Diploma Thesis

Comparison of currently available real-time PCR assays for detection of human herpesviruses DNA in the routine diagnostic laboratory

submitted by

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AFFIDAVIT

Herewith I, Sophie Heyszl, declare that I have written the present diploma thesis fully on my own and without any assistance from third parties.
Furthermore, I confirm that no sources have been used in the preparation of the Thesis other than those indicated in the thesis itself.

Graz, October 2009 Sophie Heyszl

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I dedicate this thesis to my grandparents Dr. med. univ. Gerhard Heyszl and
Dr. med. univ. Hedwig Heyszl, who passed their passion for medicine on to me.

„We act as though comfort and luxury were the chief requirements of life, when all that we need to make us happy is something to be enthusiastic about." *Albert Einstein*

ABSTRACT

In routine molecular diagnostics, detection of herpesviruses has made a major impact. Infection with herpesviruses is indicated by demonstrating the presence of the virus in selected specimens. Rapid and reliable detection of herpesvirus DNA helps to decrease the lethality as well as the sequelae of herpesvirus infection in patients at risk. This work discusses specimen types and both laboratory-developed and commercially available assays useful for molecular detection of herpesviruses. To meet the need for reliable laboratory results, it is advisable to employ maximum automated and standardized kits based on reagents and standards of reproducible high quality. In the routine diagnostic laboratory, introduction of IVD/CE and/or FDA labeled tests is preferred.

KURZFASSUNG

Der Nachweis von Herpesviren hat in der molekularen Diagnostik eine enorme Bedeutung bekommen. Die Infektion mit einem Herpesvirus kann durch die Präsenz des Virus in bestimmten Proben nachgewiesen werden. Der schnelle und verlässliche Nachweis von Herpesvirus-DNA hilft, sowohl die Letalität, als auch Folgen einer Herpesvirusinfektion, besonders in PatientInnen mit Risikofaktoren zu senken. Das Ziel der vorliegenden Arbeit ist eine umfassende Darstellung von kommerziellen und selbst entwickelten Tests zum molekularen Nachweis von Herpesviren, sowie von den dafür erforderlichen Probematerialien. Durch die Verwendung möglichst standardisierter Methoden mit hoher Reproduzierbarkeit können verlässliche Laborergebnisse garantiert werden. Im diagnostischen Routinelabor wird die Einführung IVD/CE und/oder FDA gekennzeichneter molekularer Tests empfohlen.

TABLE OF CONTENTS

1 Introduction 9
2 Molecular Detection of Herpesviruses 11
2.1 Cytomegalovirus (CMV) 14
2.1.1 Morphology 14
2.1.2 Viral Replication and Pathogenesis 15
2.1.3 Epidemiology and Transmission 17
2.1.4 Clinical Manifestations 17
2.1.5 CMV Infection in the Immunocompromised Host 19
2.1.6 Diagnosis 21
2.1.7 Therapeutical Management 21
2.1.8 Specimen Types for the Detection of CMV DNA 22
2.1.9 Laboratory-developed Assays for the Quantitation of CMV DNA 23
2.1.10 Currently Frequently Used Commercially Available Assays for the Quantitation of CMV DNA 24
2.2 Herpes Simplex Virus (HSV) 25
2.2.1 Morphology 26
2.2.2 Viral Replication and Pathogenesis 27
2.2.3 Epidemiology and Transmission 29
2.2.4 Clinical Manifestations 31
2.2.5 HSV Infection in the Immunocompromised Host 34
2.2.6 Diagnosis 35
2.2.7 Therapeutical Management 35
2.2.8 Specimen Types for the Detection of HSV DNA 36
2.2.9 Laboratory-developed Assays for the Qualitative Detection / Quantitation of HSV DNA 36
2.2.10 Currently Frequently Used Commercially Available Assays for the Qualitative Detection/Quantitation of HSV DNA 36
2.3 Epstein-Barr Virus (EBV) 38
2.3.1 Morphology 39
2.3.2 Viral Replication and Pathogenesis 40
2.3.3 Transmission and Epidemiology 41

2.3.4 Clinic Manifestations ... 41
2.3.5 Therapeutical Management ... 43
2.3.6 Diagnosis ... 43
2.3.7 Specimen Types for the Detection of EBV DNA ... 44
2.3.8 Laboratory-developed Assays for the Quantitation of EBV DNA ... 44
2.3.9 Currently Frequently Used Commercially Available Assays for the Quantitation of EBV DNA ... 44
2.4 Varicella Zoster Virus (VZV) ... 45
2.4.1 Morphology ... 47
2.4.2 Viral Replication and Pathology ... 47
2.4.3 Epidemiology and Transmission ... 48
2.4.4 Clinical Manifestations ... 49
2.4.5 Diagnosis ... 50
2.4.6 Therapeutical Management ... 51
2.4.7 Specimen Types for the Detection of VZV DNA ... 51
2.4.8 Laboratory-developed Assays for the Qualitative Detection/Quantitation of VZV DNA ... 52
2.4.9 Currently Frequently Used Commercially Available Assays for the Qualitative Detection/Quantitation of VZV DNA ... 52
2.5 Human Herpesvirus 6 (HHV-6) ... 53
2.5.1 Morphology ... 54
2.5.2 Epidemiology and Transmission ... 54
2.5.3 Clinical Manifestations ... 56
2.5.4 Diagnosis ... 57
2.5.5 Therapeutical Management ... 57
2.5.6 Specimen Types for the Detection of HHV-6 DNA ... 57
2.5.7 Laboratory-developed Assays for the Quantitation of HHV-6 DNA ... 57
2.5.8 Currently Frequently Used Commercially Available Assays for the Quantitation of HHV-6 DNA ... 58
2.6 Human Herpesvirus 7 (HHV-7) and Human Herpesvirus 8 (HHV-8) ... 58
2.6.1 Morphology ... 60
2.6.2 Epidemiology and Transmission ... 60
2.6.3 Viral Replication and Pathogenesis ... 61

2.6.4 Clinical Manifestation 62
2.6.5 Diagnosis 63
2.6.6 Therapeutical Management 63
2.6.7 Specimen Types for the Detection of HHV-7 DNA and HHV-8 DNA 64
2.6.8 Laboratory-developed Assays for the Quantitation of HHV-7 DNA and HHV-8 DNA 64
2.6.9 Commercially Available Assay for the Qualitative Detection of HHV-7 DNA and HHV-8 DNA 64
3 Conclusion 65

1 Introduction

The herpesvirus family also called *Herpesviridae* is a large family of viruses that produce diseases in animals including humans (Pellett et al. 2006). Members of the *Herpesviridae* family include the cytomegalovirus (CMV), herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), Epstein-Barr virus (EBV), varicella zoster virus (VZV), human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7), and human herpesvirus 8 (HHV-8). Depending on their biological characteristics the *Herpesviridae* have been divided into 3 subgroups, the alpha, beta and gamma herpesviruses, by the „International Commitee on the Taxonomie of Viruses“ (ICTV; Roizman 1993). Members of the alpha herpesvirus subfamily are classified on the basis of their rather short reproductive cycle, a variable host range, competent destruction of infected cells and their ability to establish latent infections chiefly in sensory ganglia. Members of the beta herpesviruses have a long reproductive cycle and a limited host range. They have the tendency to infect mononuclear cells and lymphocytes. Infected cells often become enlarged. Latency can be maintained in the white cells of the blood, kidneys, secretory glands and other tissues. The gamma herpesvirus subfamily is specific for either T or B lymphocytes, and latency is often shown in lymphoid tissue (IHMF 2008).

All members of the herpesvirus family share a common structure. The virions are composed of relatively large double-stranded, linear DNA genomes encased within an icosahedral capsid which is itself wrapped in a lipid bilayer envelope. Infections with herpesviruses most commonly occur during the first two decades of life. If immunocompetent, the infected individuals are asymptomatic or may present with benign mostly febrile illness. However, in individuals with compromised immunity, such as transplant recipients under immunosuppressive therapy and patients with human immunodeficiency virus (HIV) infection as well as in newborns with immature immune system, clinical disease with high morbidity may develop and, in some cases, may lead to death. After primary infection, herpesvirus DNA becomes latent in several host cells; reactivation usually emerges with immune suppression or deficit of the host. The actual mechanisms for reactivation are unknown but are apparently initiated through multiple causes,

such as periods of stress, traumata, solar radiation, fever, various infections, and immunosuppression.

Parts of this work will appear as chapter entitled "Molecular Detection of Herpesviruses" of the book “Diagnostic Virology” (2nd Edition).

2 Molecular Detection of Herpesviruses

Rapid laboratory diagnosis is essential for decreasing the lethality as well as the sequelae of herpesvirus infection in patients at risk. Infection with herpesviruses is indicated by demonstrating the presence of the virus in selected specimens. Molecular techniques have become an important tool in routine laboratory diagnostics of herpesvirus infection.

Reliable detection of herpesvirus DNA depends on pre-analytical issues, such as choice of the correct specimen type, optimal sampling time with regard to the progress of disease, and both time and conditions of the sample transport to the laboratory. Specimen types appropriate to molecular detection of each relevant member of the herpesvirus family are discussed below.

Prior to amplification, the target nucleic acid must be extracted. Nucleic acid extraction, also called sample preparation, is a crucial step in molecular diagnostics. It includes lysis of the nucleic acid-containing specimen and removal of substances which might inhibit subsequent amplification while protecting herpesvirus DNA from degradation. Furthermore, the risk of contamination and potential hazards caused by toxic reagents must be kept to a minimum during sample preparation. Classic manual nucleic acids extraction protocols based on phenol-chloroform extraction have been used in molecular diagnostic laboratories around the world. However, manual methods have usually been time-consuming, labor-intensive, and susceptible to contamination. Today, commercially available kits on automated platforms achieve effective recovery of herpesvirus DNA and have largely replaced the classic protocols.

The introduction of real-time PCR has significantly simplified routine molecular diagnostics (Higuchi et al. 1992). Compared to the conventional PCR procedure, real-time PCR offers several important advantages (Kessler 2007). Real-time PCR combines amplification of target DNA with detection of amplification products in the same closed vessel. Therefore, the potential for contamination is significantly reduced. With real-time PCR, the analytical turnaround time is significantly shorter than that required for assays utilizing conventional PCR. In contrast to conventional PCR, real-time PCR allows for log-phase analysis. Therefore, the quantitation range for assays based on real-time PCR is significantly greater (5-6 logs) than that for assays based on conventional PCR (2-3 logs).

To guarantee accurate and reliable results with molecular assays, several issues must be addressed. Major issues include introduction of an internal control, the detection format, and evaluation of the assay. Because amplification may fail in a reaction due to interference from inhibitors, an internal control must be incorporated in every molecular assay to exclude false-negative results. To ensure an accurate control of the entire molecular assay, the internal control should be added to the sample before the start of the nucleic acid extraction procedure. Either a homologous or a heterologous IC can be employed. With regard to the detection format, introduction of a probe detection format is required to guarantee analyte-specific testing. Today, major probe detection formats include hybridization probes, TaqMan probes, molecular beacons, and scorpions. To obtain additional information, e.g., the genotype of the DNA product, the generation of a melting curve may be useful (Haas et al. 2004). Melting curve analysis can be performed for all detection formats, except for the TaqMan probe format, because signal generation depends on the hydrolysis of the probe. Unexpected melting peaks may indicate primer: primer or primer: probe dimers or sequence variants **(Fig. 1)**.

When introducing a new molecular assay for detection of a member of the Herpesviridae family, it is advisable to employ an IVD/CE and/or FDA labeled test. For such a test, the manufacturer is responsible that the IVD achieves the performance as stated. Nevertheless, the user must verify that performance characteristics, such as accuracy and imprecision are achieved in the laboratory (Rabenau et al. 2007).

The following sections include general information on the specific virus and the adequate specimen types for molecular detection and provide an overview on both laboratory-developed and commercially available molecular assays. Currently frequently used commercial assays in Europe are listed in Tables. It is important to note that ranges of linearity are influenced strongly by both the sample preparation protocol and the amplification and detection system employed. Therefore, data provided herein must always be compared with those provided in the latest version of the manufacturer’s package insert. Furthermore, the “real” upper limit of linearity may be unknown because of the lack of extremely high concentrated samples. Finally, it must be observed that different assays may have different units of

reporting making the comparison between values obtained very difficult or even impossible. For serial or sequential specimens obtained from the same patient, the identical molecular assay should thus be employed.

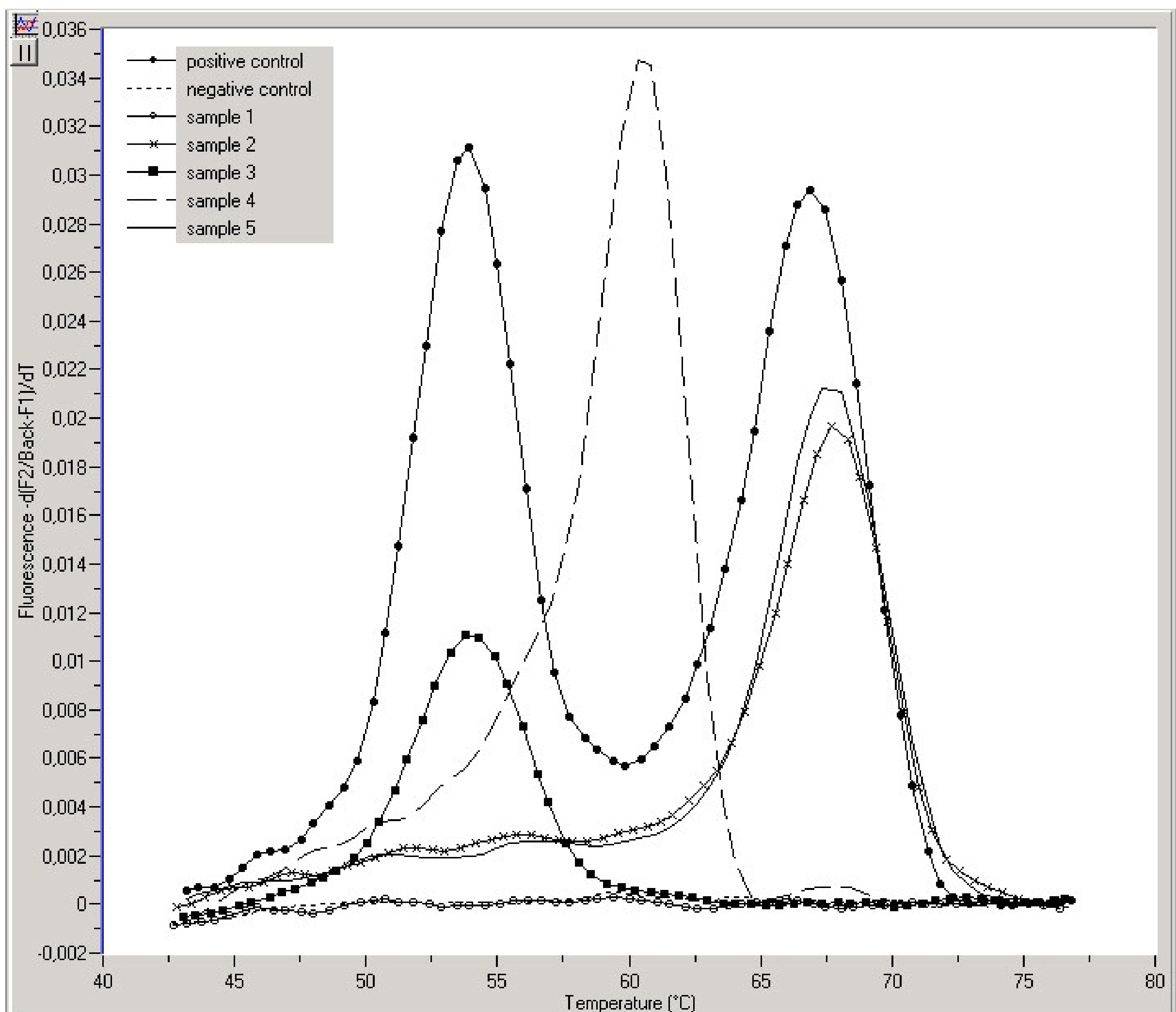

Fig. 1. Detection of herpes simplex virus (HSV) type 1 and type 2 DNA by real-time PCR. Melting curves of clinical samples (genital swabs). In sample 3, HSV-1 was detected; in samples 2 and 5, HSV-2 was found. The positive control contained both HSV-1 and HSV-2. Sample 4 shows an unexpected melting peak at 60.5°C. Sequence variation in the HSV DNA polymerase gene may produce melting peak values that differ from expected values for HSV type 1 or type 2. (Modified from Haas 2004)

