection	.12114	.15273	1.000	-.3198	.5621
		Primary from immunocompromized	.02560	.11106	1.000	-.2950	.3462
		Recurrent Infections	-.00714	.10799	1.000	-.3189	.3046
	Primary from immunocompromized	congenital infection	-.00596	.09103	1.000	-.2688	.2568
		Not known infection	.09554	.12737	1.000	-.2722	.4632

		Primary from Immunocompetent	-.02560	.11106	1.000	-.3462	.2950
		Recurrent Infections	-.03275	.06753	1.000	-.2277	.1622
	Recurrent Infections	congenital infection	.02679	.08725	1.000	-.2251	.2787
		Not known infection	.12829	.12470	1.000	-.2317	.4883
		Primary from Immunocompetent	.00714	.10799	1.000	-.3046	.3189
		Primary from immunocompromized	.03275	.06753	1.000	-.1622	.2277
Lectin16b	congenital infection	Not known infection	.07550	.24218	1.000	-.6237	.7747
		Primary from Immunocompetent	.47750	.21639	.302	-.1472	1.1022
		Primary from immunocompromized	.41058	.15879	.115	-.0478	.8690
		Recurrent Infections	.35493	.15220	.222	-.0845	.7943
	Not known infection	congenital infection	-.07550	.24218	1.000	-.7747	.6237
		Primary from Immunocompetent	.40200	.26641	1.000	-.3671	1.1711
		Primary from immunocompromized	.33508	.22218	1.000	-.3064	.9765
		Recurrent Infections	.27943	.21752	1.000	-.3486	.9074
	Primary from Immunocompetent	congenital infection	-.47750	.21639	.302	-1.1022	.1472
		Not known infection	-.40200	.26641	1.000	-1.1711	.3671
		Primary from immunocompromized	-.06692	.19374	1.000	-.6262	.4924
		Recurrent Infections	-.12257	.18838	1.000	-.6664	.4213
	Primary from immunocompromized	congenital infection	-.41058	.15879	.115	-.8690	.0478
		Not known infection	-.33508	.22218	1.000	-.9765	.3064
		Primary from Immunocompetent	.06692	.19374	1.000	-.4924	.6262
		Recurrent Infections	-.05565	.11780	1.000	-.3957	.2844
	Recurrent Infections	congenital infection	-.35493	.15220	.222	-.7943	.0845
		Not known infection	-.27943	.21752	1.000	-.9074	.3486
		Primary from Immunocompetent	.12257	.18838	1.000	-.4213	.6664
		Primary from immunocompromized	.05565	.11780	1.000	-.2844	.3957
Lectin17b	congenital infection	Not known infection	-.01050	.16299	1.000	-.4810	.4600
		Primary from Immunocompetent	.08321	.14563	1.000	-.3372	.5036
		Primary from immunocompromized	-.00135	.10686	1.000	-.3099	.3072
		Recurrent Infections	-.06136	.10243	1.000	-.3571	.2344
	Not known infection	congenital infection	.01050	.16299	1.000	-.4600	.4810
		Primary from Immunocompetent	.09371	.17929	1.000	-.4239	.6113
		Primary from immunocompromized	.00915	.14953	1.000	-.4225	.4408

