

NATIONAL STANDARD METHOD

GUIDANCE NOTE

# INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS

QSOP 48

Issued by Standards Unit, Evaluations and Standards Laboratory  
Specialist and Reference Microbiology Division



UK Clinical Virology Network

*Association of Medical Microbiologists*  
*Association of Medical Microbiologists*  
*Association of Medical Microbiologists*



**THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS**

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory

Page 1 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

# STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

Whereas every care has been taken in the preparation of this publication, the Health Protection Agency or any supporting organisation cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of or alteration to any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field, operating in the UK, and specialist advice should be obtained where necessary. If you make any changes to this publication, it must be made clear where changes have been made to the original document. The Health Protection Agency (HPA) should at all times be acknowledged.

The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at [www.hpa.org.uk](http://www.hpa.org.uk).

The HPA aims to be a fully Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions<sup>1</sup>.

More details can be found on the website at [www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk). Contributions to the development of the documents can be made by contacting [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

*Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.*

## **Suggested citation for this document:**

Health Protection Agency (2004). *Investigation of viral encephalitis and meningitis*. National Standard Method QSOP 48 Issue 2. [http://www.hpa-standardmethods.org.uk/pdf\\_sops.asp](http://www.hpa-standardmethods.org.uk/pdf_sops.asp).

## **THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS**

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 2 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

# INDEX

STATUS OF NATIONAL STANDARD METHODS .....	2
INDEX.....	3
AMENDMENT PROCEDURE .....	4
1 INTRODUCTION .....	5
2 MENINGITIS.....	5
2.1 DIAGNOSIS OF VIRAL MENINGITIS.....	5
3 ENCEPHALITIS.....	6
3.1 DIAGNOSIS OF ACUTE VIRAL ENCEPHALITIS .....	6
3.2 DIAGNOSIS OF POST INFECTIOUS ENCEPHALITIS.....	7
3.3 DIAGNOSIS OF SSPE .....	7
3.4 RELEVANT NATIONAL STANDARD METHODS.....	8
4 ADDITIONAL NOTES ON SELECTED VIRAL AGENTS OF CNS DISEASE.....	9
4.1 ADENOVIRUS.....	9
4.2 CENTRAL EUROPEAN ENCEPHALITIS .....	9
4.3 CYTOMEGALOVIRUS .....	9
4.4 DENGUE VIRUS.....	9
4.5 ENTEROVIRUSES AND PARECHOVIRUSES.....	9
4.6 EPSTEIN-BARR VIRUS.....	11
4.7 HERPES SIMPLEX VIRUS.....	11
4.8 CERCOPITHECINE HERPESVIRUS .....	11
4.9 HUMAN HERPESVIRUS 6 (HHV6).....	12
4.10 HUMAN IMMUNODEFICIENCY VIRUS .....	12
4.11 INFLUENZA.....	12
4.12 JAPANESE ENCEPHALITIS .....	12
4.13 JC POLYOMAVIRUS .....	13
4.14 LA CROSSE VIRUS .....	14
4.15 LOUPING ILL.....	14
4.16 LYMPHOCYTIC CHORIOMENINGITIS.....	14
4.17 MEASLES.....	14
4.18 MUMPS .....	15
4.19 MURRAY VALLEY ENCEPHALITIS .....	15
4.20 NIPAH VIRUS .....	16
4.21 OMSK HAEMORRHAGIC FEVER VIRUS .....	16
4.22 PARECHOVIRUSES.....	16
4.23 PARVOVIRUS B19 (ERYTHROVIRUS).....	16
4.24 RABIES .....	16
4.25 ROTAVIRUS.....	17
4.26 RUSSIAN SPRING-SUMMER ENCEPHALITIS.....	17
4.27 TICKBORNE ENCEPHALITIS (TBE) .....	17
4.28 VARICELLA ZOSTER .....	17
4.29 WEST NILE FEVER (WN) .....	18
5 SAFETY CONSIDERATIONS.....	18
5.1 SAFE HANDLING OF SPECIMENS .....	18
5.2 SPECIMEN COLLECTION TRANSPORT AND STORAGE .....	20
5.3 REFERRAL TO REFERENCE LABORATORIES.....	21
5.4 REPORTING TO THE HPA (LOCAL AND REGIONAL SERVICES AND CDSC CENTRE FOR INFECTIONS).....	21
CONTACT .....	21
REFERENCES .....	22

## THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 3 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

# AMENDMENT PROCEDURE

<b>Controlled document reference</b>	<b>QSOP 48</b>
<b>Controlled document title</b>	<b>Standard Operating Procedure for Investigation of viral encephalitis and meningitis</b>

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
22.08.05	2	2.1	1	<b>Front page</b>	Redesigned
			2	<b>Status of document</b>	Reworded
			4	<b>Amendment page</b>	Redesigned

## THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 4 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

# GUIDANCE NOTE FOR THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS

**Types of specimen:** Cerebrospinal fluid, serum, plasma, brain biopsy, swabs, faeces and urine

## 1 INTRODUCTION

A large number of infectious agents cause disease of the central nervous system (CNS). These include bacteria, fungi, parasites, viruses and prions. This Guidance Note describes the viral pathogens responsible for viral encephalitis and meningitis. For more extensive information on clinical presentation and CSF findings reference should be made to standard textbooks of infectious diseases.

## 2 MENINGITIS

Meningitis is defined as inflammation of the meninges.

Symptoms of *acute* meningitis include fever, headache, neck stiffness and photophobia. The headache is usually severe and global, often throbbing. Nausea and vomiting may be associated. Sensitivity of headache as a diagnostic criterion for meningitis is low in adults at 50%. Patients suffering from nausea and vomiting is equally low at 30%. In babies there may only be non-specific signs such as poor feeding, irritability, lethargy and irritation. Rapid recognition of acute meningitis is vital, and the differentiation between bacterial, partly treated bacterial and viral meningitis is critical to allow appropriate treatment of the infected individual and management of contacts.

*Chronic* meningitis is defined as persistence of meningeal inflammation for over 4 weeks. Mumps and lymphocytic choriomeningitis (LCM) virus infections occasionally persist for 3 to 4 weeks but true chronic viral meningitis is rare except in patients who are immunodeficient. Chronic enterovirus meningitis occurs in patients with hypogammaglobulinaemia, particularly X-linked agammaglobulinaemia<sup>2</sup>. Virus can be detected by molecular methods (but not by culture) if immunoglobulin therapy is given, and can be positive for long periods<sup>3,4</sup>. Meningitis due to HIV infection can be prolonged and occurs in the acute retroviral syndrome<sup>5</sup>.