2.1 Cytomegalovirus (CMV)

In 1881, Ribbert first detected large cells containing inclusions and hence identified histopathological evidence of CMV, but he didn´t know about the causing agent yet.
1921 Goodpasteure correctly postulated the viral etiology of those inclusions and used the term cytomegalia referring to the characteristically enlarged cells (Goodpasture 1921, Classen et al. 2004).
Finally, in 1955, CMV was isolated as responsible agent for Cytomegalic Inclusion Disease (CID) – the most severe appearance of congenital CMV infection.

The CMV is a ubiquitous virus which infects up to 100% individuals, especially in the first two decades of life. In immunocompetent individuals, primary infection is usually asymptomatic or mild. But the CMV has the ability to establish lifelong latent infection following primary exposure. Sites of latency are white blood cells, endothelial cells and cells in a variety of different organs (Forbes et al. 2007). Under certain conditions, CMV can reactivate, resulting in asymptomatic viral shedding or development of disease. While in the immunocompetent individual the infection is held in check by the host's immune response, CMV disease is generally restricted to the immunocompromised or immunologically immature host (Vancikova et al. 2001). The CMV is thus the most important opportunistic viral pathogen for immunocompromised patients, such as AIDS patients and solid organ and bone marrow recipients, with CMV seronegative transplant recipients at highest risk of developing CMV and its complications. CMV causes the most morbidity and mortality of all herpesviruses and thus is the most important cause of congenital infection in western countries (Schleiss 2008).

2.1.1 Morphology

The CMV, also called human herpesvirus 5 (HHV-5), is a double stranded DNA beta-herpesvirus which also contains 4 species of mRNA. It is the largest of the known herpesviruses, with a genome of over 235 kb and more than 200 potential open reading frames. The function of these proteins remains unclear so far. CMV measures about 150-200nm and has an icosahedral protein complex called capsid, which is designated nucleocapsid with the DNA genome implemented. The

nucleocapsid is coated with a layer of protein named tegument. The tegumented nucleocapsid is then surrounded by an envelope consisting of a lipid bilayer and within embedded glycoproteins. These glycoproteins are important for the attachment to the host cell (UC SanDiego 2009).
In vivo cytomegalic cells are 2 to 4 times larger than their surrounding non infected cells. Formation of large cells is due to the fusion of 4 to 8 normal cells (Gerok et al. 2007).
The histopathological appearance of “owl´s eyes” occurs because of the eccentrically placed intranuclear inclusions that are surrounded by a clear halo. These cells can be found in many different tissues like salivary glands, lung, liver, kidneys, intestine, pancreas, adrenal glands, blood components and central nervous system (Fauci et al. 2008).

2.1.2 Viral Replication and Pathogenesis

The replication takes place in the cell nucleus and goes along with production of large intranuclear and smaller cytoplasmatic inclusions. This type of cytopathology is pathognomic for CMV and makes it possible to distinguish CMV from other herpesviruses. The viral life cycle takes approximately seventy-two hours and is temporally divided into the following 3 regulated classes: immediate early, early, and late. After the initial fusion of the viral envelope with the plasma membrane of the cell, the encapsided virus particle is released into the cytoplasm and within minutes, transits to the nucleus via active transport through the nuclear pore. The capsid acquires entry and the viral DNA is positioned. Immediate early gene transcription proceeds within the first 4 hours after viral infection with the aim to make key regulatory proteins that allow the virus to take control of the cell. The immediate early genes are required for both early and late gene expression. After the immediate part of replication, the transcription of early genes occurs, which generally encode for DNA replication and also structural proteins. Finally, about 24 hours after infection, late gene expression occurs once the virus has begun replicating its genome and these genes encode primarily for structural proteins of the virion´s assembly and egress. Nucleocapsid particles are assembled within the nucleus and then acquire their tegument and envelope. Completely assembled virus particles bud off from the cell into the surrounding area and can then go on to infect other permissive cells (UC SanDiego 2009).

The synthesis of the late genes can be blocked by inhibitors of viral DNA polymerase, one of those is Gancyclovir.
A study by Emery et al. showed that the replication of CMV has a viral doubling time of one day in vivo and hence might not be a slowly replicating virus, as previously thought.
In vivo CMV seems to replicate in a variety of cell types like fibroblasts, leucocytes, cells of kidney and adrenal glands, whereas in vitro it prefers fibroblasts. It was found that the virus also transforms these cells in vitro, whereas it is not assumed to be oncogenic in vivo. Once settled it can either lead to a latent or lytic and productive infection. (Fauci et al. 2008)

Basically, both humoral and cellular immune response to CMV infection exists (Gerok et al. 2007).
The lipid bilayer outer envelope contains the virally encoded glycoproteins, which are the major targets of host neutralizing antibody responses. These glycoproteins are targets for human vaccine design. The layer of proteins between the envelope and the inner capsid, the viral tegument, contains proteins that are major targets of host cell-mediated (cytotoxic t-lymphocytes) immune response. The most important of these tegument proteins is the so-called major tegument protein, UL83, phosphoprotein 65 (pp65).
The cellular immune response consists of granulocytes, monocyte-macrophages, lymphocytes and plasma cells. Glycoprotein B complex is the most common component of the envelope, it is an immunologically active protein, that induces antibodies for virus neutralization. Unfortunately these inflammatory immunopathologic responses of the host also lead to symptoms of CMV disease (Fauci et al 2008).
CMV evades the host´s defense function via several pathways that are not yet fully understood and reactivates with either development of disease or asymptomatic viral shedding. Viral shedding occurs in all kinds of hosts but is especially common in immunocompromised patients.
Reactivation of CMV often occurs when T-lymphocyte-mediated immunity is compromised (organ transplantation, HIV infection, lymphoid neoplasms, acquired immunodeficiencies, antithymocyte globulin).

Initial replication of CMV leads to lysis of the host cell or stays latent in endothelial cells of salivary and intestinal glands as well as in monocytes.

2.1.3 Epidemiology and Transmission

There is a worldwide distribution of CMV with a prevalence of 40-100%.
Throughout the world, 1% of newborns are infected with CMV, in early adulthood this rate has already reached 50% on average, with higher percentages in many less-developed countries due to communal living and poor personal hygiene (Gerok et al. 2007).
Transmission happens in utero, perinatal, via airborne infection, smear infection or iatrogen and is possible via breast milk, saliva, feces, urine, whole blood or blood products. It does not happen through casual contact but through repeated and prolonged intimate contact.
Sexual transmission via semen and cervical secretion in adolescence is a usual way of infection and detection of several different strains at the same time is rather common.
Lifelong intermittent viral excretion occurs in 1-30% of latently infected individuals, most often in children and also in pregnant women and most likely via breast milk (Classen et al. 2004).
There is just one serotype of the virus, but an enormous variability concerning DNA (95% homology). This is the reason for identification of patient-specific isolates. As an explanation for exogen reinfections it is possible that the same patient hosts CM-viruses of different genotypes. Analyses of antigens show that 20% of population had have contact with more than one CMV strain (Classen et al. 2004).

2.1.4 Clinical Manifestations

Today congenital CMV infection is the most common reason for embryopathy due to infection. Per definition diagnosis of congenital CMV infection requires identification of the virus in a cultural specimen acquired before the age of 3 weeks because perinatally acquired infections may also begin to manifest at this time.
Congenital infections mainly happen because of primary infection (in 1-4% of all pregnancies) of the mother during pregnancy. Despite the fact that the transmission rate is rather high then, reaching 35-50% (Classen et al. 2004), the

majority of babies are clinically inapparent at birth. But up to 25% of them develop psychomotor, hearing, ocular or dental abnormalities over years.

Reactivation of CMV during pregnancy is rather common (3-40%) and the transmission rate is only 0,2 -2% (Classen et al. 2004).

But sadly, 5% of infected foetuses born to mothers who had primary infection during pregnancy suffer from “Cytomegalic Inclusion Disease”- a severe and disseminated form of CMV disease with thrombocytopenia related petechiae , hepatosplenomegaly, jaundice, microcephaly, intrauterine growth retardation, prematurity, elevated alanine aminotransferase levels, conjugated hyperbilirubinemia, hemolysis and elevated cerebrospinal fluid protein levels.

Mortality is high, with 20-30% and usual sequelae are intellectual and hearing difficulties.

In this case, PCR viral load can help to predict neurodevelopment outcomes (Fauci et al. 2008).

Perinatal infection happens during delivery through an infected birth canal or through postnatal contact with maternal secretions like saliva or breast milk. Local reactivation in mammary glands of CMV seropositive mothers reaches 90% with transmission rates of 40% because of breastfeeding.

Most of the infected babies stay asymptomatic, but poor weight gain, adenopathy, rash, hepatitis, anemia, atypical lymphocytosis and virus excretion for years is possible (Fauci et al. 2008). For estimation of the baby´s risk it is important to distinguish between primary- and reinfection of the pregnant mother. Therefore the detecion of IgM antibodies doesn’t help because IgM production is also possible during reinfection. Synthesis of anti-gB appears immediately during reinfection, but needs 50-100 days for emerging after primary infection (Classen et al. 2004).

In normal hosts without impaired immune system 90% of CMV infections are asymptomatic (Gerok et al. 2007), the rest of immunologically healthy hosts develope a heterophile antibody-negative mononucleosis syndrome, also called ”EBV negative mononucleosis”. Basically it is estimated that 8% of clinically diagnosed mononucleosis syndroms are due to CMV (Classen et al. 2004). This syndrome occurs at all ages, mainly in sexually active young adults. The incubation period is 20-60 days before CMV titer in blood rises to 10^4-10^5/ml; most of it cell bound (Gerok et al. 2007).

The symptoms last for 2-6 weeks with prolonged high fever, chills, profound fatigue, malaise, myalgia, headache, hepatosplenomegaly, pharyngitis and cervical lymphadenopathy as seen in EBV infection but less severe. Rash after exposure to ampicillin happens too, as well as leukopenia, relative lymphocytosis, atypical lymphocytes/monocytes with atypical nuclear structure (up to 10% of leukocytes) and thrombopenia. But there is no appearance of heterophile antibodies (as in EBV infection) and recovery takes place without any sequelae. Excretion of virus in urine, genital secretions and saliva at least is possible for months or years.
Endogen reactivations with secretion of virus DNA happen from time to time also in healthy individuals- depending on the current immunological situation- but are normally not recognized in the immunocompetent individual (Fauci et al. 2008).

2.1.5 CMV Infection in the Immunocompromised Host

CMV is a major problem for the immunocompromised host (premature newborns of low birthweight, tumor patients, transplanted patients under immune suppression, HIV-coinfected patients) and is the most common and important viral pathogen complicating organ transplantation.
CMV disease in these patients leads to fever, leucopenia, hepatitis, interstitial pneumonia (pathognomic for CMV), esophagitis, gastritis, colitis, meningoencephalitis, retinitis (as important cause of blindness in HIV-infected patients), acute graft rejection, loss of organ(especially after transplantation of the kidney) and death (Fauci et al. 2008). But CMV also has so-called “indirect effects” in transplant patients like bacterial or fungal superinfection (Razonable et al. 2004).
Risk is at maximum 1-4 months after transplantation and after the breakpoint of prophylactic therapy with thymidinekinase inhibitors like Ganciclovir, Valganciclovir, Famciclovir and oral acyclovir which is less effective than the others. In more than 60% of these patients viremia, which is often asymptomatic but shows elevated mortality rate, is not preventable. But prevalence of HCV disease decreased from 20-60% to 5% since implementation of routinely prophylactic therapy with Ganciclovir. Selection of virus strains which are resistant to therapy is possible during long-term therapy and is a negative aspect which must be accepted. Risk of disease development is highest and symptoms are

more severe if a CMV negative patient gets a CMV positive organ or CMV positive blood products (Schmetzer 2009). But mainly, reactivation of pre-existing latent infection is the cause of CMV associated symptoms (Gerok et al. 2007). The transplanted organ itself is especially susceptible for CMV infection, which often happens after kidney transplantation. Severity of disease depends on many different factors like degree of immunosuppression, type of immunosuppressive therapy, development of graft versus host disease, patient´s age, evidence of viremia, pretransplantation seropositivity and co-infections.

In advanced HIV infection, when CD4+ T cell count is below 100/ul, retinitis (pathognomic for CMV) occurs, as well as disseminated CMV disease (gastrointestinal destruction, encephalitis).

First signs of CMV infection in immunocompromised patients are prolonged fever, malaise, anorexia, fatigue, night sweat, arthralgia, myalgia, elevated liver encymes, leucopenia, thrombocytopenia, atypical lymphocytosis and respiratory involvement (high mortality of 80%, tachypnoe, hypoxia, unproductive cough).

Persistent viremia and multiple organ system involvement can be assumed to be fatal for these patients (Fauci et al. 2008).