		Recurrent Infections	-.05086	.14639	1.000	-.4735	.3718
	Primary from Immunocompetent	congenital infection	-.08321	.14563	1.000	-.5036	.3372
		Not known infection	-.09371	.17929	1.000	-.6113	.4239
		Primary from immunocompromized	-.08456	.13038	1.000	-.4610	.2919
		Recurrent Infections	-.14457	.12678	1.000	-.5106	.2214
	Primary from immunocompromized	congenital infection	.00135	.10686	1.000	-.3072	.3099
		Not known infection	-.00915	.14953	1.000	-.4408	.4225
		Primary from Immunocompetent	.08456	.13038	1.000	-.2919	.4610
		Recurrent Infections	-.06001	.07928	1.000	-.2889	.1689
	Recurrent Infections	congenital infection	.06136	.10243	1.000	-.2344	.3571
		Not known infection	.05086	.14639	1.000	-.3718	.4735
		Primary from Immunocompetent	.14457	.12678	1.000	-.2214	.5106
		Primary from immunocompromized	.06001	.07928	1.000	-.1689	.2889
Lectin18b	congenital infection	Not known infection	.03700	.15414	1.000	-.4080	.4820
		Primary from Immunocompetent	-.08357	.13772	1.000	-.4812	.3140
		Primary from immunocompromized	.02577	.10106	1.000	-.2660	.3175
		Recurrent Infections	-.03757	.09687	1.000	-.3172	.2421
	Not known infection	congenital infection	-.03700	.15414	1.000	-.4820	.4080
		Primary from Immunocompetent	-.12057	.16956	1.000	-.6101	.3689
		Primary from immunocompromized	-.01123	.14141	1.000	-.4195	.3970
		Recurrent Infections	-.07457	.13845	1.000	-.4743	.3251
	Primary from Immunocompetent	congenital infection	.08357	.13772	1.000	-.3140	.4812
		Not known infection	.12057	.16956	1.000	-.3689	.6101
		Primary from immunocompromized	.10934	.12331	1.000	-.2466	.4653
		Recurrent Infections	.04600	.11990	1.000	-.3001	.3921
	Primary from immunocompromized	congenital infection	-.02577	.10106	1.000	-.3175	.2660
		Not known infection	.01123	.14141	1.000	-.3970	.4195
		Primary from Immunocompetent	-.10934	.12331	1.000	-.4653	.2466
		Recurrent Infections	-.06334	.07497	1.000	-.2798	.1531
	Recurrent Infections	congenital infection	.03757	.09687	1.000	-.2421	.3172
		Not known infection	.07457	.13845	1.000	-.3251	.4743
		Primary from Immunocompetent	-.04600	.11990	1.000	-.3921	.3001
		Primary from immunocompromized	.06334	.07497	1.000	-.1531	.2798

Lectin19b	congenital infection	Not known infection	.03317	.05579	1.000	-.1279	.1942
		Primary from Immunocompetent	.03488	.04985	1.000	-.1090	.1788
		Primary from immunocompromized	-.01199	.03658	1.000	-.1176	.0936
		Recurrent Infections	-.04969	.03506	1.000	-.1509	.0515
	Not known infection	congenital infection	-.03317	.05579	1.000	-.1942	.1279
		Primary from Immunocompetent	.00171	.06137	1.000	-.1755	.1789
		Primary from immunocompromized	-.04515	.05118	1.000	-.1929	.1026
		Recurrent Infections	-.08286	.05011	1.000	-.2275	.0618
	Primary from Immunocompetent	congenital infection	-.03488	.04985	1.000	-.1788	.1090
		Not known infection	-.00171	.06137	1.000	-.1789	.1755
		Primary from immunocompromized	-.04687	.04463	1.000	-.1757	.0820
		Recurrent Infections	-.08457	.04340	.548	-.2099	.0407
	Primary from immunocompromized	congenital infection	.01199	.03658	1.000	-.0936	.1176
		Not known infection	.04515	.05118	1.000	-.1026	.1929
		Primary from Immunocompetent	.04687	.04463	1.000	-.0820	.1757
		Recurrent Infections	-.03770	.02714	1.000	-.1160	.0406
	Recurrent Infections	congenital infection	.04969	.03506	1.000	-.0515	.1509
		Not known infection	.08286	.05011	1.000	-.0618	.2275
		Primary from Immunocompetent	.08457	.04340	.548	-.0407	.2099
		Primary from immunocompromized	.03770	.02714	1.000	-.0406	.1160
Lectin20b	congenital infection	Not known infection	-.07767	.11051	1.000	-.3967	.2414
		Primary from Immunocompetent	-.10738	.09874	1.000	-.3924	.1777
		Primary from immunocompromized	-.07244	.07246	1.000	-.2816	.1367
		Recurrent Infections	-.09424	.06945	1.000	-.2947	.1063
	Not known infection	congenital infection	.07767	.11051	1.000	-.2414	.3967
		Primary from Immunocompetent	-.02971	.12157	1.000	-.3807	.3212
		Primary from immunocompromized	.00523	.10138	1.000	-.2875	.2979
		Recurrent Infections	-.01657	.09926	1.000	-.3031	.2700
	Primary from Immunocompetent	congenital infection	.10738	.09874	1.000	-.1777	.3924
		Not known infection	.02971	.12157	1.000	-.3212	.3807
		Primary from immunocompromized	.03495	.08841	1.000	-.2203	.2902
		Recurrent Infections	.01314	.08596	1.000	-.2350	.2613