*Recurrent* meningitis is characterised by resolution of clinical illness and the return to normal of CSF changes. It is occasionally seen in association with recurrences of genital herpes simplex type 2 lesions. Mollaret's meningitis is a rare form of recurrent meningitis associated with herpes viruses, generally HSV type 2, although infection due to HSV type 1 has been reported<sup>6</sup>. Recurrent episodes of fever, meningism, and severe headache occur, often associated with transient neurological deficits eg cranial nerve palsies, and fits. Lymphocytic pleocytosis is seen in the CSF and large 'endothelial' cells ('Mollaret' cells - probably monocytes) may be present. Attacks are separated by weeks or months and are self-limiting.

### 2.1 Diagnosis of viral meningitis

Viral meningitis may be confirmed by examination of cerebrospinal fluid (CSF). In viral meningitis there is typically a CSF lymphocytosis of <500 cells, however a negative cell count cannot exclude the diagnosis<sup>7</sup>. Very early in infection a raised CSF neutrophil count is common. Protein levels may be a little raised but glucose levels are generally normal (glucose may be low in mumps infection, and lymphocytic choriomeningitis virus infection). Bacterial meningitis on the other hand is characterised by high CSF white cell count (>500 X 10<sup>6</sup> cells/ L), high protein, and low glucose levels. Use of acute phase reactants such as C-reactive protein and procalcitonin in differentiating viral from bacterial meningitis remains unproven.

Identification of viruses in brain tissue or cerebrospinal fluid (CSF), or demonstration of intrathecal antibody production, is diagnostic in an appropriate clinical setting. In infections of the CNS where brain tissue or CSF cannot be obtained it may be possible to gain results that suggest a neurotropic virus is implicated by virus culture or PCR from another specimen

#### THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory

Page 5 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

such as faeces or plasma. Also detection in serum of IgM antibody to a virus known to cause CNS infection or demonstration of a significant rise in serum antibody titre to such a virus is possible.

Culture at present remains a useful adjunct to PCR in allowing rapid typing of viruses. Conventional typing schemes based on immunofluorescence or neutralisation are more readily available than molecular typing schemes for many viruses, especially adenoviruses and enteroviruses.

### 3 ENCEPHALITIS

Encephalitis is an inflammatory process involving the parenchyma of the brain, although it is often associated with meningitis (meningoencephalitis).

*Acute* viral encephalitis usually begins with a fever and headache, similar to acute meningitis but with accompanying signs of cerebral involvement - altered level of consciousness (ranging from mild lethargy to coma), confusion, disorientation, mental changes such as delirium, personality change, agitation, hallucinations, focal neurological changes, cranial nerve deficits, and in over 50% of cases, fits. Headaches, typically global, are caused by raised intracranial pressure and meningeal involvement. Headaches may occur early and present as the only symptom.

Herpes simplex is the commonest cause of sporadic viral encephalitis. However in many typical cases no aetiological agent is identified<sup>8</sup>. Increased foreign travel makes it necessary to consider the possibility of imported infections, especially arboviral infections, in cases of aseptic meningitis or encephalitis in the UK. The range of agents implicated in encephalitis varies considerably in different parts of the world with the major agents in South America<sup>9</sup> quite different from those of South East Asia<sup>10</sup>.

*Postinfectious* encephalitis is an encephalopathy which occurs relatively soon after an acute viral infection. It is an acute disseminated encephalomyelitis associated with an autoimmune process with demyelination due to myelin-reactive T cells. A classic example is that seen after vaccination against smallpox. This occurred at a rate of 3 per million primary vaccinations, seen 8 to 15 days after vaccination and had a 15 to 25% death rate<sup>11</sup>. Similar complications can occur with measles (about 1 per 1000 cases), mumps, rubella and EBV.

*Subacute encephalitis* occurs years after the initial infection. Examples are progressive rubella panencephalitis, a rare condition seen some 8 to 19 years after congenital or perinatal rubella<sup>12</sup> and subacute sclerosing panencephalitis associated with measles infection (see section 4.17 Measles).

#### 3.1 Diagnosis of acute viral encephalitis

Clinical findings (such as a typical rash) or epidemiological features (travel to endemic area, part of outbreak, animal exposure), are rarely adequate to make a diagnosis. Laboratory investigations are almost always required.

The initial investigation often requires a CT scan of the brain, to exclude space-occupying lesions prior to lumbar puncture. Herpes simplex encephalitis is typically focal, with CT showing focal temporal lobe changes in over 70% of cases and MR scan and EEG showing temporal lobe abnormality in over 90% of cases. Three infections are associated with MR scan abnormalities of basal ganglia and thalamus – eastern equine encephalitis, Japanese encephalitis and tick-borne encephalitis<sup>13</sup>. Other agents tend to cause more diffuse encephalitis, although La Crosse virus infection can produce focal changes similar to HSV<sup>14</sup>.

The diagnosis of acute viral encephalitis can be confirmed by CSF examination. CSF changes in encephalitis are similar to those of viral meningitis ie slightly raised protein levels, with a raised lymphocyte count (>5 but <500 X 10<sup>6</sup> cells/L). Lymphocytosis may be absent in immunocompetent individuals early in the infection<sup>15</sup> as well as in patients who are immunocompromised, including those with HIV infection.

Glucose levels are generally normal but may be low in mumps infection, and in advanced herpes simplex and lymphocytic choriomeningitis virus infection. A low glucose level should

alert the clinician to exclude the possibility of a non-viral cause for the CNS signs, eg fungal infection, leptospirosis, mycobacterial infection, syphilis, sarcoid or neoplasia.

Red cells may be present due to a traumatic tap or in advanced herpes simplex encephalitis.

Virus culture, histology and electron microscopic examination of brain biopsy are seldom required nowadays to diagnose viral encephalitis. The surgical procedure for obtaining a brain biopsy is invasive and is associated with significant morbidity. The procedure also suffers from the problem of sampling error.

PCR assays on CSF have long been used for diagnosis of CNS infection<sup>16</sup> and in general give high diagnostic yields<sup>17,18</sup>. Caution may be required in interpretation where there is evidence of CNS viral latency, as with HHV6<sup>19</sup>. Intravenous aciclovir is usually administered to patients with suspected herpes simplex viral encephalitis pending CSF PCR results.

PCR assays for herpes simplex types 1 and 2, enteroviruses and parechoviruses, and other agents such as VZ, EBV, HHV6 and CMV are readily available in specialist virology laboratories, sometimes in multiplex PCR formats. Multiplex PCR assays may be available to detect several different common viruses associated with specific presentations or patient groups (for example, patients who are immunocompromised). They may be valuable where CSF volume is limited, but the sensitivity and specificity for each virus should be similar or better than that achieved using single-target assays.