There is a clear relationship between high CMV DNA levels in blood and the clinical progression of CMV infection not only in solid organ but also in bone marrow recipients. Monitoring the viral load at biweekly intervals allows identification of patients at risk for developing CMV disease (Table 9.7). Both the CMV DNA load at the onset of active infection and the increase of viral load correlate with CMV disease in transplant recipients. This information is essential for the start of antiviral therapy whose efficacy is best monitored through quantitative real-time PCR. However, it must be stated that a clear-cut threshold value regarding prediction of disease progression and response to treatment has not yet been defined. If the CMV DNA load increases continuously during antiviral therapy, nucleotide mutations may be present and thus responsible for the lack of therapeutic efficacy. In this case, sequencing of the target gene (especially, UL97 and UL54) should be done for confirmation or exclusion.

2.1.6 Diagnosis

Evidently clinical signs alone are not reliable for diagnosis of CMV infection. Detection and quantification of CMV DNA via PCR has been implemented and has replaced the pp65-antigenemia testing in the routine diagnostic laboratory.
Nevertheless some now already obsolete diagnostic methods are mentioned.
Isolation and quantitation of immediate early protein (antigen) pp65 and CMV DNA in appropriate clinical specimens (blood leucocytes) during viremia adduces evidence of a florid CMV infection and can be used to forecast systemic reactivation.
Specimen is inoculated onto human fibroblast monolayers. It may take days or weeks, but during this period cell culture is monitored for development of the characteristic CMV-associated cythopathic effects: Presence of enlarged cells containing intranuclear inclusions designated “owl´s eyes” definitely indicates productive infection. Although specificity for this diagnostic method is rather high, sensitivity is very low (Classen et al. 2004).
Isolation of CMV out of urine and saliva neither gives evidence about cause of disease nor about activity of CMV (Gerok et al. 2007) as prolonged egration of virus via saliva and urine is very common. But nevertheless more frequent or persistent urinal CMV excretion is a sign for prospective CMV manifestation.
Concerning serologic assays (immunofluorscence, ELISA): 4 times increase in titers of IgG / IgM antibodies (after 4 weeks) proofs evidence of infection whereas single-sample antibody determination says nothing about acuteness of disease as antibody titers can remain high for years. But, as in all herpesviruses, detection of CMV in biopsy provides evidence of infectivity of blood and tissues.
It is necessary to do a lung biopsy or bronchoalveolar lavage to confirm CMV pneumonitis, as well as liver biopsy for confirmation of hepatitis and a lumbal punction of cerebrospinal fluid for detection of encephalopathy or polyradiculopathy (Schleiss 2008, Gerok et al. 2007).

2.1.7 Therapeutical Management

Treatment in individuals with normal state of immune system is supportive.
In patients under immunosuppressive therapy it can become necessary to decrease immune suppression (Forbes et al. 2007).

Ganciclovir, Foscarnet and Cidovir can be used for CMV treatment, whereas Aciclovir has no therapeutic potency but can be used in a prophylactic setting (Classen et al. 2004).

Current opinion is that non-immunocompromised patients don't need antiviral treatment and can be cured with symptomatic therapy only because CMV infection in these hosts is considered of being benign and self-limiting. But nevertheless efforts to evaluate the risks and benefits of special antiviral treatment for severely ill patients exist.

Additionally, a recent systematic review of Rafailidis et al. came to the conclusion that severe life-threatening complications of CMV infection in non immunocompromised individuals might not be as seldom as previously thought (Rafailidis et al. 2008).

To avoid lethal outcome of CMV disease in the immunocompromised patient, the start of treatment at the earliest stage is of extreme significance (Boriskin et al. 2002, Meijer et al. 2003). The level of CMV DNA has been found to be an important prognostic marker for the ongoing disease (Allice et al. 2006, Boeckh et al. 2004. Caliendo et al.2007). The preventive usage of CMV antibody-negative blood and tissue in blood transfusions and transplantations, avoiding contact with mucous membrane and the use of condoms during sexual intercourse can also reduce rates of transmission.

2.1.8 Specimen Types for the Detection of CMV DNA

There is currently no consensus on the optimal blood compartment for routine molecular CMV DNA testing. EDTA whole blood, peripheral blood leukocytes, and plasma have been used for routine diagnosis (Razonable et al. 2002, Mengelle et al. 2003). In several studies, EDTA whole blood has been found to be superior to peripheral blood leucocytes or plasma (Meijer et al. 2003, Mengelle et al. 2003, Deback et al. 2007, Garrigue et al. 2006, Koidl et al. 2008, Ljungman et al. 2004, Razonable et al. 2004, Sia et al. 2000, Weinberg et al. 2002). With a high-sensitive molecular assay, latent virus may be detected but the clinical relevance of low-level CMV DNA in whole blood is unresolved. Nevertheless, the early detection of CMV DNA has the advantage of warning clinicians to be aware of the evolution of infection and to carefully follow up viral load kinetics (Garrigue et al. 2008).

Further specimen types for detection of CMV DNA in the immunocompromised patient include cerebrospinal fluid, bronchoalveolar lavage, aqueous humor, and bone marrow. For prenatal issues, amniotic fluid may be an additional specimen type.

2.1.9 Laboratory-developed Assays for the Quantitation of CMV DNA

Following the era of laboratory-developed assays based on conventional PCR in the nineties, several assays based on real-time PCR have been established more recently. Molecular assays based on quantitative real-time PCR have been shown to provide several important advantages to detection of CMV antigen even though some studies indicated general agreement between the two methods. Advantages of molecular assays based on quantitative real-time PCR include increased sensitivity for early detection of CMV infection or reactivation, utility for patients with neutropenia, wide range of linearity (up to 8 $\log_{10}$), ability to process large number of specimens, and the potential for increased accuracy of results through precision instrumentation (Allice et al. 2006, Mengelle et al. 2003, Funato et al. 2001, Nitsche et al. 2003, Yakushiji et al. 2002)

However, laboratory-developed molecular assays for the detection of CMV DNA are almost unique for each laboratory usually lacking standardization. In a recent review, more than 10 different target regions of the CMV genome and at least three different units of result reports have been described (Espy et al. 2006***)***. In this context, it must be emphasized that the choice of the target region requires special attention. In several laboratory-developed molecular assays, the glycoprotein B gene (UL55) has been used as the target region. However, CMV variants that could not be quantified using quantitative real-time PCR targeting the glycoprotein B gene have been reported (Schaade et al. 2001). Sequence analysis revealed a single base pair mutation in the target sequence of the down-stream probe. More seriously, several additional sequence differences with the probes used in the glycoprotein B gene assay have been reported recently (Nye et al. 2005).

2.1.10 Currently Frequently Used Commercially Available Assays for the Quantitation of CMV DNA

Several commercial assays have been developed for the quantitation of CMV DNA. A summary of currently frequently used commercial assays in Europe is provided in **Table 1**. The majority of those assays employ the real-time PCR method while the COBAS Amplicor CMV Monitor Test is based on the conventional PCR technology thus being impaired by a limited range of linearity. The COBAS Amplicor CMV Monitor Test has been evaluated in numerous studies including comparisons with detection of CMV antigen and other molecular assays and has been found to be suitable for routine diagnostic testing of plasma samples obtained from patients at risk of or suffering from CMV infection (Aitken et al. 1999, Boivin et al. 2000, Pellegrin et al. 1999, Preiser et al. 2002). To improve the limit of quantitation, this assay may be performed in an ultrasensitive version by addition of a high-speed pre-centrifugation step to the standard procedure (Kaiser et al. 2002). However, in comparison to molecular assays based on real-time PCR, the COBAS Amplicor CMV Monitor Test was reported to underestimate the viral DNA concentration in plasma samples (Pang et al. 2003, Sassenscheidt et al. 2006, Yun et al. 2000).

Currently, the COBAS Amplicor CMV Monitor Test is more and more replaced by alternative commercially available molecular assays based on real-time PCR. Those assays show a wide range of linearity and several of them have been evaluated including comparisons with detection of CMV antigen and other molecular assays and have been found to be suitable for the detection and quantitation of CMV DNA in the routine diagnostic laboratory (Koidl et al. 2008, Allice et al. 2008, Gouarin et al. 2007, Hanson et al. 2007, Michelin et al. 2008, Tang et al. 2008).

Table 1: Comparison of currently frequently used commercially available assays for the quantitation of cytomegalovirus (CMV) DNA.

Characteristics	Manufacturer and details				
	Argene	Nanogen Advanced Diagnostics	Qiagen GmbH	Roche Molecular Diagnostics	Roche Molecular Diagnostics
Kit name	CMV R-gene	CMV Real Time Complete Kit	artus CMV PCR Kit	COBAS Amplicor CMV Monitor Test	LightCycler CMV Quant Kit
Target sequence	UL83 (lower matrix Phosphoprotein 65)	UL123 (major immediate early protein)	UL122 (immediate early protein)	UL54 (DNA polymerase)	UL54 (DNA polymerase)
Amplification method	Real-time PCR	Real-time PCR	Real-time PCR	Conventional PCR	Real-time PCR
Detection method	Fluorescence	Fluorescence	Fluorescence	EIA	Fluorescence
Internal control	Heterologous	Heterologous	Heterologous	Homologous	Heterologous
Standards	Four EQS	Four EQS	Four EQS	One IQS	Four EQS
Range of linearity	$5.0 \times 10^2 – 2.5 \times 10^6$ copies/mL	$3.2 \times 10^2 – 1.3 \times 10^7$ genome equivalents/mL	$3.2 \times 10^2 – 1.0 \times 10^7$ copies/mL	$6.0 \times 10^2 – 1.0 \times 10^5$ copies/mL	$1.0 \times 10^3 – 2.0 \times 10^7$ copies/mL

EQS: external quantitation standards; IQS: internal quantitation standard.

2.2 Herpes Simplex Virus (HSV)

The term “herpes” has been used in medicine for many centuries and is deduced from the Greek word for “creeping” used by the ancient Greeks to describe spreading cutaneous lesions of varied etiology. During the twentieth century, HSV research was very popular and histopathologic studies characterized the multinucleated giant cells associated with herpesvirus infection. In 1919, Lowenstein experimentally confirmed that the HSV was indeed spread from one infected person to an uninfected one. In the 1920's and 1930's, the natural history of HSV was widely studied and it was known that HSV not only infects the skin, but also the central nervous system. Also host immune responses to HSV were exhaustively examined and the specific feature of HSV known as latency was characterized. By the 1940's and 1950's, research was concentrated on the diseases caused by HSV (Evans et al. 1997).

More recent research has focused on antiviral research, differences between HSV strains, and using HSV vectors for use in vaccines.

The HSV causes a wide spectrum of clinical manifestations. The HSV type 1 (HSV-1) is commonly associated with oropharyngeal infections, keratoconjunctivitis, and infections of the central nervous system (CNS) whereas the HSV type 2 (HSV-2) commonly produces genital infections. Basically, both subtypes may cause genital and/or oral-facial infections, where the clinical appearance is indistinguishable and needs further diagnostic methods to be verified.

Neonatal HSV infection including HSV encephalitis and retinitis is predominantly caused by HSV-2, whereas an increasing proportion of genital infections are attributable to HSV-1 rather than to HSV-2 (Lafferty et al. 2000, Lamey et al. 1999).

Today, effective therapeutic management exists; however, especially for CNS disease, antiviral drugs must be administered at a very early stage. Rapid laboratory diagnosis is thus essential for decreasing the lethality as well as the sequelae of HSV infection.

2.2.1 Morphology

The HSV is a linear, double stranded DNA alpha-herpesvirus, with a genome of over 152 kb and more than 90 potential open reading frames, under which 84 identified proteins. HSV measures about 100-110nm and DNA is packaged in an icosahedral protein shell called capsid (reassembled of 162 capsomeres), which is designated nucleocapsid with the DNA genome implemented. The nucleocapsid is coated with a layer of protein named tegument. The tegumented nucleocapsid is then surrounded by an envelope consisting of a lipid bilayer and within embedded glycoprotein that is acquired as the nucleocapsid buds through the inner nuclear membrane of the host cell (Fauci et al. 2008, Skern 2006).

The two subtypes, HSV1 and HSV2, are very similar regarding genomic issues. The homology in genomic sequence is around 50%, in proteome homology over 80% (Fauci et al. 2008). Nevertheless there are many type specific regions unique for either HSV-1 or HSV-2 virus, among those some are encoding for proteins which are important in host immunity. Based on these type-specific regions, routinely used serologic assays that distinguish between the two viral subtypes have been developed. By genomic sequencing or restriction endonuclease

analysis of viral DNA acquired from two individuals epidemiologically related origins, such as mother-infant or sexual partners can be inferred from these patterns (Fauci et al. 2008).

2.2.2 Viral Replication and Pathogenesis

Viral replication cycle consists of highly regulated stages, taking place both within the nucleus and the cytoplasm. The attachment involves HSV-surface glycoproteins that first bind to cellular glycosaminoglycanes like heparin-like surface receptors and then induces fusion of viral and cellular membrane. During uncoating the capsid enters the nucleus releasing DNA and tegument proteins are released into cytoplasma and nucleus which affect both viral and cellular processes. Some proteins can increase cellular RNA degradation and therefore shut off the cell´s protein synthesis while others initiate the transcription of early genes of HSV replication via the cellular RNA polymerase II. This transcription of viral genetic material leads to the expression of immediate early, early and late transcripts. Proteins encoded by early genes are responsible for the replication of genetic material. Late proteins on the other hand are involved in the assembly and maturation. In the nucleus DNA is packaged into capsid and passes through the nuclear membrane into cytoplasm where more tegument proteins are added. The viral envelope and glycoproteins are collected during passage through the golgi apparatus. Finally, mature HSV is released from the infected cell by exocytosis.
(Fauci et al. 2008, Skern 2006)

HSV-1 and HSV-2 can productively infect epithelial cells and latently infect neuronal cells. In epithelial cells the full program of viral gene expression is carried out, large amounts of new virus are made and thus cells are destroyed. This infection is cleared by adaptive immunity. Whereas in neuronal cells almost no gene expression takes place and hardly any HSV proteins are produced, subsequently host´s defense is not on alert and infected neurons are never cleared. However, there is one characteristic RNA molecule termed LAT (latency associated transcript) detectable which function is yet unknown. HSV is invisible to the innate and adaptive immune system, it interferes with and hides from immune system and therefore can cause long-term infections. Some strategies of HSV are: There are surface glycoproteins which inactivate complement C3b and antibodies. The virus infection reduces expression of major histocompatibility complex class I

molecules and thus reduces efficiency of adaptive immune system. However, this reduction can be sensed by natural killer cells. There are several factors that can cause HSV to leave the latent state and become active like systemic or local immunosuppression, physical or mental stress, hormonal changes, UV-light and trauma to the skin or ganglia. However, the exact mechanisms of the establishment, maintenance, or interruption of latency are incompletely understood and also how reactivation takes place in molecular terms is yet unknown. During reactivation virus is released from neurons without their lysis, transported intra-axonally from the nerve cell bodies in ganglia to the epithelial ending of the nerval cell, leading to productive infection of neighboring epithelial cells and reappearance of virus on mucosal surfaces. (Fauci et al. 2008, Skern 2006)

During primary HSV infection, natural killer cells activation as a result of the production of several cytokines like interferon-gamma, are the most important effectors of defense. The efficiency of the immune response to primary infection may have an considerable impact on the quantity of virus established in the ganglia and frequency of recurrences. When subsequent recurrences appear and as the immune system matures CD4+ and CD8+ T cells take over the position of main effector in resolution of infection (Runge et al. 2009).