	Primary from immunocompromized	congenital infection	.07244	.07246	1.000	-.1367	.2816
		Not known infection	-.00523	.10138	1.000	-.2979	.2875
		Primary from Immunocompetent	-.03495	.08841	1.000	-.2902	.2203
		Recurrent Infections	-.02180	.05375	1.000	-.1770	.1334
	Recurrent Infections	congenital infection	.09424	.06945	1.000	-.1063	.2947
		Not known infection	.01657	.09926	1.000	-.2700	.3031
		Primary from Immunocompetent	-.01314	.08596	1.000	-.2613	.2350
		Primary from immunocompromized	.02180	.05375	1.000	-.1334	.1770

Appendix 8: Tables of One-way ANOVA results for the relation between HCMV sample type and the glycosylation of the glycoproteins.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Lectin1b	respiratory	4	.2400	.04320	.02160	.1713	.3087	.18	.28
	blood	77	.2247	.12159	.01386	.1971	.2523	.09	.88
	Urine	8	.1662	.04033	.01426	.1325	.2000	.11	.22
	Total	89	.2201	.11515	.01221	.1959	.2444	.09	.88
Lectin2b	respiratory	4	.5950	.21237	.10618	.2571	.9329	.45	.90
	blood	77	.5677	.19179	.02186	.5241	.6112	.29	1.17
	Urine	8	.4413	.15851	.05604	.3087	.5738	.20	.65
	Total	89	.5575	.19154	.02030	.5172	.5979	.20	1.17
Lectin3b	respiratory	4	.6200	.06880	.03440	.5105	.7295	.57	.72
	blood	77	.7218	.26023	.02966	.6628	.7809	.32	1.48
	Urine	8	.5462	.11463	.04053	.4504	.6421	.40	.74
	Total	89	.7015	.25009	.02651	.6488	.7541	.32	1.48
Lectin4b	respiratory	4	.8175	.14385	.07192	.5886	1.0464	.72	1.03
	blood	77	.7813	.32442	.03697	.7077	.8549	.30	2.27
	Urine	8	.5725	.13090	.04628	.4631	.6819	.41	.79
	Total	89	.7642	.31095	.03296	.6987	.8297	.30	2.27
Lectin5b	respiratory	4	.3425	.15861	.07931	.0901	.5949	.18	.55
	blood	77	.2158	.13293	.01515	.1857	.2460	.07	.78
	Urine	8	.2663	.37925	.13408	-.0508	.5833	.08	1.20
	Total	89	.2261	.16856	.01787	.1906	.2616	.07	1.20
Lectin6b	respiratory	4	.9875	.27195	.13598	.5548	1.4202	.63	1.26
	blood	77	.8542	.30495	.03475	.7849	.9234	.35	1.57
	Urine	8	.7425	.26623	.09413	.5199	.9651	.50	1.22
	Total	89	.8501	.30066	.03187	.7868	.9134	.35	1.57
Lectin7b	respiratory	4	1.1875	.29680	.14840	.7152	1.6598	.82	1.52
	blood	77	1.1008	.53266	.06070	.9799	1.2217	.17	2.50
	Urine	8	.8200	.34472	.12188	.5318	1.1082	.35	1.55
	Total	89	1.0794	.51434	.05452	.9711	1.1878	.17	2.50
Lectin8b	respiratory	4	.8100	.58793	.29397	-.1255	1.7455	.28	1.47
	blood	77	.3991	.15105	.01721	.3648	.4334	.12	.95
	Urine	8	.3513	.08560	.03026	.2797	.4228	.24	.48
	Total	89	.4133	.19938	.02113	.3713	.4553	.12	1.47
Lectin9b	respiratory	4	.7325	.24514	.12257	.3424	1.1226	.41	.96
	blood	77	.6852	.27734	.03161	.6222	.7481	.32	1.71
	Urine	8	.4788	.08871	.03136	.4046	.5529	.35	.64
	Total	89	.6688	.26982	.02860	.6119	.7256	.32	1.71
Lectin10b	respiratory	4	.8550	.44792	.22396	.1423	1.5677	.49	1.45
	blood	77	.6290	.23833	.02716	.5749	.6831	.15	1.26
	Urine	8	.5150	.17105	.06047	.3720	.6580	.34	.84
	Total	89	.6289	.24844	.02633	.5765	.6812	.15	1.45