It is essential to be aware of the sensitivity and specificity of any PCR assay used. It may not be possible to exclude specific viral causes based upon a single negative PCR result, and in the presence of on-going clinical evidence of infection, a further CSF should be requested.

PCR for arboviruses and LCM may require referral to reference centres such as CAMR, Porton Down.

Serological assays continue to have an important role in diagnosis. Rising titres or IgM reactivity in sera can support a specific diagnosis while serum:CSF antibody ratio estimations can be diagnostic by demonstrating intrathecal production of antibodies.

### **3.2 Diagnosis of post infectious encephalitis**

Diagnosis of post infectious encephalitis is dependent on making the temporal link between the neurological illness and the antecedent infection. Antibody tests are useful in confirming the viral diagnosis, particularly IgM assays on serum. CSF does not always show intrathecal production of antibody to the triggering virus, and PCR is generally negative.

### **3.3 Diagnosis of SSPE**

A characteristic periodic pattern is seen on EEG, with bursts every 3 to 8 seconds of high-voltage, sharp slow waves followed by periods of attenuated ("flat") background<sup>20</sup>. CSF is acellular. Protein level is normal or slightly elevated but there is a markedly elevated gamma-globulin level (>20% of total CSF protein). CSF antimeasles antibody levels are high, and oligoclonal antimeasles antibodies are often present. Cerebral imaging shows multifocal white matter lesions, cortical atrophy, and ventricular enlargement. Measles virus can be cultured from brain tissue using special cocultivation techniques. Viral antigen can be identified by immunohistochemistry. The measles virus genome can be detected by in situ hybridisation and by PCR.

**Table I: Viral causes of meningitis and/or encephalitis***Viruses in italics are endemic in the UK*

<b>Direct infection</b>	
Alphaviruses-	Eastern Equine, Western Equine, Venezuelan Equine
Flaviviridae-	St Louis, Murray Valley, West Nile <sup>a</sup> , Japanese, Dengue, Tick-borne encephalitis <sup>b</sup> , Central European encephalitis <sup>b</sup> , <i>Louping ill</i> <sup>c</sup>
Bunyaviridae-	La Crosse, Rift Valley, Toscana
Paramyxoviridae-	<i>Mumps</i> , <i>Measles</i> , Hendra, Nipah
Arenaviridae-	Machupo, Junin, Lassa
Picornaviridae-	Poliovirus <sup>d</sup> , <i>Coxsackieviruses</i> , <i>Echoviruses</i> , <i>Parechoviruses</i> , Enterovirus 71
Reoviridae-	Colorado tick fever
Rhabdoviridae-	Lyssavirus <sup>b</sup> , Rabies <sup>b</sup>
Filoviridae-	Ebola, Marburg
Retroviridae-	<i>HIV</i>
Herpesviridae-	<i>HSV 1 and 2</i> , <i>VZV</i> , <i>EBV</i> , <i>CMV</i> , <i>HHV6</i> , <i>Herpes B</i>
Adenoviridae-	<i>Adenovirus</i>
Parvoviridae	<i>Parvovirus B19</i>
<b>Post infectious</b>	
Togaviridae-	<i>Rubella</i>
Orthomyxoviridae-	<i>Influenza</i>
Paramyxoviridae-	<i>Mumps</i> , <i>Measles</i>
Poxviridae	<i>Vaccinia</i> <sup>e</sup>
Herpesviridae-	<i>VZV</i> , <i>EBV</i>

a - UK surveillance in progress

b - Central/Western Europe

c - Very rare

d - In the UK as part of vaccine associated disease (wild type virus eradicated)

e - Post-immunisation

### 3.4 Relevant National Standard Methods

For additional details on specific areas of diagnosis refer to the following SOPs available through the Evaluations and Standards Laboratory web page ([www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)) of the Health Protection Agency website ([www.hpa.org.uk](http://www.hpa.org.uk)):

VSOP 19 – Investigation of Herpes Simplex Encephalitis by Idaho LightCycler PCR

VSOP 20 – Nucleic Acid Extraction

VSOP 23 – Investigation of Human Herpes Viruses (excluding herpes genitalis)

VSOP 36 – Operation of the Roche MagNA Pure LC automated nucleic acid extraction robot

BSOP 27 – Investigation of Cerebrospinal fluid

BSOP 40 – Investigation of specimens for Mycobacterium species

#### THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 8 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

## **4 ADDITIONAL NOTES ON SELECTED VIRAL AGENTS OF CNS DISEASE**

### **4.1 Adenovirus**

Adenovirus encephalitis is relatively uncommon even in patients who are immunocompromised<sup>21</sup>. The onset is acute and the outcome generally but not invariably benign<sup>22</sup>. Higher rates of adenovirus encephalitis have been described in an adenovirus 7 outbreak in hospitalised children<sup>23</sup>. A syndrome of transient encephalopathy in association with upper respiratory symptoms has recently been described in children under 3 years of age<sup>24</sup>.

#### **4.1.1 Diagnosis of adenovirus infection**

Serological diagnosis is generally made by looking for rising titres to adenovirus antigen in CFT tests. Diagnosis is often made by immunofluorescent detection of antigen in an appropriate specimen eg nasopharyngeal aspirate if there are respiratory symptoms, and by virus culture, often using the shell vial technique (DEAFF), in HeLa, Hep2, A549 (most sensitive), fibroblasts (least sensitive), or 293 cells<sup>25</sup>. PCR is more sensitive than culture in most studies and can be done on the same types of samples used for virus culture including CSF, throat and nose swabs, nasal washes as well as on anticoagulated blood<sup>26</sup>.

### **4.2 Central European encephalitis**

See tickborne encephalitis

### **4.3 Cytomegalovirus**

Encephalitis, typically with periventricular lesions, occurs as part of the congenital CMV syndrome. In patients who are immunocompromised with AIDS it was until recently one of the most common causes of CNS disease (16%)<sup>27</sup>. CMV encephalitis also occurs post-transplantation but is believed to be uncommon. However CMV DNA has been described in 50% of brains from liver transplant patients with CNS symptomatology<sup>28</sup>. Occasional cases are described in individuals who are immunocompromised<sup>29</sup>. The clinical presentation of CMV encephalitis is often non-specific<sup>30</sup>.

#### **4.3.1 Diagnosis of CMV encephalitis**

CMV PCR on CSF is the best test for diagnosis, with sensitivity around 79%<sup>27</sup> or higher<sup>30</sup>.

Supporting evidence can come from serum CMV IgM reactivity blood, urine, or throat swab CMV culture and PCR. However, only CSF CMV PCR or intrathecal CMV antibody production are diagnostic.