But anyway, in immunologically healthy people HSV generally does not spread throughout the body. The only imaginable scenario where HSV causes a systemic infection is when an immunosuppressed host and a whole body contact with HSV are brought together. These conditions are fulfilled during delivery of a child from a mother with genital herpes infection for example. In the immunocompetent individual strong cytotoxic T cells and natural killer cell response limit infection and the cellular attachment molecule for HSV is heparin sulphate, a very abundant part of the extracellular matrix which absorbs the virus before it can spread. (Skern 2006)

Primary infection occurs when mucosal surfaces or abraded skin is exposed to HSV and thus the virus is permitted to entry epithelial cells and initiates its replication. Dermal vesicles containing infectious virus particles or body fluids like saliva of an infected individual may be transmitters. This may happen without symptoms. The virus then infects sensory or autonomic nerve endings and is subsequently transported intra-axonally to the nerve cell bodies in ganglia where

they enter a latent state but can reactivate at any mucosal site innervated by the infected ganglia (Runge et al. 2009). Contiguous local spread of virus infection from one epithelial cell to the other also allows further extension of disease. HSV viremia also takes place in a certain amount of infected patients and may lead to the extension of infection throughout the body (Fauci et al. 2008).

2.2.3 Epidemiology and Transmission

HSV infections are documented worldwide. Nowadays serologic assays allow differentiating uninfected seronegative persons from HSV positive persons and also distinguish between the two viral subtypes, HSV-1 and HSV-2. Also multiple strains of the same viral subtype are detected in one individual and proof the possibility of exogen infection with different strains at the same time.

The incidence of first-episode genital HSV-1 infection is increasing and incidence of HSV-1 genital infection varies between 10% and 30% in the USA and 50% in the UK and Japan (Malkin 2004).

As the two different types of viruses have different requirements they both have their preferred localization and thus genital HSV-2 twice as likely reactivates and recurs 8-10 times more frequently than HSV-1 genital infection. Consequently oral-labial HSV-1 infection recurs more frequently than oral-labial HSV-2 infection.

The same applies to asymptomatic viral shedding, which is most likely to occur if oral-facial mucocutanous areas are infected with HSV-1 and genital areas are infected with HSV-2 (Fauci et al. 2008).

Infection with HSV-1 occurs more frequently and earlier than infection with HSV-2, usually in early childhood.

Over 90% of adults have antibodies against HSV-1 by fifth decade of their life and earlier, before third decade of life, in countries of low socioeconomic status (Fauci et al. 2008).

There is sufficient evidence that seroprevalence of HSV-1 induces a partly cross immunity so that in countries where acquiring of HSV-1 has decreased, HSV-2 seroprevalence has increased (Robert Koch Institut 2009).

Usually, antibodies to HSV-2 are not detected until puberty, correlating with past sexual activity. Prevalence of HSV-2 is higher in women than in men, but there are mostly the same independent risk factors of acquiring HSV-2 in both genders, like older age, higher lifetime number of sexual partners, positive HIV serology and

positive syphilis serology. In developing countries, prevalence can vary widely from 2-74% according to the country, age, sex or urban versus rural areas (WHO 2009). A recent study investigating socio-economic differentials in HSV-2 seroprevalence in Australian men and women showed that HSV-2 seroprevalence was significantly lower in areas of low socio-economic status (SES) than in high SES areas among both men and women for all ages. There was a lower rate of HSV-2 in men with lower educational achievement but HSV-2 prevalence was higher for women with lower individual levels of education for all ages (Page et al. 2009).

About 15-20% of the US population carry HSV-2 antibodies, in most obstetrics clinics 25% of women are tested positive and in sexual transmitted disease clinics even 50% of heterosexual adults carry antibodies to HSV-2, although not all of them have a history of genital lesions (Fauci et al. 2008). In other countries like Central America, South America (39% in Brazil), Europe (7% in the UK) or Africa the situation is similar or even worse (Smith 2002). Actually, on the one hand HSV-2 is an independent risk factor for acquisition and transmission of infection with the HI-Virus which can be shed from herpetic lesions of co-infected patients. As a result epidemiology shows that HSV-2 infection is associated with double or 4fold increase in HIV acquiring (Fauci et al. 2008). And on the other hand among co-infected persons HSV-2 is reactivated and transmitted more frequently than among persons without HIV infection (Fauci et al. 2008).

Transmission of HSV is possible from contact with mucocutaneous surfaces of persons suffering from active ulcerative lesions or from contact with persons asymptomatically shedding virus at the time.

Among immunocompetent persons HSV-2 DNA can be detected from genital tract on 20-30% of days tested by PCR, regardless of the presence or absence of clinical symptoms. This is similar with HSV-1 in oral secretions (Fauci et al. 2008).

The highest rates of HSV shedding, with 30-50% of days tested in immunological healthy persons and 20-80% of days tested in immunosuppressed patients, occur during the initial years after acquiring of infection (Fauci et al. 2008).

There is obviously a large reservoir of carriers for transmission, who might not even know about their illness and the fact that they might endanger their fellows.

Actually 70% of transmission is attributable to asymptomatic viral shedding (Runge et al. 2009).

In a monogamous couple, a seronegative female has a greater than 30% per year risk of acquiring an HSV infection from a seropositive male partner. If an oral HSV-1 infection is contracted first, seroconversion needs a period of 6 weeks to provide protective antibodies against a future genital HSV-1 infection (Mertz 1993). But prior to seroconversion it is possible for the virus to spread to other sites of the host´s body either through an additional physical contact to an infected patient or through autoinoculation.

As exposure to HSV is very common in everyday-life (sexual contact, kissing, sharing glasses etc.) continuous spread and high seroprevalence of HSV is being observed worldwide (Fauci et al. 2008).

2.2.4 Clinical Manifestations

Primary infections (there are no HSV antibodies in host serum) generally happen without noticing of the host or appear as gingivostomatitis and pharyngitis, mainly among children. Occasionally primary infection may be accompanied by systemic signs and symptoms, that may include fever, malaise, cervical adenopathy, exsudative or ulcerative lesions on hard and soft palate, posterior pharynx, tonsillar pillars, gingiva, tongue, lip and facial area. In this case a longer duration of healing and a more severe illness has to be assumed (Fauci et al. 2008).

After recovery from a primary herpes infection, the virus is not cleared from the host, but lies somewhat inactive in an almost non-replicating state within the cell body of the neuron. The result of primary infection is the production of antibodies to the particular type of HSV that caused the primary infection for prevention of a possible subsequent infection of the same virus type at a different site of the body.

Reactivation of HSV may occur asymptomatic viral excretion in saliva, intraoral mucosal ulcerations, herpetic ulceration on lip or external facial skin whereas recurrent herpes labialis is the most common option.

Erythema multiforme is also associated with HSV infection (Fauci et al. 2008), as well as Bell´s palsy (Lazarini 2006).

Genital infection with either HSV-1 or HSV- 2 in immunocompetent persons usually goes unrecognized.

Whereas the symptomatic primary infection with HSV in the genital area is rather severe , with multiple small painful vesicles and ulcers around the introitus or labia in women and glans or shaft of the penis in men, and the buttocks and anal area in both sexes.
After an average incubation period of 4 days crops of lesions occurs. First a papule forms, evolves into a clear or pustular vesicle within hours, and finally turns into a shallow painful ulcer. These crops of lesions emerge over 1 to 2 weeks, crusting and healing need an additional 1 to 2 weeks.
Additional systemic symptoms and complications of first episode primary genital herpes are secondary bacterial infection, aseptic meningitis, fever, headache, malaise, pain, itching, dysuria, vaginal and urethral discharge, cervicitis, flu-like symptoms, inguinal lymphadenopathy, erythematous ulcer. Cervix and urethra are even involved in more than 80% of cases of first episode (Fauci et al. 2008).
When patients already have had prior infection with either HSV, first episode of genital herpes shows a faster healing and is less severe because of a preexisting humoral and cellular immune response; all in all it can be mistaken for a recurrent episode. (Runge et al. 2009)
After recovery the virus lies dormant in the root ganglia and in most patients reactivation occurs. 12 months recurrence rate is 90% with HSV-2 and 55% with HSV-1, hence the median number of recurrences is 4 versus less than one episodes per year (Fauci et al. 2008).
In recurrent episodes a neuropathic prodrome like dysesthesia may occur 6 to 24 hours before the appearance of lesions, which occasionally may be mistaken for excoriation or irritation because of their atypical appearance (Runge et al. 2009).
The detection of virus in the absence of visible lesions is attributed to minor but continuous subclinical viral shedding, which can be detected by PCR in about 20% of days (Runge et al. 2009). HSV isolation in urine without any coexisting external genital lesions for example is characteristic for HSV urethritis. The frequency of subclinical shedding is greatest in the first 6-12 months after requiring genital herpes, decreases in frequency over time and is generally less common in HSV-1 than in HSV-2 (Runge et al. 2009). The episodes of viral shedding are temporally associated around symptomatic outbreaks, detectable more than a week preceding or following symptomatic recurrences (Leone 2005). It is assumed that

the development of symptoms or lesions may be strongly related to duration of viral shedding (Runge et al. 2009).

Symptomatic and asymptomatic rectal and perianal infections and also subclinical perianal shedding may occur without skin to skin contact but due to prior genital tract infection and reactivation in epithelial cells perianal after latency in the sacral dermatome.

Other manifestations of HSV are the herpetic whitlow and the herpes gladiatorum, both due to inoculation of the virus through a break in the epidermal surface and potentially affecting almost any area of skin after some kind of dermal trauma.

(Fauci et al. 2008)

HSV induced keratitis is the most common cause of corneal blindness in the United States and becomes manifest in acute onset of pain, blurred vision, chemosis, conjunctivitis and characteristic dendritic lesions of the cornea. The uncommon but severe and widely feared chorioretinitis usually occurs within disseminated infections in neonates.

HSV, mostly type HSV-1, accounts for 10-20% of all cases of sporadic viral encephalitis in the United States (Fauci et al. 2008). There is no consistent pathogenesis of HSV encephalitis. While the virus enters CNS by neurotropic spread from the periphery via the olfactory bulb in primary infection of children, most adults have clinical evidence of mucocutaneous HSV-1 infection before the onset of CNS symptoms. Thus reactivation of latent HSV infection in trigeminal or autonomic ganglia and following extension into the CNS via nerves may be one option in developing HSV encephalitis. The reactivation of a long existing latent CNS infection may be another explanation (Fauci et al. 2008). Acute onset of fever and focal neurologic failure especially concerning the temporal lobe are symptoms and signs of HSV encephalitis. Recent studies indicate genetic polymorphisms in two separate genes among children with HSV encephalitis where mononuclear cells secrete reduced levels of IFN in response to HSV. This suggests that sporadic HSV encephalitis may be related to a variety of host genetic determinants (Fauci et al. 2008). Antiviral therapy started on suspicion reduces death rate but neurologic sequelae are common nevertheless.

HSV induced encephalitis has to be distinguished from HSV meningitis which is an acute but self-limiting illness that implicates headache, fever and mild

photophobia for 2-7 days but no neurologic abnormities occur and no sequelae result.
Visceral infection with HSV resulting from viremia rarely leads to multiple-organ involvement in immunocompetent patients but may occur in one organ only, like the esophagus, the lung, the liver and leads to monarticular arthritis, adrenal necrosis, idiopathic thrombocytopenia or glomerulonephritis. The disseminated infection is best detected by the presence of HSV DNA in plasma or blood.
(Fauci et al. 2008)
HSV infection in the newborn is rare, the incidence in the US is only 20-50/100 000 live births, it is not known as being teratogenic and the rate of HSV-2 infection is 85%. Presentation of infection in the newborn varies greatly from localized lesions of the skin and eyes until severe encephalitis or even disseminated infection involving organ failure and death. (Lissauer et al. 2006)
Vertical transmission generally occurs from contact with infected genital secretions at vaginal delivery particularly if vesicles are present. The risk of transmission is high if the mother recently (in the third trimester) acquired infection because the fetus will not have passive immunity from maternal antibodies yet. Thus, caesarean section is recommended for those delivering within 6 weeks of a primary episode or with genital lesions due to primary infection at the time of delivery. The risk of transmission is surprisingly low in women with recurrent herpes who have vesicles present at the time of delivery. (Impey et al. 2008) Daily acyclovir in late pregnancy may reduce the frequency of recurrences at term. Once exposed, neonates are given acyclovir in a high dose, which reduces mortality rate to 15% (Fauci et al. 2008)
Neonates younger than 6 weeks, present the highest frequency of visceral and CNS HSV infection of all infected populations. Without therapy rate of death is high with 65% and less than 10% of infected babies develop normally if CNS is involved. (Fauci et al. 2008)

2.2.5 HSV Infection in the Immunocompromised Host

Extend into mucosal and deep cutaneous layers may occur and persistent ulcerative HSV infections accompanied by candida are common in patients with AIDS. Systemic antiviral therapy speeds rate of healing and relieves pain in immunosuppressed individuals. The rate of HSV reactivation during early phases

of transplantation or induction chemotherapy is high, with 50-90% (Fauci et al. 2008). In a weakened immune system HSV can cause unusual skin lesions like clean linear erosions looking like knife cuts (Fleming 2008).
Atopic eczema “eczema herpeticum” involve extensive areas of skin and may disseminate to visceral organs (James et al. 2006).

2.2.6 Diagnosis

The diagnosis of HSV is established from examination and with viral swabs (Impey et al. 2008).
Viral culture had once been the gold standard in diagnosis, but PCR for HSV DNA is 3 to 4 times more sensitive than viral culture and thus can increase viral detection from lesions by 11% to 70% compared with cell culture. Cultures are most sensitive while lesions are in vesicular-pustular stage and sensitivity declines rapidly as lesions ulcerate and crust (Fauci et al. 2008).
IgM antibody is often present with recurrent HSV outbreaks and does not indicate recent infection, thus is only indicated for the diagnosis of neonatal herpes infection (Runge et al. 2009).
In some cases herpes is effortlessly indentified by clinical examination. The appearance of multiple round and superficial vesicular lesions on an erythematous base is rather typical for oral herpes. Patients with non-typical presentations are much more difficult to diagnose and anyway, the final diagnosis is best confirmed in laboratory by detection of virus, viral antigen or viral DNA in scrapings from lesions. PCR is most sensitive, achieves higher sensitivity in vesicular than in ulcerative lesions and also higher sensitivity in first than in recurrent episodes. Finally, more antigen and DNA can be detected in immunosuppressed patients (Fauci et al. 2008).