Lectin11b	respiratory	4	.2800	.11195	.05598	.1019	.4581	.18	.42
	blood	77	.2381	.12431	.01417	.2098	.2663	.08	.62
	Urine	8	.1875	.08730	.03087	.1145	.2605	.10	.37
	Total	89	.2354	.12118	.01284	.2099	.2609	.08	.62
Lectin12b	respiratory	4	.1825	.04500	.02250	.1109	.2541	.12	.22
	blood	77	.1848	.17049	.01943	.1461	.2235	.07	1.39
	Urine	8	.1463	.04984	.01762	.1046	.1879	.09	.22
	Total	89	.1812	.15966	.01692	.1476	.2149	.07	1.39
Lectin13b	respiratory	4	.5375	.18025	.09013	.2507	.8243	.39	.77
	blood	77	.4384	.25260	.02879	.3811	.4958	.17	2.35
	Urine	8	.3550	.05237	.01852	.3112	.3988	.29	.47
	Total	89	.4354	.23980	.02542	.3849	.4859	.17	2.35
Lectin14b	respiratory	4	.7375	.24019	.12010	.3553	1.1197	.55	1.09
	blood	77	.5458	.21672	.02470	.4967	.5950	.21	1.39
	Urine	8	.4438	.12872	.04551	.3361	.5514	.28	.67
	Total	89	.5453	.21556	.02285	.4999	.5907	.21	1.39
Lectin15b	respiratory	4	.9275	.25474	.12737	.5222	1.3328	.64	1.22
	blood	77	.6774	.26212	.02987	.6179	.7369	.11	1.47
	Urine	8	.5950	.19647	.06946	.4307	.7593	.41	1.02
	Total	89	.6812	.26090	.02766	.6263	.7362	.11	1.47
Lectin16b	respiratory	4	.8625	.35994	.17997	.2898	1.4352	.65	1.40
	blood	77	.3695	.45600	.05197	.2660	.4730	.07	2.80
	Urine	8	.4088	.50632	.17901	-.0145	.8320	.08	1.46
	Total	89	.3952	.46359	.04914	.2975	.4928	.07	2.80
Lectin17b	respiratory	4	1.1450	.21810	.10905	.7980	1.4920	1.01	1.47
	blood	77	.8560	.30855	.03516	.7859	.9260	.38	2.15
	Urine	8	.6600	.20543	.07263	.4883	.8317	.46	1.14
	Total	89	.8513	.30735	.03258	.7866	.9161	.38	2.15
Lectin18b	respiratory	4	.8925	.15586	.07793	.6445	1.1405	.71	1.09
	blood	77	.7932	.30036	.03423	.7251	.8614	.22	1.91
	Urine	8	.6550	.11439	.04044	.5594	.7506	.43	.76
	Total	89	.7853	.28619	.03034	.7250	.8456	.22	1.91
Lectin19b	respiratory	4	.3300	.05831	.02915	.2372	.4228	.25	.39
	blood	77	.2696	.10878	.01240	.2449	.2943	.08	.57
	Urine	8	.1975	.06274	.02218	.1451	.2499	.12	.29
	Total	89	.2658	.10617	.01125	.2435	.2882	.08	.57
Lectin20b	respiratory	4	.4450	.04041	.02021	.3807	.5093	.41	.50
	blood	77	.5373	.21435	.02443	.4886	.5859	.14	1.44
	Urine	8	.4188	.06578	.02326	.3638	.4737	.32	.54
	Total	89	.5225	.20377	.02160	.4795	.5654	.14	1.44