### **4.4 Dengue virus**

The incidence of CNS disease in dengue infections may be underestimated as other encephalitogenic arboviruses often coexist with dengue virus. Dengue virus should be considered in the differential diagnosis of encephalitis in areas where it is endemic. In a study of encephalitis in Vietnam 4% of cases seemed to be associated with the dengue virus<sup>31</sup>.

#### **4.4.1 Diagnosis of dengue virus infection**

Detection of serum or CSF antibody by IgM capture ELISA is recommended although similar immunoassays show satisfactory performance<sup>32</sup>. Virus isolation is undertaken in mosquito cells, with immunofluorescence detection. Virus isolation from serum taken within 5 days of onset gives over 60% yield<sup>33</sup>.

### **4.5 Enteroviruses and Parechoviruses**

Enterovirus infections account for most cases of viral meningitis<sup>34</sup>. Infections with poliomyelitis resulted in neurological disease in about 1% of cases, with aseptic meningitis more common than paralytic poliomyelitis. Aseptic meningitis is commonly due to enteroviruses in children, and usually follows a benign course. Coxsackie A7, A9, B1-6, echovirus 4, 6, 11, 14, 16, 25, 30, 31 are commonly involved. In the hypogammaglobulinaemic chronic meningeal irritation or encephalitis can occur due to chronic enteroviral infection. Enteroviral meningitis tends to be seasonal with a peak in the summer months. Enteroviral encephalitis is uncommon and may or may not be associated with meningitis. It is relatively benign although occasional neurological sequelae can occur, including hypothalamic-pituitary damage<sup>35</sup>.

Enterovirus 71 appears to have a particular propensity for the central nervous system, producing aseptic meningitis, acute flaccid paralysis or brain stem encephalitis<sup>36</sup>. During outbreaks most cases occur in children under 5 years of age<sup>37</sup>. Hand, foot and mouth disease can also be a feature of epidemics with a high rate of neurological illness<sup>38</sup>.

The human parechoviruses 1 and 2, formerly echovirus 22 and 23, are more commonly associated with diarrhoea and respiratory illness than with CNS disease<sup>39</sup>. Cases of meningitis and encephalitis occasionally occur with these agents<sup>40</sup>.

#### **4.5.1 Diagnosis of enterovirus infection**

Virus culture is relatively sensitive (50%) in enterovirus meningitis<sup>7</sup> but increased diagnostic yields can be obtained with rt-PCR testing<sup>18 41,42</sup>. Separate probes are required for enterovirus and parechovirus detection<sup>40</sup>. Enterovirus IgM ELISA testing of serum may support a diagnosis of recent enteroviral infection.

## 4.6 Epstein-Barr virus

Encephalitis and meningitis are recognised as uncommon complications of glandular fever<sup>43</sup>. Acute EBV accounts for 4 to 5% of infection-related neurological disease, while reactivated EBV is reported to be an important cause of neurological complications in paediatric patients<sup>44</sup>.

### 4.6.1 Diagnosis of EBV encephalitis

Detection of EBV DNA PCR on CSF is diagnostic for CNS disease (provided there has not been a traumatic tap). When quantitative PCR is done levels are relatively high in both EBV CNS lymphoma and acute EBV encephalitis cases, and lower in post infectious EBV-related syndromes such as Guillain-Barre syndrome, acute demyelinating encephalitis and transverse myelitis<sup>45</sup>. Antibody studies on serum may support a diagnosis of acute EBV encephalitis, for example when EBV VCA IgM is positive and anti-EBNA negative.

## 4.7 Herpes simplex virus

Herpes simplex virus affects the CNS under several circumstances. In the rare congenital herpes simplex infection CNS damage results from intrauterine herpes encephalitis. This can occur at any gestational age<sup>46</sup>. In the neonatal period HSV acquired from the mother peripartum may present as localised vesicles, encephalitis with or without skin or mucosal involvement, and as disseminated HSV infection involving the CNS and other organs<sup>46</sup>.

The mortality rate for untreated neonatal CNS disease is 50 to 80%. Survivors of neonatal herpes encephalitis treated with aciclovir or vidarabine are more likely to suffer neurological sequelae with HSV type 2 infection than with HSV type 1<sup>47</sup>. MR scanning frequently shows haemorrhagic lesions in the cerebral cortex in the infected neonate<sup>48</sup>.

In sexually active individuals, the primary attack of genital HSV type 2 infection is not infrequently associated with evidence of meningitis, particularly in women. Genital lesions are however present only in a minority of cases of HSV type 2 meningitis<sup>49</sup>. Although herpes simplex meningitis is usually self-limiting with spontaneous recovery, severe cases can occur particularly in patients who are immunocompromised<sup>50</sup>.

Herpes simplex encephalitis is the most common cause of sporadic encephalitis, affecting 1 to 4 per million population per year. Over 95% of cases are due to HSV type 1<sup>51</sup>, including both primary HSV infection and reactivation of latent HSV. The disease presents with a flu-like illness in the two weeks preceding the onset of neurological symptoms. Lesions are characteristically in the temporal lobes<sup>52</sup>. Early administration of aciclovir is crucial in improving outcome<sup>53</sup>.

### 4.7.1 Diagnosis of herpes simplex meningitis and encephalitis

A combination of detection of intrathecal production of antibody and PCR on CSF is regarded as the most reliable diagnostic strategy<sup>54</sup>. In HSV encephalitis a second CSF sample for PCR is advised 14 days after treatment. If the HSV PCR is positive retreatment is recommended. It should be noted that in the first few days after the onset of HSV encephalitis CSF PCR may be negative in a significant number of patients<sup>55</sup> (over 25%). Quantitative PCR may have a role in assessing prognosis and monitoring response to therapy<sup>56</sup>.

Introduction of HSV PCR has resulted in many atypical or mild cases being recognised, particularly with HSV type 2<sup>57</sup>.

In neonatal herpes encephalitis CSF HSV PCR has high sensitivity<sup>48,58</sup>. Further sensitivity can be gained by testing blood as well as CSF by PCR<sup>58,59</sup>. HSV DNA detected from neonatal dried blood spots may allow early diagnosis where symptoms are vague<sup>60</sup>. Virus isolation or PCR from other sites such as skin vesicles, are also relevant<sup>48</sup>.

## 4.8 Cercopithecine herpesvirus

The cercopithecine herpesvirus 1 (monkey B virus) is enzootic among macaque monkeys, in which it causes mild localised lesions and remains latent. Some 70% of captive adult

macaques carry B virus, which is shed periodically in urogenital secretions, saliva and conjunctival fluid. Contact may result in vesicular lesions and ascending acute demyelinating encephalomyelitis<sup>61</sup>. Case fatality rate from B virus encephalitis is 50 to 70%<sup>62</sup>.