2.2.7 Therapeutical Management

Oral or iv Acyclovir, Valacyclovir and Famcyclovir are being used.
Antiviral therapy for initial herpes genitalis prevents new lesions formation and rapidly reduces viral shedding, infectivity and the risk for autoinoculation. If antiviral therapy is taken continuously this may suppress recurrences and reduce subclinical shedding and thus risk for transmission between sexual partners by about 50 % (Fauci et al. 2008). In HSV infected newborns high dose acyclovir

therapy and continued suppressive oral treatment for the first year of live is recommended to prevent relapse (Lissauer et al. 2006).

2.2.8 Specimen Types for the Detection of HSV DNA

For CNS disease, the detection of HSV DNA in cerebrospinal fluid specimens has been recognized as the gold standard. HSV DNA is detectable in cerebrospinal fluid as early as one day after onset of clinical symptoms (Tang et al. 1999). For suspected dermal or genital HSV infection, detection of HSV DNA in swabs is the standard method. Further specimen types for detection of HSV DNA include aqueous humor (in case of retinitis and/or conjunctivitis) and EDTA whole blood (in case of generalized HSV infection).

2.2.9 Laboratory-developed Assays for the Qualitative Detection / Quantitation of HSV DNA

Assays for the qualitative detection/quantitation of HSV DNA based on real-time PCR have been established recently. Several gene targets have been selected including genes coding for glycoproteins B, C, D, and G, thymidine kinase, DNA polymerase, and DNA binding protein (Espy et al. 2006). Molecular assays based on real-time PCR have shown increased detection rates when compared to cell culture methods for diagnosis of HSV infections.
While quantitation of HSV DNA is usually unnecessary, the recognition of HSV-1 and/or HSV-2 in a specimen must be warranted in the laboratory-developed molecular assay (Kessler et al. 2000, Peter et al. 2001, Schalasta et al. 2000). However, with real-time PCR assays utilizing the melting curve analysis, amplified DNA of HSV may exhibit atypical melting temperatures that occur between the predicted melting temperatures obtained with HSV-1 and HSV-2 (Anderson et al. 2003, Issa et al. 2005). According to different polymorphism patterns, these so-called intermediate strains may be designated type A and type B (Issa et al. 2005).

2.2.10 Currently Frequently Used Commercially Available Assays for the Qualitative Detection/Quantitation of HSV DNA

Several commercial assays have been developed for the qualitative detection/quantitation of HSV DNA. A summary of currently frequently used commercial assays in Europe is provided in **Table 2**. The majority of those assays

employ the real-time PCR method while the NucliSens EasyQ HSV-1/2 test is based on the nucleic acid sequence-based amplification (NASBA) technology. Originally, the NASBA was designed for the amplification of RNA targets. For this qualitative DNA NASBA assay, restriction enzyme digestion has been incorporated into the NASBA reaction allowing efficient amplification of DNA targets (Deiman et al. 2008). Recently, evaluation studies on commercially available molecular assays for the detection of HSV DNA have been published. Investigations include comparisons with alternative molecular assays and the newly introduced assays were found to be reliable for the detection and quantitation of HSV DNA in the routine diagnostic laboratory (Podzorski et al. 2006, Reil et al. 2008, Rose et al. 2008).

Table 2: Comparison of currently frequently used commercially available assays for the quantitation of cytomegalovirus (CMV) DNA.

Characteristics	Manufacturer and details				
	Argene	Nanogen Advanced Diagnostics	Qiagen GmbH	Roche Molecular Diagnostics	Roche Molecular Diagnostics
Kit name	CMV R-gene	CMV Real Time Complete Kit	artus CMV PCR Kit	COBAS Amplicor CMV Monitor Test	LightCycler CMV Quant Kit
Target sequence	UL83 (lower matrix Phosphoprotein 65)	UL123 (major immediate early protein)	UL122 (immediate early protein)	UL54 (DNA polymerase)	UL54 (DNA polymerase)
Amplification method	Real-time PCR	Real-time PCR	Real-time PCR	Conventional PCR	Real-time PCR
Detection method	Fluorescence	Fluorescence	Fluorescence	EIA	Fluorescence
Internal control	Heterologous	Heterologous	Heterologous	Homologous	Heterologous
Standards	Four EQS	Four EQS	Four EQS	One IQS	Four EQS
Range of linearity	$5.0 \times 10^2 – 2.5 \times 10^6$ copies/mL	$3.2 \times 10^2 – 1.3 \times 10^7$ genome equivalents/mL	$3.2 \times 10^2 – 1.0 \times 10^7$ copies/mL	$6.0 \times 10^2 – 1.0 \times 10^5$ copies/mL	$1.0 \times 10^3 – 2.0 \times 10^7$ copies/mL

EQS: external quantitation standards; IQS: internal quantitation standard.

2.3 Epstein-Barr Virus (EBV)

In the 1950s Denis Burkitt identified a previously unrecognized form of cancer (B-cell lymphomas), which mainly occurred in the jaws of young African children from malaria endemic areas, as being influenced by climatic factors as temperature and elevation and assumed an infectious agent to be associated etiologically (Biomed 2009).

This infectious agent was named after Anthony Epstein and Yvonne Barr, who identified the virus in cell (B-lymphocyte cells) cultures by electron microscopy from tumor sent from Uganda by Denis Burkitt in 1964 (Epstein 1964).

This novel herpesvirus named EBV had the ability to confer unlimited growth on peripheral blood B lymphocytes in tissue culture, the so called immortalization (Biomed 2009).

Gertrud and Werner Henle then demonstrated that EBV is ubiquitous in the human population and usually the cause of non apparent infections but that the more severe result is malignant transformation and cancer development (Robert Koch Institut 2009).

The EBV is a gamma-herpesvirus which has the ability to remain latent in B-cell lymphocytes following primary exposure and thus has major importance in immunocompromised patients, especially in transplant recipients and AIDS patients. Quantitation of EBV DNA in these patients may provide information for initiating treatment and monitoring response to therapy. Information on quantitative viral load may guide a preemptive strategy to reduce the incidence and level of EBV reactivation in transplant recipients. However, EBV is also associated with development of certain malignancies, including posttransplant lymphoproliferative disease (PTLD) and lymphomas. Quantitation of EBV DNA may thus not only reduce the incidence of EBV reactivation but also the subsequent development of PTLD and lymphomas. Recently, a correlation between the EBV DNA level and the likelihood of development of PTLD was demonstrated (Niesters et al. 2002). In transplant recipients, development of PTLD was found to be associated with a rapid increase of EBV genomes per peripheral blood mononuclear cell (Orentas et al. 2003).

The introduction of quantitative real-time PCR for detection of EBV DNA in plasma may reduce serious problems through. Furthermore, it has improved the management of PTLD. High EBV DNA levels obtained from consecutive samples are considered strong predictors for the development of PTLD and require immediate treatment. In contrast, a low EBV DNA load is frequently detected after transplantation but may vanish without treatment. Because viral loads are detected on average not earlier than two months after transplantation EBV DNA monitoring at monthly intervals allows the identification of patients at high risk of developing PTLD (Table 9.7).

The lack of universal standards for quantitative real-time PCR makes it difficult to correlate viral load and disease, and to establish common criteria for diagnosis and treatment of PTLD. A standardization of the methods is then necessary.

2.3.1 Morphology

The Morphology of EBV is mainly identical to other herpes viruses.

EBV is a complex virus with a linear double stranded DNA genome molecule of more than 172 kb in length that encodes some 85 genes (Skern 2006). It is surrounded by an icosahedral capsid and envelope that contains glycoproteins. Concerning viral attachment and entry, virus receptors have been identified for B lymphocytes to facilitate membrane fusion. The complement receptor 2 (CR2/CD21) binds EBV, co-receptor is the HLA class II molecule. (Biomed 2009)

The linear DNA is the infectious form of the virus, whereas upon entry into cells the genome circularizes within the cell nucleus to form extrachromosomal episomes via homologous recombination of terminal repeat sequences.

EBV multiplies in the nucleus of the host cell. The virus is restricted to infecting epithelial cells of the pharynx and B lymphocytes. Primary infection first occurs at the mucosal layer, subsequently the virus infects the locally circulating B cells, followed by lytic replication producing many lytic viral proteins that are highly immunogenic. The alternative viral life cycle is called latent replication, where the expression of latent (less immunogenic) viral genes occurs and hence gives the virus the position to escape from the host´s immune surveillance.

EBV is a member of lymphocryptovirus genus, that all share a tropism for B lymphocytes and the propensity to oncogenicity. It infects the lymphocytes latently

with very high efficiency in vitro and in vivo. The infected cells start to proliferate and don’t stop proliferating due to expression of specific growth-promoting genes.
There are two types of EBV that are largely spread but are not distinguishable by conventional serologic tests (Fauci et al. 2008, Biomed 2009).

2.3.2 Viral Replication and Pathogenesis

EBV infects epithelial cells of the throat productively and salivary glands of the oropharynx where the virus is also shed from. After having contact with epithelial cells circulating B lymphocytes are infected, whereas lymphocytes in the tonsillar crypts might be infected directly. Then the virus spreads through the bloodstream and viremia occurs.
Replication in B lymphocytes causes them to proliferate and during active infection this, together with reactive T cells, results in enlargement of the lymphoid tissue.
Most infected cells are cleared by immune system, but some of them escape immune surveillance, because they are not recognized by cytotoxic T cell response. This allows latent infection of memory B cells, where only some few EBV nuclear antigens and membrane proteins are expressed, some of them for immortalization. The memory cells present the reservoir for EBV in the body. Reservoir is never eradicated and periodically asymptomatic reactivation and viral shedding of new virions that may infect a new host occur; the signals responsible for reactivation and mechanisms of reactivation are still unclear though.
Subsequently Infected individuals may produce virions, produce EBV-specific antibody and harbor latently infected memory B-cells. The predominant role of latent EBV infection is in the development of a variety of malignancies.

Many of the clinical manifestations of EBV infection occur as a result of immune mechanisms, targeting infected circulating B cells. Large numbers of activated CD8+ cells which eradicate lytically infected cells are produced and lead to an inverted CD4+/CD8+ T cell ratio. Actually, the atypical cells seen on peripheral blood smears are these cytotoxic T cells. Symptoms of acute disease arise during the T cell response and resolve as the infection is controlled. (Fauci et al. 2008, Skern 2006)

2.3.3 Transmission and Epidemiology

EBV infections occur worldwide. By young adulthood, more than 95% of the world´s population is infected and thus has antibodies to the virus (Biomed 2009).

Based on serology, in the US and the UK 50% of children are infected before age of 5 (Biomed 2009) whereas in areas with lower standards of hygiene like in developing countries, the rate of infected children is assumed to be higher. In western countries stricter hygiene prevents exposure to the virus until adolescence.

Viral excretion from oropharynx occurs in over 90% of asymptomatic seropositive individuals for months after primary infection and intermittently throughout life (Biomed 2009) The viral shedding is increased in patients with active EBV infection and in immunocompromised individuals (Fauci et al. 2008). Transmission frequently occurs by transfer of saliva during kissing and from an asymptomatic adult to an infant, it has also happened by blood transfusion and tissue transplantation (Fauci et al. 2009, Biomed 2009).

In African countries such as Uganda, in the so-called lymphoma belt, the association of Burkitt Lymphoma with EBV is very strong (97%) whereas it is just 85% in Algeria and only 10-15% in France and the USA (WHO 2009).

2.3.4 Clinic Manifestations

Infectious Mononucleosis (IM) is the typical clinical presentation of acute primary Epstein-Barr virus infection, defined by the clinical triad of fever, pharyngitis and cervical lymphadenopathy.

But most EBV infections take place in infants and young children and are asymptomatic or present as mild and unspecific pharyngitis. If the infection is delayed until adolescence, up to 75% of these infections present as Infectious Mononucleosis (Fauci et al. 2008).

The incubation period is about 4-6 weeks, a prodrome of fatigue and malaise may last for 1 to 2 weeks, the duration of disease is variable but may last for weeks or even more than a month, whereas the disease itself is self limiting.

Transmission normally occurs through saliva during kissing where the viral inoculum through exchange of saliva is quite large and leaded to designations like “Kissing disease” or “Glandular fever” , but probably can also occur sexually.

Sore throat, malaise, fever, generalized lymphadenopathy, hepatosplenomegaly, intense asthenia, hyper-lymphocytosis with atypical lymphocytes and elevated transaminase levels are the most common symptoms and signs of disease. A prominent pharyngitis accompanied by enlarged tonsils and an impressive exudate resembling that of streptococcal angina may be misleading. Subsequently treatment with antibiotics during acute infection often results in a diffuse maculopapular rash, which is strongly associated with beta-lactam antibiotics like Ampicillin and Amoxicillin, but may occur in association with other antibiotics as well. It ought to be kept in mind that this kind of rash is not predictive of future unfavorable reactions to Penicillins.

Complications of IM might be splenic rupture, thrombocytopenia, agranulocytosis, hemolytic anemia, encephalitis, meningitis, bacterial superinfection, upper airway obstruction, orchitis or myocarditis.

The occurring inflammation of vascular reticuloendothelial organs produces mild hepatitis and splenomegaly. This is the reason why it is advised to avoid contact sports or similar activities with risk of trauma for some time after suffering from IM. Splenic rupture due to minimal trauma has been frequently reported and is one of the few causes of mortality associated with EBV. (Biomed 2009, Fauci et al. 2008, Skern 2006, WHO 2009)

Some EBV associated human tumors and tumor like diseases are the Posttransplant lymphoproliferative disease (PTLD), the Primary central nervous system lymphoma of AIDS (PCNSL), the X-linked lymphoproliferative disease (Duncan´s syndrome), the Hodgkin´s disease, Nasopharyngeal carcinoma, the Oral Hair Leukoplakia of AIDS and the Burkitt Lymphoma.

The Burkitt Lymphoma (BL) is a malignant tumor of B lymphocytes that is associated with EBV and some kinds of immunosuppression like the HIV infection.

It is sporadic in the US and endemic to New Guinea and central parts of Africa where it accounts for 80% of all childhood cancers there (Biomed 2009). Also, there is an annual incidence of 6-7 cases per 100 000 and a peak incidence at 6 to 7 years of age (WHO 2009). The anatomic distribution is characteristic and mainly

affects the jaw with 72% of cases and the abdomen with 56% of cases (Biomed 2009).

The Risk factors for developing BL are EBV infection in early life, repeated infection with plasmodium falciparum (causative agent of malaria, cause immune suppression and incorrect T cell response) life below 1000metres in high rainfall areas and an elevated antibody titer against EBV viral capsid antigen (VCA) (WHO 2009, Skern 2006).

