

**Molecular Detection of Human Herpes
Viruses 1, 2, 3 and 6 in the Cerebrospinal
Fluid of Patients with Central Nervous
System Infections**

Thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

صدق الله العظيم

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List of Abbreviations

Abb.	Full term
ACIP	Advisory Committee on Immunization Practices
ADEM	Acute Disseminated Encephalomyelitis
AdV	Adenovirus
AIDS	Acquired Immune Deficiency Syndrome
AM	Aseptic meningitis
ANE	Acute Nectrotising Encephalopathy
CEP	California Encephalitis Project
CMV	Cytomegalovirus
CNS	Central nervous system
CPE	Cytopathic effect
CSF	Cerebrospinal fluid
Ct	Cycle threshold
CVA	Cerebrovascular accident
DNA	Deoxyribonucleic acid
EEG	Electroencephalography
EIA	Enzyme immunoassay
EBV	Epstein–Barr virus
EV	Enterovirus
FA	FilmArray
FA/ME	FilmArray Meningitis/Encephalitis panel
FDA	Food and Drug Administration
gD2	Glycoprotein D2
HCMV	Human Cytomegalovirus

 List of Abbreviations

Abb.	Full term
HEp-2	Human epithelial type 2 cell line
HHV	Human herpes virus
HHV-6	Human herpes virus 6
HIV	Human immunodeficiency virus
HPV	human papillomavirus
HSV	Herpes simplex virus
HSV-1	Herpes simplex virus 1
HSV-2	Herpes simplex virus 2
HSVE	Herpes simplex virus encephalitis
HZ	Herpes zoster
ICP	Intracranial pressure
ICU	Intensive care unit
IF	Immunofluorescence
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
IM	Intramuscular
IV	Intravenous
IQR	Interquartile range
JEV	Japanese encephalitis virus
LCMV	Lymphocytic choriomeningitis virus
LP	Lumbar puncture
ME	Meningitis/encephalitis
miRNA	Micro Ribonucleic acid
MRI	Magnetic Resonance Imaging
MRC-5	Medical Research Council cell strain 5

 List of Abbreviations

Abb.	Full term
MS	Multiple sclerosis
MV	Mumps virus
NAT	Nucleic acid test
NMDAR	Anti-N-methyl-D-Aspartate receptor
NS	Non-significant
PCR	Polymerase chain reaction
PCT	Procalcitonin
RNA	Ribonucleic acid
RNase	Ribonuclease
S	Significant
S. pneumoniae	Streptococcus pneumoniae
TB	Tuberculosis
TBE	Tick Borne Encephalitis
TK	Thymidine kinase
TL	Temporal lobe
Tm	Melting temperature
TNF	Tumour necrosis factor
TOSV	Toscana virus
VZIG	Varicella-zoster immunoglobulin
VZV	Varicella-zoster virus
WBC	White blood cells
WNV	West Nile virus
ZN	Ziehl-Neelsen

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Introduction

Infections of central nervous system (CNS), such as encephalitis, meningitis, and meningoencephalitis, remain a major global cause of morbidity and mortality (**Liu et al., 2015**). In the United States, each year, there are approximately 7.3 hospitalizations per 100,000 population due to encephalitis. However, the syndromes encephalitis and meningitis are not nationally notifiable infectious conditions and therefore the incidence of these diseases is not well documented (**Dupuis et al., 2011**).

Acute encephalitis is characterized by a triad of fever, headache, and altered mental status. Diffuse or focal neurologic signs such as cranial nerve dysfunction, hemiparesis, or seizures are commonly encountered. Capillary and endothelial inflammation of cortical vessels is a striking pathologic finding, occurring primarily in the gray matter or the gray–white junction. Aseptic meningitis refers to a disease with acute onset of symptoms and obvious signs of meningeal involvement, such as fever, headache, photophobia, stiff neck, irritability, nausea, vomiting, and rash. Negative bacterial culture of the cerebrospinal fluid (CSF) is suggestive of aseptic meningitis. Although the etiologic agent is not identified

for most cases, viral infection has been reported as a major cause (**Kelly et al., 2013**).

Herpes simplex virus-1 (HSV-1) and varicella-zoster virus (VZV) are the first and second most common causes of viral encephalitis, respectively. These viruses are associated with mortality and morbidity, especially when treatment is delayed (**Pillet et al., 2015**). HSV-1 is responsible for approximately 10% of all cases of encephalitis and is the most common cause of fatal sporadic viral encephalitis worldwide. The mortality rate of HSV encephalitis (HSVE) may be more than 70% if untreated, and more than 95% of untreated survivors will suffer lifelong sequelae. Varicella-zoster encephalitis affects 0.1 to 0.2% of persons with primary varicella. Before the use of varicella vaccine it was the most common cause of encephalitis in children in Europe. In a recent UK study, it remains second only to HSV among infectious causes of encephalitis (5%), and occurs mainly in the immunocompromised; 10% of whom were less than 20 years of age (**Britton and Jones, 2013**). Infection with HSV-2 may result in meningitis or meningoencephalitis, which may recur despite therapy. Neonatal infection with HSV-2 is especially devastating, and disseminated disease may occur in approximately 25% of cases (**Binnicker et**

al., 2014). Human herpes virus-6 (HHV-6) has two distinct variants; HHV-6A and HHV-6B. Primary infection with HHV-6B in infants causes a non-specific febrile illness or most commonly exanthem subitum (roseola or 6th disease), and is associated with febrile convulsions. Up to 7% of children with encephalitis syndrome of otherwise unknown cause have HHV-6 DNA detectable in CSF with or without specific antibody responses **(Britton and Jones, 2013).**

A distinction must be made between acute viral encephalitis (VE) and autoimmune/post-infectious encephalitis, which can occur with a variable latent phase between acute illness and the onset of neurologic symptoms. This distinction is critical because the management and prognosis are often quite different. Evaluation of cerebrospinal fluid (CSF) following lumbar puncture is essential for accurately diagnosing disease, unless its collection is contraindicated because of high intracranial pressure **(Kelly et al., 2013).**

Due to similarities in the clinical presentations of the diseases caused by these herpesviruses and other pathogens, virological testing is often needed **(Pillet et al., 2015).** The detection of herpesviruses in CSF using real-time PCR is now recognized as the gold-standard approach for diagnosing herpes encephalitis and herpes meningitis

(Binnicker et al., 2014). Studies suggest screening CSF cell count and protein values before performing HSV PCR in order to rationalize the use of this test. The only criteria proposed have been elevated CSF leukocyte counts (≥ 5 cells/mm³) and/or protein levels (> 50 mg/dl) **(Roa et al., 2013).**