#### **4.8.1 Diagnosis of cercopithecine herpesvirus 1**

Virus culture has been regarded as the gold standard but is now being superseded by PCR. A recently developed Taqman assay is twice as sensitive as culture<sup>63</sup>.

#### **4.9 Human herpesvirus 6 (HHV6)**

The association of HHV6 with febrile convulsions in children under 2 years of age is well recognised<sup>64</sup>.

HHV6 is also a cause of meningitis and encephalitis in patients who are immunocompetent<sup>65,66</sup> as well as patients who are immunocompromised<sup>66</sup>. In the bone marrow transplant recipient, encephalitis presentation occurs between 10 days to 15 months (median 45 days) after transplantation. The predominant clinical features are mental changes, with 25% of patients suffering from fits and headache, and 17% with neurological focal changes. CSF cell count may be normal, and neuro-imaging is seldom helpful<sup>67</sup>.

#### **4.9.1 Diagnosis of HHV6 encephalitis**

Positive anti-HHV6 IgM serology in CSF is diagnostic. A fourfold rise in antibody in serum supports the diagnosis but only reflects reactivation of virus. Virus culture from CSF is insensitive, PCR from CSF is more appropriate<sup>65,68</sup>. Although interpretation may require caution, given the reported high prevalence (over 30%) of latent virus in brain detectable by PCR<sup>69</sup>, it is doubtful whether HHV6 DNA is detectable in CSF except in active CNS infection<sup>67</sup>.

#### **4.10 Human immunodeficiency virus**

Direct HIV infection of the CNS occurs in 10 to 50% of patients with AIDS, ultimately producing dementia. Early signs are subtle such as reduced ability to concentrate, progressing to apathy, motor symptoms such as weakness and clumsiness, and worsening cognitive function including memory loss. Multinucleated giant cells are the pathognomonic feature in deep grey and central white matter<sup>70</sup>. HIV encephalitis may be more common in drug users than in HIV-infected homosexuals<sup>71</sup>. The incidence of HIV encephalitis, which had fallen with more effective therapy, has risen in recent years<sup>72</sup> perhaps due to longer survival and greater exposure of brain to HIV. HIV lymphocytic meningitis and HIV encephalitis can be found at various stages of HIV infection<sup>72</sup> and tend to develop before opportunistic infections.

#### **4.10.1 Diagnosis of HIV CNS disease**

Detection of HIV RNA in CSF is consistent with this diagnosis<sup>73</sup>, in the presence of compatible serum antibody findings.

#### **4.11 Influenza**

Influenza A and B have been described in association with both acute and postinfectious encephalitis. Incidence is believed to be low except in particular epidemics<sup>74,75</sup>.

Young children are most affected. CSF protein and glucose levels are usually normal. CSF white cell count may be normal or show lymphocytosis<sup>76</sup>.

#### **4.11.1 Diagnosis of influenza encephalitis**

Most cases have been diagnosed by positive CSF PCR for influenza A or B<sup>74,76</sup>. Support may come from virus isolation, antigen detection or PCR on respiratory secretions as well as from rising titres in CFT, HI or other serological assay.

#### **4.12 Japanese encephalitis**

##### **THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS**

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 12 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

Japanese encephalitis (JE) is the most widespread of the arboviral encephalitides, affecting rural areas of China (where over 20000 clinical cases are reported annually), Japan, India, Pakistan eastern Russia, and the Philippines. It has been reported to have spread south to the Torres Strait islands of Australia<sup>77</sup>.

JE is caused by a mosquito-borne flavivirus carried by *Culex* species mosquitoes. Infection occurs in the summer months in the northerly areas involved but can occur throughout the year in more southern areas. Children are most affected, and are much more likely than adults to have fits. Most infections are asymptomatic<sup>62,78</sup>.

#### **4.12.1 Diagnosis of Japanese encephalitis**

Detection of IgM antibody is the mainstay of diagnosis. This can be done by either capture ELISA or particle agglutination assay. Both have high sensitivity<sup>79</sup> but specificity is less satisfactory because of cross reactivity with other flaviviruses. Antigen detection tests such as reverse passive haemagglutination and immunofluorescence, as well as PCR detection are also available<sup>78</sup>.

#### **4.13 JC polyomavirus**

JC virus, latent in most individuals from childhood, may be associated with chronic meningoencephalitis and progressive multifocal leukoencephalopathy (PML) in patients who are immunocompromised. The insidious onset of a central nervous system illness should alert the clinician to the possibility of PML. Visual field defects and mental impairment (ranging from subtle personality changes to dementia) are typical. AIDS-associated PML progresses rapidly, with a median survival of 6 months<sup>80</sup>. Cerebral CT scanning may show hypodense, non enhancing white matter lesions without associated oedema. MRI scanning may show multifocal asymmetric white matter lesions, particularly in the occipital and parietal lobes, without contrast enhancement or mass effect.

#### 4.13.1 Diagnosis of JC polyomavirus infection

Diagnosis of PML can be made on CSF by positive JC PCR<sup>81</sup> with sensitivity estimated at 75% and 98% specificity<sup>82</sup>. Diagnosis can also be made on a serum:CSF pair demonstrating intrathecal production of JC antibody (97% sensitive, 79% specific)<sup>83</sup>.

#### 4.14 La Crosse virus

This is the most prevalent North American arbovirus infection of children. Cases of aseptic meningitis and encephalitis are seen mainly in school-age children<sup>78</sup>. It is caused by a bunyavirus in the California encephalitis serogroup carried by the *Aedes triseriatus* mosquito. Presentation can be very similar to herpes simplex – focal seizures, focal signs, and focal EEG changes are common and found in up to 50% of cases. Mortality in clinical cases is of the order of 10%. Neurological and intellectual deficits are common in survivors. Differential diagnosis also includes partially treated bacterial meningitis, as peripheral blood white cell count and CRP are often raised<sup>14</sup>.

#### 4.15 Louping ill

The only indigenous British arbovirus, louping ill disease affects mainly red grouse and sheep, and can also affect cattle and goats. It is thought that there may be other reservoirs such as the red deer and mountain hare in Scotland. It is a hazard group 3 pathogen. The louping ill virus is a flavivirus transmitted by the hard tick *Ixodes ricinus*, the commonest tick in Northern Europe. Human cases are few and seldom fatal, but should be considered in certain occupational groups in Scotland<sup>84</sup>.