The area of the so called “lymphoma belt” is characterized by high temperature and humidity, which may lead to the assumption that there is an association of malaria with Burkitt Lymphoma (Mutalima et al. 2008, Carpenter et al. 2007).

It is assumed that continuous reinfection with malaria causes polyclonal B lymphocyte stimulation which together with malnutrition suppresses T cell responses with a subsequently higher probability of developing cytogenetic abnormalities (Biomed 2009, WHO 2009) .

2.3.5 Therapeutical Management

Treatment with beta-lactam antibiotics should be avoided in IM.

Acyclovir is capable of suppressing oropharyngeal shedding, but not indicated for treatment of acute disease as there is no proofed evidence. For uncomplicated IM no specific intervention is recommended as the disease is self limiting. As many of the EBV associated symptoms are due to the exuberant T cell response, treatment with steroids might help but has not been proved useful (Runge et al. 2009).

2.3.6 Diagnosis

EBV frequently is diagnosed simply from clinical presentation and serologic test results that include a reactive lymphocytosis, greater than 10% of atypical lymphocytes and a positive reaction to a “mono spot” test. If clinic presentation seems to be very clear, a positive Paul-Bunnel heterophile antibody test result is diagnostic. Serologic antibody tests for EBV can measure the presence and concentration of several different specific EBV antibodies. By interpretation of the results, which is highly complex, the stage of EBV infection can be determined.

(CDC 2009) For differential diagnosis a streptococcal test is indicated in some cases because treatment of streptococcal pharyngitis, which is a potential

differential diagnosis of IM, would be important but avoidance of an amoxicillin with a negative test may spare the patient an EBV associated related beta-lactam rash.

2.3.7 Specimen Types for the Detection of EBV DNA

There is currently no consensus on the optimal blood compartment for routine molecular EBV DNA testing. The most meaningful results may be obtained by quantitation of EBV DNA in EDTA whole blood including both the cellular and the cell-free compartments from serial or sequential specimens obtained from the same patient (Stevens et al. 2001, Fafi-Kremer et al. 2004). For CNS disease, detection of EBV DNA in cerebrospinal fluid specimens of patients with meningitis and encephalitis is the standard method. EBV DNA was successfully detected in cerebrospinal fluid specimens of patients with AIDS-related lymphomas (Bossolasco et al. 2002).

2.3.8 Laboratory-developed Assays for the Quantitation of EBV DNA

Assays for the quantitation of EBV DNA based on real-time PCR have been established recently. Several gene targets have been selected including genes coding for the DNA polymerase, the thymidin kinase, the Epstein-Barr nucleic antigen type 1, the glycoprotein B, the ZEBRA protein, and a nonglycosylated membrane protein (Espy et al. 2006). Molecular assays based on real-time PCR have shown superior performance when compared to assays based on conventional PCR (Brengel-Pesce et al. 2002, Stevens et al. 2002).
Similar to the detection of CMV, the choice of the target region requires attention as EBV sequence variation is a recognized phenomenon. Especially, if the latent membrane protein genes are used as the target region, sequence variation may lead to an underestimation of the EBV viral load or even to a false-negative result (Walling et al. 2004, Mainou et al. 2006, Shibata et al. 2006).

2.3.9 Currently Frequently Used Commercially Available Assays for the Quantitation of EBV DNA

Several commercial assays have been developed for the quantitation of EBV DNA. A summary of currently frequently used commercial assays in Europe is provided in **Table 3**. All of those assays are based on real-time PCR. Recently, evaluation studies on commercially available molecular assays for the quantitation

of EBV DNA have been published. Assays investigated were found to be reliable and suitable for routine EBV-associated disease monitoring (Ruiz et al. 2005, Fafi-Kremer et al. 2008, Mengelle et al, 2008). However, it must be mentioned that one of those assays (the LightCycler EBV Quant Kit) amplifies part of the EBV latent membrane protein 2 gene. In presence of the recognized EBV sequence variation (see above) a shifted melting curve may result because of an altered binding of one of the fluorescent hybridization probes. Therefore, careful analysis of the melting curve is of paramount importance to avoid false-negatives when using this assay.

Table 3: Comparison of currently frequently used commercially available assays for the quantitation of Epstein-Barr virus (EBV) DNA.

Characteristics	Manufacturer and details				
	Argene	Cepheid AB	Nanogen Advanced Diagnostics	Qiagen GmbH	Roche Molecular Diagnostics
Kit name	EBV R-gene	affigene EBV trender	EBV Q-PCR Alert Kit	artus EBV PCR Kit	LightCycler EBV Quant Kit
Target sequence	BXLF1 (thymidine kinase)	BKRF1 (Epstein-Barr nucleic antigen 1)	BKRF1 (Epstein-Barr nucleic antigen 1)	BKRF1 (Epstein-Barr nucleic antigen 1)	LMP2 (latent membrane protein 2)
Amplification method	Real-time PCR	Real-time PCR	Real-time PCR	Real-time PCR	Real-time PCR
Detection method	Fluorescence	Fluorescence	Fluorescence	Fluorescence	Fluorescence
Internal control	Heterologous	Heterologous	Heterologous	Heterologous	Heterologous
Standards	Four EQS	Two EQS	Four EQS	Four EQS	Four EQS
Range of linearity	5.0×10^2 – 5.0×10^5 copies/mL	1.0×10^3 – 2.0×10^7 copies/mL (EDTA plasma); 3.5×10^3 – 2×10^7 copies/mL (EDTA whole blood)	4.0×10^1 – 4.0×10^6 genome equivalents/10^5 cells	1.0×10^3 – 1.0×10^6 copies/mL	1.0×10^3 – 2.0×10^7 copies/mL (EDTA plasma); 2×10^3 – 2×10^7 copies/mL (EDTA whole blood)

NA: data not available; EQS: external quantitation standards.

2.4 Varicella Zoster Virus (VZV)

The VZV is an alpha-herpesvirus. After primary infection, the VZV persists in latent form and may reactivate in the advanced age producing vesicles that are typically confined to a single dermatome of the skin. However, VZV infections can also

produce systemic infections of the central nervous and respiratory systems, mainly in immunocompromised patients, especially in transplant recipients and AIDS patients (Gilden et al. 2000). Because the clinical presentation of VZV dermal disease can be confused with that produced by HSV, laboratory diagnosis is of major importance for distinguishing HSV from VZV infections (Rubben et al. 1997). In immunocompromised patients, quantitation of VZV DNA may provide information for initiating treatment and monitoring response to therapy. Information on quantitative viral load may guide a preemptive strategy to reduce the incidence and level of VZV reactivation in transplant recipients.

Quantitation of VZV-DNA levels has been a valuable diagnostic approach in immunosuppressed patients. Although no consensus has been reached on VZV-DNA load cut-off values, several studies suggested the viral load a marker for the severity and a predictor for the outcome of the disease. High viral loads were associated with clinical symptoms and poor clinical outcome. Indeed, it was reported that immunosuppressed patients having >10^5 VZV DNA copies/ml in the peripheral blood showed organ involvement such as pneumonia, hepatitis, and generalized infection and some of those patients died. In addition, detection of VZV DNA in CSF correlates strongly with meningitis and encephalitis. Frequent monitoring of VZV DNA by quantitative real-time PCR may be used to diagnose primary infections and reactivations and to monitor patients undergoing antiviral therapy.

VZV has a long recorded history. In the late 18th century Heberden clinically differentiated between small pox and chickenpox, which were often mixed up until then because of their similar clinical appearance. 1888 von Bokay indicated that chickenpox and herpes zoster were due to the same causal agent and 1943 Garland suggested that Herpes zoster was due to the reactivation of latent VZV. 1958 Weller and Stoddard confirmed this connection by isolating virus from chickenpox and zoster and comparing them: It turned out that they were indeed due to the same virus and neither biologic nor immunologic differences between the viral agents isolated could have been found. Examination of viral DNA by restriction endonuclease analysis verified molecular identity of these two viruses.
(Mandell et al. 2005)

2.4.1 Morphology

The Varicella Zoster Virus, or Human Herpes Virus Type 3 (HHV-3), as a member of the herpes virus family shares structural characteristics with the other members of the family. The VZV is a linear, double stranded DNA alpha-herpesvirus which is about 125 000 bp in length encoding for about 75 proteins. There is a lipid envelope surrounding a nucleocapsid of 162 capsomeres with icosahedral symmetry and a virus total size of about 150-200 nm. (Fauci et al. 2008)
The nucleocapsid is coated with a layer of protein named tegument, which play a major role in initiating the process of virus reproduction. The tegumented nucleocapsid is then surrounded by an envelope consisting of a lipid bilayer and with glycoprotein spikes (Mandell et a. 2005).
VZV is closely related to HSV, sharing much genome homology and the envelope glycoproteins, representing the primary markers for both humoral and cell-mediated immune response, mainly corresponding with those in HSV. VZV is susceptible to disinfectants as the envelope is sensitive to detergent and air drying and only enveloped virions are infectious. (Mandell et al. 2005)

2.4.2 Viral Replication and Pathology

Local of infection of the oral- and nasopharyngeal area occurs through airborne transmission of infectious droplets of the respiratory tract or of vesicular skin lesions. Subsequent localized replication in regional lymph nodes occurs, leading to seeding of the reticuloendothelial system and ultimately viremia. Finally infection of endothelial cells of the skin and the epidermis leads to a disseminated rash which is reflected in the diffuse and scattered nature of the lesions and may be verified in selected cases by recovery of VZV from blood or detection of viral DNA in blood or lesions by PCR. Vesicles that involve corium and dermis are clear at the beginning and later become pustular. They either rupture and release infectious fluid or are gradually reabsorbed. (Mandell et al. 2005)
Mechanisms of reactivation and subsequent development of clinical herpes zoster are unknown, presumably the virus infects dorsal root ganglia during chickenpox, becomes latent and may occur at any time. Active replication in other organs like lung and brain might occur but is very uncommon in immunocompetent patients. (Fauci et al. 2008, Mandell et al. 2005, Runge et al. 2009)

2.4.3 Epidemiology and Transmission

Chickenpox, which represents the primary form of infection, is highly contagious. The attack rate among seronegative individuals is at least 90% (Fauci et al. 2008) and both sexes and all races are affected equally often. It is a common infection of childhood and most commonly children between 5 and 9 years old acquire primary infection. However, approximately 10% of the US population over the age of 15 is seronegative. The incubation period of chickenpox is assumed to be 10 to 20 days, and infectiousness occurs about 48 hours before appearance of the rash and may last until the last vesicle is crusted. (Fauci et al. 2008, Mandell et al 2005) Reported sporadic cases of second attack rates might be because of imprecise diagnosis where actually a different etiologic cause has to be assumed.

A live attenuated VZV vaccine is available and recommended for all children and adolescents, has approximately 99% efficacy (Runge et al. 2009). Due to the implementation of vaccination the annually incidence of chickenpox is decreasing drastically. However, the medical importance of chickenpox should be stressed, as in the US there have been approximately 250 deaths per year from this infection, even in recent years when a vaccine has been available (Mandell et al. 2005). The chickenpox associated mortality rate in normal children is 2 per 100.000 and the risk increases enormously for adults (Mandell et al. 2005).

Reactivation of VZV after primary infection and subsequent appearance of chickenpox, leads to herpes zoster, a sporadic disease, that occurs at all ages, but mainly among the elderly persons aged 60 years and older, who are seropositive for VZV and have had chickenpox. The reactivation of VZV presumably depends on the balance between viral factors and factors of the host´s immune system. Herpes zoster has a lifetime incidence of approximately 20% (RKI 2009).

However, second periods of herpes zoster are very rare, in fact recurrent dermatomal lesions are usually caused by HSV (Mandell et al. 2005).

There is also a vaccine for prevention of VZV among people, which decreased the incidence of both herpes zoster and postherpetic neuralgia in a large clinical trial (Runge et al. 2009). The virus can be transmitted to seronegative individuals with consequent chickenpox but rate of infectivity is low because the only infectious agent is the vesical fluid (Fauci et al. 2008).

2.4.4 Clinical Manifestations

Primary VZV infection results in chickenpox, which generally is a rather mild disease in children but might be rather severe among adults and immunocompromised patients. The main symptoms are low-grade fever, malaise and a rash. The hallmark of disease is the dermal rash that occurs over several days and spreads from the head to the trunk and finally to the extremities. Most lesions are small, have an erythematous base and might be located on the mucosa of the pharynx and genital area. Incubation period of chickenpox usually is about 14-21 days; however, some develop a prodrome 1-2 days before onset of exanthema.

However, the severity of disease varies from one individual to the other. Generally younger children tend to have milder symptoms whereas immunocompromised are at higher risk for visceral complications, some of which are fatal, especially in patients with leukemia. Very common complications are secondary skin infections, caused by streptococcus pyogenes or staphylococcus aureus through inoculation of infectious particles by scratching lesions.

The most common extracutaneous complication is involvement of CNS (acute cerebellar ataxia, meningeal inflammation) which is a benign complication of VZV infection in children. A serious and potentially life-threatening complication especially in adults and pregnant women is varicella pneumonia, other complications are rather rare but may involve myocarditis, nephritis, bleeding diathesis and hepatitis.

Perinatal varicella in the neonate is associated with high mortality 30% (RKI 2009) because in this case newborns do not receive protective transplacental antibodies and have an immature immune system.

In a previously immunized person a usually mild illness and rash different from tipical varicella might occur, but nevertheless is infectious to others. Congenital varicella, which occurs when a pregnant woman develops chickenpox disease in the first trimester, causes severe fetal malformations (skin scarring, limb hypoplasia, microcephaly, eye abnormalities) but is extreme uncommon. (Fauci et al. 2008, Runge et al. 2009, Mandell et al. 2005)

Herpes zoster is the result of the reactivation of dormant VZV and a sporadic disease where most patients presenting herpes zoster have no history of recent exposure to other individuals with VZV infection.

VZV occurs at all ages, but incidence rises with old age and waning of cell-mediated immunity. Usually pain is the first symptom and within 48 to 72 hours followed by eruption of unilateral maculopapular rash in a dermatomal distribution, mainly thoracic and lumbal dermatomes are affected. The duration of disease is usually 10-15 days. The most burden on patients is associated painful acute neuritis and postherpetic neuralgia, that may be constant pain or intermittent pain with peaks at night or when temperature changes.

In children reactivation of VZV and subsequent herpes zoster is usually benign and not associated with progressive pain. Also, postherpetic neuralgia is uncommon in young people but may occur in up to 50% of patients older than 50 years (Mandell et al. 2005).