ANOVA						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Lectin1b	Between Groups	.026	2	.013	.995	.374
	Within Groups	1.141	86	.013		
	Total	1.167	88			
Lectin2b	Between Groups	.122	2	.061	1.684	.192
	Within Groups	3.107	86	.036		
	Total	3.228	88			
Lectin3b	Between Groups	.251	2	.126	2.056	.134
	Within Groups	5.253	86	.061		
	Total	5.504	88			
Lectin4b	Between Groups	.328	2	.164	1.723	.185
	Within Groups	8.181	86	.095		
	Total	8.509	88			
Lectin5b	Between Groups	.075	2	.038	1.333	.269
	Within Groups	2.425	86	.028		
	Total	2.500	88			
Lectin6b	Between Groups	.169	2	.085	.936	.396
	Within Groups	7.786	86	.091		
	Total	7.955	88			
Lectin7b	Between Groups	.620	2	.310	1.177	.313
	Within Groups	22.659	86	.263		
	Total	23.280	88			
Lectin8b	Between Groups	.676	2	.338	10.297	.000
	Within Groups	2.822	86	.033		
	Total	3.498	88			
Lectin9b	Between Groups	.326	2	.163	2.304	.106
	Within Groups	6.081	86	.071		
	Total	6.407	88			
Lectin10b	Between Groups	.308	2	.154	2.587	.081
	Within Groups	5.123	86	.060		
	Total	5.432	88			
Lectin11b	Between Groups	.027	2	.013	.913	.405
	Within Groups	1.265	86	.015		
	Total	1.292	88			
Lectin12b	Between Groups	.011	2	.005	.208	.813
	Within Groups	2.233	86	.026		
	Total	2.243	88			

Lectin13b	Between Groups	.094	2	.047	.815	.446
	Within Groups	4.966	86	.058		
	Total	5.060	88			
Lectin14b	Between Groups	.230	2	.115	2.566	.083
	Within Groups	3.859	86	.045		
	Total	4.089	88			
Lectin15b	Between Groups	.303	2	.152	2.293	.107
	Within Groups	5.687	86	.066		
	Total	5.990	88			
Lectin16b	Between Groups	.926	2	.463	2.214	.116
	Within Groups	17.986	86	.209		
	Total	18.912	88			
Lectin17b	Between Groups	.639	2	.320	3.583	.032
	Within Groups	7.674	86	.089		
	Total	8.313	88			
Lectin18b	Between Groups	.187	2	.093	1.143	.324
	Within Groups	7.021	86	.082		
	Total	7.208	88			
Lectin19b	Between Groups	.055	2	.027	2.520	.086
	Within Groups	.937	86	.011		
	Total	.992	88			
Lectin20b	Between Groups	.127	2	.063	1.548	.219
	Within Groups	3.527	86	.041		
	Total	3.654	88			

Multiple Comparisons

Bonferroni

Dependent Variable	(I) spec.type.r	(J) spec.type.r	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Lectin1b	respiratory	blood	.01532	.05906	1.000	-.1289	.1595
		Urine	.07375	.07052	.896	-.0984	.2459
	blood	respiratory	-.01532	.05906	1.000	-.1595	.1289
		Urine	.05843	.04278	.527	-.0460	.1629
	Urine	respiratory	-.07375	.07052	.896	-.2459	.0984
		blood	-.05843	.04278	.527	-.1629	.0460
Lectin2b	respiratory	blood	.02734	.09747	1.000	-.2107	.2653
		Urine	.15375	.11639	.570	-.1304	.4379
	blood	respiratory	-.02734	.09747	1.000	-.2653	.2107
		Urine	.12641	.07060	.231	-.0460	.2988
	Urine	respiratory	-.15375	.11639	.570	-.4379	.1304
		blood	-.12641	.07060	.231	-.2988	.0460