#### 4.15.1 Diagnosis of louping ill

Diagnosis is based on detection of rising haemagglutination-inhibiting antibody titres in serum. Early in the illness the virus may be detected in anticoagulated blood by rt-PCR.

#### 4.16 Lymphocytic choriomeningitis

This arenavirus infection contracted from exposure to the urine of chronically infected rodents presents as a flu-like illness. Leukopenia followed by lymphocytosis is typical. Coryza, retroorbital pain, anorexia and nausea are common and precede symptoms of meningitis<sup>85</sup>.

#### 4.16.1 Diagnosis of LCM

Diagnosis of LCM is usually demonstrated serologically with rising titres detected by immunofluorescence, by ELISA IgG or by neutralisation. PCR has been used to examine CSF but confirms that the incidence of LCM is currently very low<sup>86</sup>.

#### 4.17 Measles

Measles causes three types of encephalitis, postinfectious encephalitis 5 to 14 days after the rash, progressive infectious encephalitis 3 to 6 months after the initial infection and subacute sclerosing panencephalitis (SSPE) 5 to 10 years after the initial infection<sup>20</sup>. Postinfectious encephalitis is an autoimmune response characterised by perivascular inflammation and demyelination. Clinical features are abrupt onset of fever, with seizures and multifocal neurological signs<sup>20</sup>. The incidence is about 1 in 1000 cases of measles and there is a 10 to 20% death rate.

Progressive infectious encephalitis occurs in children who are immunodeficient or immunosuppressed and is due to uncontrolled measles virus replication. This is evidenced by the presence of intranuclear and intracytoplasmic inclusion bodies in glial cells and neurons. There is a generalised progressive neurological deterioration.

SSPE occurred at a rate of 1 per million per year but its incidence has fallen with widespread measles vaccination. Measles vaccine virus rarely causes SSPE. It is more likely to follow natural measles infection in children under 2 years of age. Some 5 to 10 years after the attack of measles mental deterioration starts to occur, with rapid deterioration marked by blindness and akinetic mutism. Myoclonus is a feature. SSPE is due to the

#### THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 14 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

presence in the brain of a defective virus. Defects in M protein and/or membrane-associated F or H proteins may destabilise envelope maturation and lead to failure of immune surveillance<sup>20</sup>. Treatment with intraventricular interferon plus IV ribavirin may be of benefit<sup>87</sup>.

#### 4.17.1 Diagnosis of measles encephalitis

Serology, particularly detection of IgM by ELISA is sensitive<sup>88</sup> and supports the diagnosis of measles encephalitis in the appropriate context. Oral fluid can be used for detection of measles IgM and of virus by rt-PCR with over 90% sensitivity, while virus can be detected by rt-PCR in urine for some 5 weeks after infection<sup>89</sup>. SSPE is diagnosed by demonstrating intrathecal production of measles IgG antibody.

#### 4.18 Mumps

Meningitis commonly occurs in mumps infection, but has a benign course with recovery in a few days. In 50% of cases of mumps there is a CSF pleocytosis. Symptoms and signs (headache, vomiting, neck stiffness) occur in 5 to 30% of mumps cases. Meningitis usually follows parotitis but may occur in its absence. The CSF cell count may have a polymorph predominance on the first day and CSF glucose level is sometimes low<sup>90</sup>. Mumps encephalitis is uncommon, perhaps 0.1% of cases of mumps, but the rate of long term sequelae is relatively high<sup>91</sup>.

##### 4.18.1 Diagnosis of mumps meningitis and encephalitis

Traditional serological assays such as CFT for mumps 'S' and 'V' antigens have largely been replaced by ELISA assays for IgG and IgM. Isolation of mumps virus from saliva or throat swab is quite sensitive (>95% using shell vial culture in Vero cells) in the first 7 to 10 days of illness, after that urine culture is more satisfactory<sup>92</sup>. Direct immunofluorescent antigen detection can give similar high yields<sup>92</sup>. Oral fluid can be used for both serodiagnosis by IgM detection and, particularly early in infection, virus detection by rt-PCR<sup>93</sup>. Sequence analysis may be required to differentiate vaccine virus from wild type virus when mumps is detected by PCR<sup>94</sup>.

#### 4.19 Murray Valley encephalitis

Originally described in South Eastern Australia, Murray Valley encephalitis (MVE) is now well recognised throughout Australia but particularly in the Northern Territory and Western Australia<sup>95</sup>.

It is caused by a mosquito-borne flavivirus, maintained through a cycle involving birds and the mosquito *Culex annulirostris*. The infection is more prevalent after high rainfall and flooding.

#### 4.19.1 **Diagnosis of Murray Valley encephalitis**

A four-fold rise in serum MVE antibody titres (eg neutralising antibody titres) in the absence of Kunjin, JE or dengue antibodies supports the diagnosis. A more definitive diagnosis can be made by detecting flavivirus IgM in CSF by isolation or rt-PCR<sup>96</sup>.

#### 4.20 **Nipah virus**

The Nipah virus, a paramyxovirus related to Hendra virus, was found to be the aetiological agent of an outbreak of severe encephalitis among pig farmers in Malaysia in 1998, and of cases the following year in Singapore and Malaysia associated with pigs. The outbreak ended after the culling of more than 1 million pigs in Malaysia and the banning of export of pigs from that country. Pigs are probably infected by food contaminated with the saliva and urine of flying foxes (fruit bats)<sup>97</sup>, the main natural host. Over 90% of patients have had direct contact with pigs, so the majority of cases have occurred in adult males<sup>98</sup>. The main features at presentation are fever and headache, with 55% having reduced level of consciousness and evidence of brain stem involvement (myoclonus, areflexia, hypotonia, hypertension, tachycardia)<sup>99</sup>. The mortality rate has been reported to be as high as 32%. Of the survivors 22% had persistent neurological deficits, and 19% had a clinical relapse. Treatment with ribavirin may be of benefit<sup>100</sup>.

#### 4.20.1 **Diagnosis of Nipah virus infection**

Diagnosis of Nipah virus can be made by virus isolation in Vero cell culture which gives rise to the formation of syncytia. Diagnosis by rt-PCR has also been used. Serological diagnosis is based on ELISA IgG<sup>101</sup>.

#### 4.21 **Omsk haemorrhagic fever virus**

see Tickborne encephalitis

#### 4.22 **Parechoviruses**

see Enteroviruses and parechoviruses

#### 4.23 **Parvovirus B19 (erythrovirus)**

The detection of parvovirus B19 DNA in CSF from about 4% of cases of meningoencephalitis has been described during an epidemic period for parvovirus B19<sup>102</sup>.

Parvovirus B19 DNA may persist in CSF, blood and brain samples<sup>103,104</sup>.