Recurrent herpes zoster is rare except in immunocompromised hosts, especially those with AIDS. In these patients cutaneous dissemination is feared especially in patients with lymphoma. Other serious complications include pneumonitis, meningoencephalitis, hepatitis and the Ramsay Hunt syndrome involving the external auditory canal with vesicles and pain, the tongue with loss of taste and an ipsilateral facial palsy. Trigeminal nerve involvement might cause herpes ophthalmicus and is a potentially sight-threatening manifestation. In case that additionally to the dorsal root ganglia, also the anterior horn cell is involved, this may lead to motor paralysis in a manner similar to poliomyelitis. Involvement of CNS might be more common than previously thought and may present simply through occasional headaches (Mandell et al. 2005).

(Fauci et al. 2008, Runge et al. 2009, Mandell et al. 2005)

2.4.5 Diagnosis

History and physical examination are usually sufficient for both chickenpox and shingles. The characteristic skin rash of chickenpox with lesions in multiple stages of development and especially in a person with a history of exposure provides the basis for diagnosis. Thus symptoms like pruritus, pain, mild fever and malaise are helping to establish diagnosis. The dermatomally distributed vesicular rash makes the diagnosis of herpes zoster very likely. Laboratory testing like viral culture of

vesicular skin lesions or antibody testing with seroconversion or diagnostic rises in titer can help to define the diagnosis. PCR assays are a useful diagnostic tool to detect VZV DNA in vesicular fluid or in cerebrospinal fluid when central nervous system is involved. (Fauci et al. 2008, Mandell et al. 2005, Runge et al. 2009)

2.4.6 Therapeutical Management

The medical therapy in the immunically healthy patient is directed toward reduction of complications of VZV infections. Good hygiene including bathing and cutting fingernails closely is important to decrease the risk for complications like secondary infections in patients with varicella. Children at high risk of complications, infected adults and immunocompromised patients should be treated with antiviral drugs like acyclovir for both chickenpox and herpes zoster within 24 hours of onset of disease, as treatment efficacy is dependent of the early initiation of antiviral therapy.

Varicella-zoster immune globulin can be given within 96 hours after exposure to individuals at high risk for complications, like seronegative pregnant women or newborns whose mother had onset of chickenpox less than five days before delivery or up to 48 hours postpartal.

Antidepressants and neuroleptics may be useful in decreasing postherpetic neuralgia. (Fauci et al. 2008, Mandell et al. 2005, Runge et al. 2009)

2.4.7 Specimen Types for the Detection of VZV DNA

For suspected dermal VZV infection, detection of VZV DNA in swabs is the standard method. In immunocompromised patients, the most meaningful results may be obtained by quantitation of VZV DNA in EDTA whole blood including both the cellular and the cell-free compartments from serial or sequential specimens obtained from the same patient. For CNS disease, detection of VZV DNA in cerebrospinal fluid specimens is the standard method. Recently, VZV DNA was the most prevalent herpesvirus DNA detected in cerebrospinal fluid specimens (Smith et al. 2004). For respiratory VZV infection, detection of VZV DNA in bronchoalveolar lavage is meaningful. Recently, detection and quantitation of VZV DNA in oral fluid proved to be useful in patients with Ramsey Hunt Syndrome (Raggam et al. 2008).

2.4.8 Laboratory-developed Assays for the Qualitative Detection/Quantitation of VZV DNA

Several laboratory-developed assays for the qualitative detection/quantitation of VZV DNA based on real-time PCR have been established. Gene targets include genes coding for the DNA polymerase, the glycoprotein B, the DNA binding protein, and the immediate-early transactivator (Espy et al. 2006). Molecular assays based on real-time PCR have shown superior performance when compared to conventional shell vial cell culture for detection of VZV (Espy et al. 2000, Schmutzhand et al. 2004, Stranska et al. 2004).

2.4.9 Currently Frequently Used Commercially Available Assays for the Qualitative Detection/Quantitation of VZV DNA

Several commercial assays have been developed for the qualitative detection/quantitation of VZV DNA. A summary of currently frequently used commercial assays in Europe is provided in **Table 4**. All of those assays are based on real-time PCR. Currently, no evaluation studies have been published so far. One of the assays, the RealArt VZV LC PCR test, was employed recently for the quantitation of VZV DNA in patients with Ramsey Hunt Syndrome and detection rates were found to be higher compared to those reported recently (Raggam et al. 2008).

Table 4: Comparison of currently frequently used commercially available assays for the qualitative detection/quantitation of varicella zoster virus (VZV) DNA

Characteristics	Manufacturer and details				
	Argene	Cepheid AB	Nanogen Advanced Diagnostics	Qiagen GmbH	Roche Molecular Diagnostics
Kit name	HSV1 HSV2 VZV R-gene	affigene VZV tracer	VZV Q-PCR Alert Kit	artus VZV PCR Kit	LightCycler VZV Qual Kit
Target sequence	ORF17 (host shutoff virion protein)	ORF62 (immediate early protein)	ORF29 (major DNA binding protein)	ORF38 (virion protein)	ORF29 (major DNA binding protein)
Amplification method	Real-time PCR	Real-time PCR	Real-time PCR	Real-time PCR	Real-time PCR
Detection method	Fluorescence	Fluorescence	Fluorescence	Fluorescence	Fluorescence
Internal control	Heterologous	Heterologous	Heterologous	Heterologous	Heterologous
Standards	Four EQS	---[a]	Four EQS	Four EQS	---[a]
Range of linearity	$1.0 \times 10^4 - 1.0 \times 10^7$ copies/mL	---[b]	$1.3 \times 10^2 - 1.3 \times 10^7$ genome equivalents/mL	NA	---[c]

[a]Qualitative test; [b]Lower limit of detection 7.2×10^1 to 3.3×10^2 copies/mL (depending on the sample preparation protocol employed); [c]Lower limit of detection 3.5×10^2. EQS: external quantitation standards; NA: data not available.

2.5 Human Herpesvirus 6 (HHV-6)

HHV-6 was first isolated in 1986 from peripheral-blood leukocytes (Fauci et al. 2008) and thus identified as etiologic cause for exanthema subitum, a near universal childhood disease. (Gerok et al. 2007)

The HHV-6 is a beta-herpesvirus and has the ability to establish lifelong latent infection following primary exposure which usually occurs in the childhood presenting as unremarkable febrile illness or resulting in roseola infantum also called exanthema subitum (Asano et al. 1994). The HHV-6 includes two the molecularly and biologically distinct variants, A and B. While variant A is not associated with any disease, variant B is associated with the symptoms mentioned above. In contrast, primary infection in an adult seems to be a rare event. However, HHV-6 may reactivate and produce systemic infections of the central nervous system including meningitis and encephalitis, mainly in

immunocompromised patients, especially in transplant recipients and AIDS patients (Hall et al. 1994). Furthermore, HHV-6 may produce hepatitis and pneumonia in those patients. The reactivation of HHV-6 in organ recipients usually occurs within the first 4 weeks after transplantation. Although there are antiviral drugs available that inhibit the replication of HHV-6 *in vitro*, evaluation of such agents in larger clinical trial has not been done yet.

Detection of HHV-6 DNA in blood correlates well with active replication and quantitation of HHV-6 DNA by real-time PCR may allow discrimination between latent and active infection but a reference value to be used as threshold value has not yet been defined. Nevertheless, reactivation of HHV-6 in the post-transplantation period is characterized by a progressive increase of viral load. A biphasic progress of the viral load may be observed with an increase within 4 weeks after transplantation, followed by a decrease afterwards. Finally, HHV-6 levels should be similar to those observed prior to transplantation. Complications such as thrombocytopenia, graft-versus-host-disease, anemia, and leucopenia may occur in patients with a high HHV-6 load. Therefore, sequential measurement of the HHV-6 load is advisable to prevent viral complications (Table 9.7).

2.5.1 Morphology

Morphology, structure, genome organization, genome expression and replication cycle is mainly identical with the CMV (Gerok et al. 2007).

The virion particle is 160 nm to 200nm and has the morphologic features typical of herpes virion particles (central double stranded viral DNA within an icosahedral capsid and a tegument layer surrounded by a an envelope). The closest homology in genome organization is to the HHV-7 and the CMV (CDC 1999).

HHV-6A and HHV-6B are two variants of the virus that share 90% sequence homology, but do differ in epidemiology, growth, cell tropism, antigenic composition, and clinical manifestations (Mandell et al. 2005).

2.5.2 Epidemiology and Transmission

The HHV-6 has a worldwide distribution and might to be transmitted by genital secretions but appears to be generally transmitted by saliva during the first year of life. The infection occurs during infancy with a peak of transmission rate at the age of 9-21 months as the maternal antibodies wane. By the age of 2, seropositivity is

assumed to be approximately 80%, and probably reaches about 95 % in adults (Mandell et al. 2005). There are no differences in prevalence according to gender, race, socioeconomic status or country (Mandell et al. 2005). HHV-6B is responsible for up to 93% of primary infections in Europe and North America (Hall et al. 1994); it is found to be occurring in children, where in some cases it causes the childhood disease exanthema subitum and in healthy adults, whereas variant A is commonly isolated from spinal fluid and from immunocompromised patients (Mandell et al. 2005). There is no specific disease manifestation as in Variant B.
Both variants are particularly pathogenic in immunocompromised individuals.
Studies suggest that congenital and perinatal transmission of HHV-6 may occur. The virus can be isolated from genital secretions, breast milk and urine (Mandell et al. 2005) and the virus DNA recovered from the blood of acutely infected children were shown to be identical to that in their mothers´ saliva. (Fauci et al. 2008, Mandell et al. 2005)
The clinical importance of HHV-6 should be stressed as approximately 10-20% of febrile seizures without rash during infancy are caused by this virus (Fauci et al. 2008); and thus it is responsible for up to 20% of infant emergency room visits in the US (Hall et al. 1994).
In 33-48% of patients who have had hematopoietic stem cell transplantation, HHV-6 reactivation occurs and may lead to CMV reactivation and severe CMV disease, hepatitis, pneumonitis, bone marrow suppression, encephalitis, fever and rash, graft versus host disease and delayed engraftment (Salvaggio 2008).
Pathology and Viral Replication
The HHV-6 has a tropism for various cell types like e.g. salivatory glands and T-cells, most of them CD4+.
Primary infection takes place in the oropharynx, proceeds through the regional lymphoid tissue to the mononuclear cells and is distributed throughout the body.
HHV-6 is a more latent than lytic infection. HHV-6 downregulates expression of CD3 and MHC class I molecules as a strategy to escape immune surveillance of the host.
During active infection replication takes place in lymphocytes, macrophages, histiocytes, endothelial cells and epithelial cells.

The HHV-6 is mainly found in circulating lymphocytes during primary infection, but it persists in the nucleus of monocyte-macrophages, from where it reactivates in immunosuppressed patients.
There is a potentially important synergy between HIV-1 and HHV-6 as both of them infect and replicate in CD4+ lymphocytes. HHV-6 infection induces CD4 expression in CD4- lymphocytes and natural killer cells, making them susceptible to HIV-1 entry. In turn HIV upregulates HHV-6 replication. Subsequently there is a more rapid progression of HIV disease in co-infected individuals. (Mandell et al. 2005)

2.5.3 Clinical Manifestations

HHV-6 generally leads to a unrecognized latent and chronic infection.
Most children are infected subclinically or develop less typical symptoms like otitis, respiratory symptoms, fussiness and mild diarrhea. In recent studies the rate of children who developed exanthema subitum/ roseola infantum was 9% versus 17% (Mandell et al. 2005).
HHV-6 is the major cause for this childhood disease; the other cause is HHV-7. This common illness is characterized by fever and rash, malaise and occasional cervical lymphadenopathy for 3-6 days. Fever is high but otherwise unremarkable and additionally there might be mild upper respiratory symptoms. The classic diffuse maculopapular rash emerges as fever vanishes and is associated with a mild atypical lymphocytosis and a relative neutropenia in the blood count. This disease is generally benign and self-limiting. However, complications might be meningitis, encephalitis and febrile seizures. Seizures not only occur because of the high fever but also because HHV-6 replicates in the central nervous system (Mandell et al. 2005).
HHV-6 is also associated with mononucleosis syndromes in adults as with focal encephalitis, pneumonia, myocarditis, myelosuppression and disseminated disease in immunocompromised hosts. In transplant recipients HHV-6 reactivation may lead to graft dysfunction, end organ disease and death (Fauci et al. 2008).
The quantity of viral load correlates well with disease in transplant recipients (Mandell et al. 2005)

2.5.4 Diagnosis

In serological diagnosis seroconversion or a fourfold or greater rise in titer is recquired, but cross-reactivity with HHV-7 occurs. HHV-6 can be cultured from peripheral blood mononuclear cells, but is generally detected by PCR where HHV-6 DNA can commonly be detected in spinal fluid even years after clinical manifestation of exanthema subitum (Mandell et al. 2005).

2.5.5 Therapeutical Management

Normally there is no need for therapy.
Acyclovir is inactive but ganciclovir and foscarnet appeare to be susceptible to HHV-6 (Fauci et al. 2008).

2.5.6 Specimen Types for the Detection of HHV-6 DNA

There is currently no consensus on the optimal blood compartment for routine molecular HHV-6 DNA testing. The most meaningful results may be obtained by quantitation of HHV-6 DNA in EDTA whole blood including both the cellular and the cell-free compartments. Similar to other herpesviruses, such as CMV and EBV, the HHV-6 can be detected in peripheral blood mononuclear cells during both active disease and latency (Palleau et al. 2006). When employing a quantitative molecular assay, it must be taken into consideration that a cutoff to distinguish active infection from latent infection has not yet been identified. Furthermore, the comparability of results obtained from serial or sequential specimens may be impaired through fluctuating leukocyte counts. In serum or plasma, HHV-6 may be found only during active disease. However, HHV-6 DNA may be detectable in serum or plasma obtained from individuals with HHV-6 sequences integrated in their cellular chromosomes, even when they are healthy (Clark et al. 2006). For CNS disease, detection of HHV-6 DNA in cerebrospinal fluid specimens is the standard method. However, false-positive results may be obtained if mononuclear cells are present in the CSF. For respiratory HHV-6 infection, detection of HHV-6 DNA in bronchoalveolar lavage is meaningful.

2.5.7 Laboratory-developed Assays for the Quantitation of HHV-6 DNA

Molecular assays based on real-time PCR have been established and used for the detection of HHV-6 DNA in plasma obtained from recipients of allogeneic stem-cell

transplantation (Locatelli et al. 2000, Ogata et al. 2006). For the quantitation of HHV-6 DNA in cerebrospinal fluid, specimens obtained from patients with CNS disease were investigated with conventional PCR; however, this assay has not been evaluated sufficiently (Birnbaum et al. 2005).