Lectin3b	respiratory	blood	-.10182	.12674	1.000	-.4113	.2076
		Urine	.07375	.15134	1.000	-.2958	.4433
	blood	respiratory	.10182	.12674	1.000	-.2076	.4113
		Urine	.17557	.09181	.177	-.0486	.3997
	Urine	respiratory	-.07375	.15134	1.000	-.4433	.2958
		blood	-.17557	.09181	.177	-.3997	.0486
Lectin4b	respiratory	blood	.03620	.15817	1.000	-.3500	.4224
		Urine	.24500	.18887	.594	-.2162	.7062
	blood	respiratory	-.03620	.15817	1.000	-.4224	.3500
		Urine	.20880	.11457	.216	-.0710	.4885
	Urine	respiratory	-.24500	.18887	.594	-.7062	.2162
		blood	-.20880	.11457	.216	-.4885	.0710
Lectin5b	respiratory	blood	.12666	.08612	.435	-.0836	.3369
		Urine	.07625	.10283	1.000	-.1748	.3273
	blood	respiratory	-.12666	.08612	.435	-.3369	.0836
		Urine	-.05041	.06238	1.000	-.2027	.1019
	Urine	respiratory	-.07625	.10283	1.000	-.3273	.1748
		blood	.05041	.06238	1.000	-.1019	.2027
Lectin6b	respiratory	blood	.13334	.15430	1.000	-.2434	.5101
		Urine	.24500	.18425	.561	-.2049	.6949
	blood	respiratory	-.13334	.15430	1.000	-.5101	.2434
		Urine	.11166	.11177	.962	-.1612	.3846
	Urine	respiratory	-.24500	.18425	.561	-.6949	.2049
		blood	-.11166	.11177	.962	-.3846	.1612
Lectin7b	respiratory	blood	.08672	.26323	1.000	-.5560	.7295
		Urine	.36750	.31433	.737	-.4000	1.1350
	blood	respiratory	-.08672	.26323	1.000	-.7295	.5560
		Urine	.28078	.19068	.434	-.1848	.7464
	Urine	respiratory	-.36750	.31433	.737	-1.1350	.4000
		blood	-.28078	.19068	.434	-.7464	.1848
Lectin8b	respiratory	blood	.41091*	.09290	.000	.1841	.6377
		Urine	.45875*	.11094	.000	.1879	.7296
	blood	respiratory	-.41091*	.09290	.000	-.6377	-.1841
		Urine	.04784	.06729	1.000	-.1165	.2122
	Urine	respiratory	-.45875*	.11094	.000	-.7296	-.1879
		blood	-.04784	.06729	1.000	-.2122	.1165
Lectin9b	respiratory	blood	.04731	.13636	1.000	-.2857	.3803
		Urine	.25375	.16284	.368	-.1438	.6513
	blood	respiratory	-.04731	.13636	1.000	-.3803	.2857
		Urine	.20644	.09878	.119	-.0347	.4476
	Urine	respiratory	-.25375	.16284	.368	-.6513	.1438
		blood	-.20644	.09878	.119	-.4476	.0347
Lectin10b	respiratory	blood	.22604	.12517	.223	-.0796	.5317
		Urine	.34000	.14947	.076	-.0250	.7050