#### 4.23.1 **Diagnosis of parvovirus B19 infection**

Support for a diagnosis of parvovirus B19 encephalitis comes from finding parvovirus IgM in serum by ELISA, while definitive evidence comes from detection of parvovirus DNA by PCR and detection of parvovirus B19 IgM in CSF specimens<sup>102</sup>.

#### 4.24 **Rabies**

Rabies is an acute progressive fatal encephalitis caused by a lyssavirus. It is transmitted to humans via the saliva of infected mammals, usually as a result of a bite. Dogs are the principal reservoir and vector. Foxes, coyotes, jackals, mongooses and skunks are also important wild reservoirs. Bat rabies has become increasingly recognised worldwide including Britain. There are 6 bat-associated lyssaviruses which are known to cause rabies<sup>105</sup>.

After an incubation period usually of 1 to 2 months (but which can be more than a year), an acute neurological illness develops which progresses to paralysis, coma, and death<sup>106</sup>.

#### 4.24.1 **Diagnosis of rabies**

Several tests should be used to attempt to diagnose rabies in a suspected case. In the UK the only laboratory accredited to undertake rabies diagnostic work is the Veterinary Laboratories Agency, New Haw, Addlestone, Surrey (telephone +44(0)1932 341111).

#### THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 16 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

Examination with fluorescent antibody to rabies antigen of the cutaneous nerves at the base of hair follicles in skin biopsies from the nape of the neck is rapid but insensitive. Virus isolation and rt-PCR (amplifying from the nucleoprotein gene)<sup>107</sup> can be employed on saliva and on skin biopsy, with the latter being a more sensitive and rapid method<sup>108</sup>. Rabies antibodies can be looked for in CSF and serum samples.

#### 4.25 Rotavirus

The association of gastroenteritis in children with seizures has been described and although they are associated less with rotavirus diarrhoea (<4%) than with bacterial gastroenteritis (7%), rotavirus RNA has been detected in CSF in a number of cases. It remains uncertain as to whether rotavirus can cause encephalitis<sup>109</sup>.

#### 4.26 Russian spring-summer encephalitis

see tickborne encephalitis

#### 4.27 Tickborne encephalitis (TBE)

This flavivirus infection occurs across central, northern and eastern Europe and is transmitted by hard ixodid ticks.

Cases occur in Austria, Belarus, Bulgaria, Croatia, Czech Republic, Estonia, Finland, Germany, Hungary, Italy, Latvia, Lithuania, Poland, Romania, Slovakia, Slovenia, Sweden, Switzerland, Ukraine and also on Bornholm<sup>110</sup>. This European type TBE, transmitted by *Ixodes ricinus* ticks, is seasonal with peak incidence from May to September. Tickborne encephalitis is a biphasic illness. An initial viraemic phase, with headache, fever, malaise, is followed by an asymptomatic period lasting about 8 days. Presentation of CNS symptoms then follows in 20 to 30% of infected individuals<sup>111</sup>.

The Far Eastern type of TBE, Omsk haemorrhagic fever, occurs mainly in muskrat trappers in the Siberian regions of Omsk, Novosibirsk, Kurgan and Tjumen. It is categorised as a hazard group 4 pathogen and has caused several cases in laboratory workers. In the field it may be transmitted to man directly from muskrats and virus-contaminated water as well as via the ticks *Dermacentor reticulatus*, *Dermacentor marginatus*, *Ixodes persulcatus*. It begins with chills, fever, headache, pain in lower and upper extremities, and severe prostration. There is usually a rash on the soft palate, as well as cervical lymphadenopathy and conjunctival injection, progressing to CNS abnormalities in 1 to 2 weeks. In severe cases haemorrhage occurs due to thrombocytopenia, with a death rate of 1 to 10%.

##### 4.27.1 Diagnosis of tickborne encephalitis

IgG ELISA assays and haemagglutination-inhibition assays are useful screening tests but specificity can be low due to cross reactivity among flaviviruses (including vaccination against yellow fever)<sup>112</sup>. Neutralisation assays may be required to confirm the flavivirus responsible for antibody reactivity<sup>113,114</sup>.

Intrathecal production of IgG against TBE is somewhat unreliable for diagnosis<sup>115</sup>. A positive CSF IgM is reliable for diagnosis of TBE. PCR and virus culture in Vero cells are positive early in the infection from blood but are not usually present when CNS features develop. Serum IgM antibodies may persist for some 10 months<sup>111</sup>.

#### 4.28 Varicella Zoster

In some series VZ virus is more common than herpes simplex or enteroviruses in suspected viral CNS infection, for meningitis, myelitis and encephalitis<sup>116</sup>.

VZ infections often appear without a rash. Varicella zoster encephalitis is a vasculopathy<sup>112</sup>.

Large vessel VZ encephalitis affects individuals who are immunocompetent and results in stroke weeks or months after zoster infection. Small vessel VZ encephalitis affects patients who are immunocompromised eg patients suffering from AIDS, transplant recipients, and may follow zoster infection weeks or months later or may occur in the absence of any skin lesions. A subacute or chronic progressive encephalitis ensues characterised by headache,

fever, vomiting, mental changes leading on to hemiplegia, aphasia, visual field deficits and death<sup>117</sup>.

Demyelinating lesions may be seen in the white matter of the MRI scan of the brain<sup>118</sup>.

#### 4.28.1 Diagnosis of Varicella-Zoster

Both PCR on CSF and antibody testing for VZ IgG and IgM (using ELISA or similar immunoassays) on both serum and CSF should be carried out in cases of CNS disease believed to be associated with VZ<sup>117</sup>.

Similar tests should also be done to exclude herpes simplex. In encephalitis associated with chickenpox VZ PCR is more likely to be positive in children, in 33% of cases. In this group 20% had VZ IgM detected in CSF, 66% had serum VZ IgM, and only 5% had evidence of intrathecal VZ IgG production<sup>118</sup>.

Intrathecal production of VZ IgG gave a greater diagnostic yield in encephalitis associated with shingles (56%) than did PCR (34%), with IgM detectable in serum in 36% of patients<sup>69</sup>. Positive findings in serum alone, such as IgM or rising antibody titres, can support but cannot confirm a diagnosis of CNS VZ infection. Similarly detection of virus by culture, IF, EM or PCR from skin sites or saliva<sup>119</sup> can lend support to the diagnosis.

#### 4.29 West Nile Fever (WN)

This flavivirus infection is very widely distributed across Africa, Asia, the Middle East and Europe<sup>120</sup>.