2.5.8 Currently Frequently Used Commercially Available Assays for the Quantitation of HHV-6 DNA

Currently, there are two molecular assays based on real-time PCR commercially available (**Table 5**). Recently, one of the assays, the CMV HHV6,7,8 R-gene test, was found useful for the routine diagnostic laboratory when compared to two HHV-6 in-house quantitative real-time PCR methods (Deback et al. 2008).

Table 5: Comparison of currently frequently used commercially available assays for the quantitation of human herpesvirus 6 (HHV-6) DNA.

Characteristics	Manufacturer and details	
	Argene	Nanogen Advanced Diagnostics
Kit name	CMV HHV6,7,8 R-gene	HHV6 Q-PCR Alert Kit
Target sequence	U57 (U42 for HHV7; ORF26 for HHV8)	ORF13R
Amplification method	Real-time PCR	Real-time PCR
Detection method	Fluorescence	Fluorescence
Internal controls	Heterologous	Heterologous
Standards	Four EQS	Four EQS
Range of linearity	$5.0 \times 10^2 – 2.5 \times 10^6$ copies/mL	$1.3 \times 10^2 – 1.3 \times 10^7$ genome equivalents/mL

EQS: external quantitation standards.

2.6 Human Herpesvirus 7 (HHV-7) and Human Herpesvirus 8 (HHV-8)

HHV-7 was isolated as lymphotropic virus in 1990 from mononuclear cells from peripheral blood of a healthy individual, (Fauci et al. 2008)

HHV-8, the 8th and most recently discovered human herpes virus, was detected because of its strong association with the Kaposi Sarcoma.

In 1872, the dermatologist Moritz Kaposi first described skin lesions in five men, which appeared to be part of some very aggressive and incurable disease that rapidly leads to death of the patient. (Mandell et al. 2005)
In the 80´s around 30-40% of homosexual men with AIDS developed KS at some point of their illness. (University of Washington 2009)
Herpesvirus-like DNA sequences were identified in 1994 in tissues derived from Kaposi´s sarcoma (KS) and lymphomas that occur in patients with AIDS (Fauci et al. 2008). Studies indicated that the KS occurred 20 times more often in men who have sex with HIV-infected men compared to those who got infected with HIV due to the blood-borne route (Mandell et al. 2005).

Both, HHV-7 and HHV-8 have the ability to establish lifelong latency. Primary infection may cause exanthema subitum and/or high fever but in general, HHV-7 is believed to be less pathogenic than HHV-6. Reactivation following liver and bone marrow transplantation has been reported and HHV-7 has been associated with the development of encephalitis and hepatitis (Ljungman et al. 2002). However, the role of HHV-7 in human disease is still not defined clearly. In contrast, infections caused by HHV-8 have been described and discussed in detail. It does not seem to be ubiquitous in the general population and the distribution appears related to behavioral and geographic factors. Infection with HHV-8 seems to occur most commonly through sexual transmission including saliva as a vehicle of transmission. Furthermore, HHV-8 may be transmitted through blood transfusion and transplantation. Activation of HHV-8 replication in the latently infected cells is responsible for viral spread and presumed to contribute to the development of HHV-8 associated diseases including Kaposi’s sarcoma, Multicentric Castleman’s Disease, primary effusion lymphoma, and diffuse large B-cell lymphoma (Vega et al. 2005). Kaposi’s sarcoma develops in 0.5–5% of solid organ transplant recipients depending on the patient’s geographic origin and the immunosuppressive regimen used. In the majority of patients, the disease is a result of HHV-8 reactivation. The risk of developing Kaposi’s sarcoma is greatest within the first 2 years following transplantation and decreases afterwards, maybe because of the reduction of immunosuppressive therapy.

2.6.1 Morphology

The HHV-7 has linear double-stranded DNA genome of about 145 kb, which shows homologies to human herpesvirus 6 (HHV-6) and cytomegalovirus (CMV). Mature HHV-7 particles measure about 170 nm in diameter, with a nucleocapsid of 90 to 95 nm and a tegument of approximately 30 nm. Its DNA encodes for 84 different proteins. There is a 21%- 76% nucleic acid homology between HHV-7 and HHV-6 (depending on the area) subsequently this antigenic similarity complicated serologic testing (Mandell et al. 2005).

HHV-8 is a large, double-stranded DNA virus, whose genome contains approximately 140kb of unique sequence that encodes nearly 100 open reading frames (ORF). It lies within a protein cover, a capsid tegument (protein layer surrounding) and a lipid envelope derived from the cell membrane.
The virus is 140nm in diameter and attaches to cells by binding to cell surface heparin sulfate. (Mandell et al. 2005)

2.6.2 Epidemiology and Transmission

Almost everybody is infected with HHV-7 by the age of 5 as the virus is spread very easily through saliva (Mandell et al. 2005) and thus frequently acquired during childhood, but at a later age than HHV-6. In contrast to HHV-6 it is also detectable in breast milk (Fauci et al. 2008).

HHV-8 is causally related to the Kaposi´s sarcoma (KS), as with an AIDS related B cell lymphoma and the Multicentric Castleman´s Disease (a lymphoproliferative disorder of B cells).
HHV-8 does not cause worldwide infection but is much more common in some geographic areas than in others. In Central and South Africa are the highest rates of infection with approximately 50% of population infected. The seroprevalence is 10% in the Mediterranean region, 30% in certain regions of Italy, 5% in the US and northern Europe and only 0.2% in Japanese individuals.
Presumably, in areas of high-prevalence the infection is transmitted in saliva, occurs in childhood and is associated with seropositiv mothers. But in low-prevalence areas, the infections generally occur in adults and are due to sexual

transmission. Transmission through organ transplantation or intravenous drug use might also be possible. (Fauci et al. 2008)
High titers of HHV-8 DNA are found in saliva, the predominant vehicle for infection, while very low titers are found in genital secretions and other sites. But solid organ transplantation has also been shown to transmit HHV-8 (Mandell et al. 2005).
It is not yet completely understood why the prevalence of HHV-8 seropositivity tends to be higher in men who have sex with men. 15-20% of HIV-negative men and 40% of HIV-positive men who have sex with men are HHV-8 positive (Mandell et al. 2005).
The risk for acquiring HHV-8 increases with a person´s number of sexual partners and one´s past sexually transmitted diseases. But it has to be stressed that it is not exactly known yet, how the virus is passed between individuals (Washington University 2009).
About 90-100% of individuals with KS are seropositive, which is compatible with the assumption of HHV-8 as the etiologic cause for KS (Mandell et al. 2005). Mainly in Africa prevalence has significantly increased since the HIV epidemic with now KS accounting for about 25% of childhood malignancies there (Mandell et al. 2005). Besides, Kaposi´s sarcoma (KS) is more frequent in men than in women, despite a similar HHV-8 prevalence in both sexes (Mandell et al. 2005).

2.6.3 Viral Replication and Pathogenesis

The HHV-7 has a tropism for various cell types like salivatory glands and T-cells. It circulates in T lymphocytes and persists in the nucleus of the cell. HHV-7 infects peripheral blood CD4+ lymphocytes and like in the HHV-6, the CD4 receptor allows HHV-7 to interfere with HIV-1. HHV-7 persists in circulating CD4+ cells and can be reactivated by T-cell activation.
HHV-7 encodes genes that can interfere with host immune responses to the virus, like through downregulation of CD4 expression on T cells (Mandell et al. 2005).

The HHV-8 infects certain B lymphocytes and endothelial cells (Fauci et al. 2008).
The infection may be both lytic and latent, but latent infection is predominant, just a small fraction of cells undergo lytic infection. During lytic infection, thousands of virus particles are produced in the cell and are then released from infected cell as soon as this cell dies. These virions then infect new cells or are transmitted to a

new host. Almost all of the genes of HHV-8 are only expressed during lytic infection and encode proteins that are important for replication. In latently infected cells the viral genome circularizes and persists as a naked extrachromosomal episome within the cell nucleus. It uses the cellular replication machinery to replicate itself. Various signals, such as inflammation, may provoke the virus to reactivate and thus enter into lytic replication. Just about 5 genes are expressed during latency. These may lead to cell survival, which is an important feature of malignancy and thus the reason for association of HHV-8 with various tumors and tumor like syndromes. (Mandell et al. 2005)

2.6.4 Clinical Manifestation

HHV-7 has been isolated in encephalitis, hepatitis and others but the extent of clinical manifestations and the clinical importance of HHV-7 is still not completely clear. (Mandell et al. 2005)

Viremia is detectable during primary infection with HHV-7 and during reactivation. Fever and seizures are presumably the most common clinical manifestations in otherwise healthy children; some may present respiratory or gastrointestinal signs and symptoms.

HHV-6, HHV-7 and CMV infections may cluster in transplant recipients, but it is difficult to sort out the role of the various agents in individual clinical symptoms (Fauci et al. 2008).

The HHV-8 is well adapted to its host and thus usually does not cause any disease during asymptomatic chronic infection (Mandell et al. 2005), but suppression of immune system disturbs this balance and disease finally occur, above all malignancies.

A primary infection syndrome has not been clearly described; the infection is presumably most asymptomatic or unrecognized. Maculopapular rash beginning on the face and spreading downwards may occur, as well as upper respiratory tract symptoms, fever for median 10 days, mild diarrhea, fatigue, localized rash and lymphadenopathy. The infection is mild and self-limited in healthy persons but might be severe and leads to consequences in immunosuppressed patients (Mandell et al. 2005).

Immunocompromised individuals with primary infection may suffer from fever, splenomegaly, lymphoid hyperplasia, pancytopenia or even rapid-onset KS (Fauci et al. 2008).
Based on Current knowledge it appears to be very unlikely for a HHV-8 positiv but otherwise healthy individual to develop Kaposi´s sarkoma (KS) or some others serious HHV-8 related problem (Washington University 2009).
The risk for development of KS in with HHV-8 and HIV double infected patients is 45%; they should be aware of their increased risk so that they can start treatment as soon as possible (Washington University 2009).
The KS mainly involves skin with lesions that enlarge from patches to plaques to nodules, with a color altering from lilaceous to brown. A highly vascular nature gives its purple color. There are different variants of the tumor. Some of which predominantly involves skin of the lower extremities and is indolent, some of which occurs in children younger than the age of 10 and is aggressive, multifocal and sometimes without dermal manifestation. Finally, in HIV-infected patients, lesions normally involve face, nose, genitalia and oral cavity in addition to lower extremities (Mandell et al. 2005).

2.6.5 Diagnosis

There is serologic antibody testing, antigenic testing and use of PCR in diagnosis of HHV-7.
Specific antibodies to HHV-8 are detected to determine who has been exposed to the virus.

2.6.6 Therapeutical Management

It is not clear what role HHV-7 plays in human disease and whether there is ever a need for treatment (Mandell et al. 2005).
However, HHV-7 appears to be susceptible to Ganciclovir and Foscarnet (Fauci et al. 2008).

Effective antiretroviral therapy for HIV-infected patients leads to a reduction in rates of KS among persons infected with both viruses, (Fauci et al. 2008) and also reduction in immunosuppression where possible, may lead to KS remission.
(Mandell et al. 2005)

However, HHV-8 appears to be susceptible to Ganciclovir, Foscarnet and Cidofovir (Fauci et al. 2008).

2.6.7 Specimen Types for the Detection of HHV-7 DNA and HHV-8 DNA

The most meaningful results for routine molecular HHV-7 and HHV-8 DNA testing may be obtained by quantitation of viral DNA in EDTA whole blood. During infection, viral DNA can be detected in both peripheral blood mononuclear cells and plasma. Detection of HHV-8 DNA in peripheral blood mononuclear cells from HIV-1 infected individuals was associated with an increased risk of subsequent development and the clinical stage of Kaposi's sarcoma (Campbell et al. 2000). Recently, it has been suggested that peripheral blood mononuclear cells and plasma are both adequate specimen types for quantitation of HHV-8 DNA (Tedeschi et al. 2008). In patients with CNS disease, detection of HHV-7 DNA in cerebrospinal fluid specimens may be meaningful.

Quantitation of the HHV-8 load in blood PCR assays may be useful for monitoring of post-transplant patients (Table 9.7). A significant correlation exists between viral load and disease progression. However, with regard to the development of Kaposi's sarcoma, no HHV-8 DNA load cut-off has been defined.

2.6.8 Laboratory-developed Assays for the Quantitation of HHV-7 DNA and HHV-8 DNA

Molecular assays based on real-time PCR have been established and used for the quantitation of HHV-7 DNA (Fernandez et al. 2002, Hara et al. 2002). Similarly, molecular assays employing the real-time PCR technique were found useful for the quantitation of HHV-8 DNA (White et al. 2000, Boivin et al. 2002).

2.6.9 Commercially Available Assay for the Qualitative Detection of HHV-7 DNA and HHV-8 DNA

The CMV HHV6,7,8 R-gene test (Argene) allows for the qualitative detection of HHV-7 and HHV-8 DNAs and was found to be an efficient and reliable tool for the diagnosis of infections produced by HHV-7 and/or HHV-8 (Deback et al. 2008).

3 Conclusion

For the rapid diagnosis of herpesvirus infection, molecular assays have been introduced in the routine molecular diagnostic laboratory. Today, the majority of assays are based on the real-time PCR technique which provides quantitation through addition of standards with calibrated levels of target nucleic acid. To meet the need for reliable laboratory results, it is advisable to use a maximum automated and standardized kit based on reagents and standards of reproducible high quality. Modern automated sample preparation allows introduction of a large variety of specimens including (EDTA) whole blood, serum, plasma, peripheral blood mononuclear cells, tissues, cerebrospinal fluid, sputa, and bronchoalveolar lavages.

Especially in immunocompromised patients, screening a panel of herpesviruses may be of importance Real-time PCR is a highly sensitive, specific reproducible technique. It may be employed to monitor the viral load in transplant recipients permitting the prediction of the progress of disease and to detect drug resistance. However, the interpretation of quantitative results obtained by molecular test systems is currently complicated through the lack of clear-cut thresholds caused by the use of non-standardized molecular assays with a great number of different specimens. It is of utmost importance to overcome this limitation as soon as possible. This requirement can be met by introduction of a set of real-time PCRs which are performed with one cycling program in parallel in a single run allowing for flexible testing of clinical samples for the presence of different herpesviruses and providing unaltered limits of detection/quantitation (Stöcher et al. 2004). Today, this principle is already included in commercially available real-time PCR kits. For instance, the CMV HHV6,7,8 R-gene test (Argene) allows for single automated extraction of each sample followed by the quantitation of CMV and HHV-6 DNAs and the qualitative detection of HHV-7 and HHV-8 DNAs with one cycling program in parallel in a single run. Furthermore, there are additional kits available for quantitation of HSV1, HSV2, EBV, and VZV with all of them using the identical cycling program allowing for screening the whole panel of herpesviruses in parallel in a single run. In contrast, assays based on multiplex real-time PCR may be impaired by worsened limits of detection/quantitation. In future, this problem may be overcome by improved detection through microarray techniques.

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