	blood	respiratory	-.22604	.12517	.223	-.5317	.0796
		Urine	.11396	.09067	.637	-.1074	.3353
	Urine	respiratory	-.34000	.14947	.076	-.7050	.0250
		blood	-.11396	.09067	.637	-.3353	.1074
Lectin11b	respiratory	blood	.04195	.06220	1.000	-.1099	.1938
		Urine	.09250	.07428	.649	-.0889	.2739
	blood	respiratory	-.04195	.06220	1.000	-.1938	.1099
		Urine	.05055	.04506	.795	-.0595	.1606
	Urine	respiratory	-.09250	.07428	.649	-.2739	.0889
		blood	-.05055	.04506	.795	-.1606	.0595
Lectin12b	respiratory	blood	-.00231	.08263	1.000	-.2041	.1994
		Urine	.03625	.09867	1.000	-.2047	.2772
	blood	respiratory	.00231	.08263	1.000	-.1994	.2041
		Urine	.03856	.05985	1.000	-.1076	.1847
	Urine	respiratory	-.03625	.09867	1.000	-.2772	.2047
		blood	-.03856	.05985	1.000	-.1847	.1076
Lectin13b	respiratory	blood	.09906	.12323	1.000	-.2018	.4000
		Urine	.18250	.14715	.655	-.1768	.5418
	blood	respiratory	-.09906	.12323	1.000	-.4000	.2018
		Urine	.08344	.08926	1.000	-.1345	.3014
	Urine	respiratory	-.18250	.14715	.655	-.5418	.1768
		blood	-.08344	.08926	1.000	-.3014	.1345
Lectin14b	respiratory	blood	.19166	.10863	.244	-.0736	.4569
		Urine	.29375	.12971	.078	-.0230	.6105
	blood	respiratory	-.19166	.10863	.244	-.4569	.0736
		Urine	.10209	.07869	.594	-.0900	.2942
	Urine	respiratory	-.29375	.12971	.078	-.6105	.0230
		blood	-.10209	.07869	.594	-.2942	.0900
Lectin15b	respiratory	blood	.25010	.13187	.184	-.0719	.5721
		Urine	.33250	.15747	.113	-.0520	.7170
	blood	respiratory	-.25010	.13187	.184	-.5721	.0719
		Urine	.08240	.09552	1.000	-.1508	.3156
	Urine	respiratory	-.33250	.15747	.113	-.7170	.0520
		blood	-.08240	.09552	1.000	-.3156	.1508
Lectin16b	respiratory	blood	.49302	.23453	.115	-.0796	1.0657
		Urine	.45375	.28005	.327	-.2301	1.1376
	blood	respiratory	-.49302	.23453	.115	-1.0657	.0796
		Urine	-.03927	.16988	1.000	-.4541	.3755
	Urine	respiratory	-.45375	.28005	.327	-1.1376	.2301
		blood	.03927	.16988	1.000	-.3755	.4541
Lectin17b	respiratory	blood	.28903	.15318	.188	-.0850	.6631
		Urine	.48500*	.18292	.029	.0384	.9316
	blood	respiratory	-.28903	.15318	.188	-.6631	.0850
		Urine	.19597	.11096	.243	-.0750	.4669

	Urine	respiratory	-.48500*	.18292	.029	-.9316	-.0384
		blood	-.19597	.11096	.243	-.4669	.0750
Lectin18b	respiratory	blood	.09925	.14653	1.000	-.2585	.4570
		Urine	.23750	.17497	.535	-.1897	.6647
	blood	respiratory	-.09925	.14653	1.000	-.4570	.2585
		Urine	.13825	.10614	.589	-.1209	.3974
	Urine	respiratory	-.23750	.17497	.535	-.6647	.1897
		blood	-.13825	.10614	.589	-.3974	.1209
Lectin19b	respiratory	blood	.06039	.05353	.787	-.0703	.1911
		Urine	.13250	.06392	.124	-.0236	.2886
	blood	respiratory	-.06039	.05353	.787	-.1911	.0703
		Urine	.07211	.03877	.199	-.0226	.1668
	Urine	respiratory	-.13250	.06392	.124	-.2886	.0236
		blood	-.07211	.03877	.199	-.1668	.0226
Lectin20b	respiratory	blood	-.09227	.10385	1.000	-.3458	.1613
		Urine	.02625	.12401	1.000	-.2766	.3291
	blood	respiratory	.09227	.10385	1.000	-.1613	.3458
		Urine	.11852	.07523	.356	-.0652	.3022
	Urine	respiratory	-.02625	.12401	1.000	-.3291	.2766
		blood	-.11852	.07523	.356	-.3022	.0652