Wild birds, which have a prolonged viraemia, are the principal hosts. Large outbreaks have occurred in recent years in Romania, Israel, and Russia. Infection has spread to the USA, where cases were first recognised in New York in 1999<sup>121</sup>. The virus is now disseminated widely across the USA. Most human infections are subclinical. Symptoms occur in about 20% of cases, typically high fever of sudden onset, headache, fatigue, arthralgia, myalgia and rash. These last 3 to 6 days. Although severe neurological disease occurs in only about 1 in 150 cases<sup>120</sup>, principally in those over 50 years of age, CNS disease is the most common reason for hospitalisation with WN infection. Among those hospitalised in the Israeli epidemic in the year 2000 58% of patients had encephalitis, 19% meningitis and 14% died. All patients were elderly. The fatality rate reached 29% in those over 70 years of age<sup>122</sup>. It is interesting to note that outbreaks in horses are characterised by high death rates from neurological disease<sup>123</sup>.

The virus is transmitted by mosquitoes, mainly bird-feeding species of the genus *Culex*. Transmission appears to have occurred to three of four recipients of organs transplanted from an infected organ donor<sup>124</sup>.

#### 4.29.1 Diagnosis of West Nile Fever

Detection of West Nile Virus IgM antibody by capture ELISA (MAC-ELISA)<sup>125</sup> is the preferred test for CSF and for serum. IgM antibody is detectable in 90% of sera taken within 8 days of onset of symptoms and in 95% of CSF samples from infected individuals<sup>121</sup>. IgM to flaviviruses can persist for over 6 months, so in endemic areas it may be important to confirm recent infection by comparing neutralising antibody levels on paired sera<sup>121</sup>. Cross reactivity occurs with other flaviviruses including St Louis encephalitis, yellow fever and Japanese encephalitis. The plaque reduction neutralisation assay may be needed to differentiate infection or vaccination with other flaviviruses. The positive-negative absorbance ratio can however often give satisfactory differentiation on a single serum<sup>126</sup>. Virus culture gives low yields and PCR for West Nile virus is positive in less than 55% on CSF and 10% of serum samples<sup>121</sup>.

## 5 SAFETY CONSIDERATIONS

### 5.1 Safe handling of specimens

Laboratories must take suitable safety precautions when handling CSF specimens. Basic precautions should be taken to minimise exposure to CSF samples, especially if the

possibility of a transmissible spongiform encephalopathy cannot be excluded. Laboratory policies that take into account the local risk assessments for transmissible spongiform encephalopathy may dictate that the use of a microbiological safety cabinet should be used when dispensing the specimen. Refer to Guidance Note QSOP 42 - Microbiological Examination of CSF that contain agents of Spongiform Encephalopathies.

For additional details refer to “Transmissible spongiform encephalopathy agents: safe working and the prevention of infection. Guidance from the Advisory Committee on Dangerous Pathogens and the Spongiform Encephalopathy Advisory Committee”, is available on [www.doh.gov.uk/cjd/tseguidance](http://www.doh.gov.uk/cjd/tseguidance).

Viruses associated with viral encephalitis fall into Hazard Groups 2, 3, and 4. Of viruses endemic to the UK most are in Hazard Group 2, but higher levels of containment must be considered when encephalitis has been acquired abroad. Refer to Table II and to “Categorisation of biological agents according to hazard and categories of containment HSE Books.4<sup>th</sup> edition,1995”.

#### **THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS**

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 19 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

**Table II. Classification of viral agents of CNS disease**

Hazard Group 2	Hazard Group 3	Hazard Group 4
Enteroviruses	LCM	Nipah
Herpesviruses other than herpes simiae	Flaviruses including dengue, Japanese encephalitis, Murray Valley encephalitis, St Louis encephalitis, West Nile fever	Russian Spring-Summer encephalitis
Mumps	Herpes simiae	Omsk haemorrhagic fever
	HIV	
	Rabies	
	Alphaviruses including Eastern equine encephalitis, Western equine encephalitis, Venezuelan encephalitis	
	Tickborne encephalitis, louping ill	

## 5.2 Specimen collection transport and storage

Samples should ideally be transported as soon as possible, usually to a Specialist Virology Centre. Packaging should conform to the UN 650 requirements and IATA Dangerous Goods Regulations (9<sup>th</sup> edition) if posted or sent via Hays DX.

### 5.2.1 Cerebrospinal fluid

CSF should be collected by lumbar tap into sterile containers. It is essential that CSF is sent for cell count, bacteriology studies and biochemistry as well as for virology. CSF may be stored at +4°C if delays in processing for virus culture or viral PCR will be less than 24 hours. If greater delays are likely CSF should be frozen at –80°C, while clotted blood samples should be separated and the serum stored at –20°C. Refer to Standard Operating Procedure BSOP 27 - Investigation of cerebrospinal fluid.

### 5.2.2 Serum

Clotted blood 7 to 10 mL, should be collected by venepuncture for serology, particularly IgM assays. Serum can also be used for enterovirus PCR and parvovirus B19 (erythrovirus) PCR.

### 5.2.3 Plasma

EDTA-anticoagulated blood 5mL should be collected into sterile tubes by venepuncture. EDTA-anticoagulated whole blood is suitable for PCR assays, especially herpesvirus assays, eg HSV in neonatal CNS disease, CMV and EBV in patients who are immunocompromised.

### 5.2.4 Brain biopsy

#### THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 20 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

Brain specimens should be collected unfixed into a sterile container. Brain smears can be used for viral antigen detection by immunofluorescent antibody staining, and for electron microscopy with negative staining. Emulsified brain tissue is suitable for tissue culture and after proteinase K treatment for PCR.

### **5.2.5 Swabs**

Swabs when taken should be put into virus transport medium. Suitable swabs include throat swabs for a range of virus cultures and PCR.

### **5.2.6 Faeces**

Faeces should be collected for enterovirus culture into clean containers.

### **5.2.7 Urine**

Ten to 20 mL of urine should be collected into sterile containers (without preservatives) for mumps virus culture and mumps PCR.

## **5.3 Referral to Reference Laboratories**

Samples of CSF and serum from patients suspected of having arbovirus infection should be referred to the Diagnosis and Reference Division, Health Protection Agency Centre for Applied Microbiology and Research (CAMR), Porton Down, Wiltshire. At least 500 µL of serum should be sent for antibody tests. Urine, CSF, and throat swab in virus transport medium for mumps virus PCR should be referred to the Enteric, Respiratory and Neurological Virus Laboratory, Health Protection Agency Specialist and Reference Microbiology Division, Colindale, London.

## **5.4 Reporting to the HPA (Local and Regional Services and CDSC Centre for Infections)<sup>127</sup>**

Any virus causing meningitis or encephalitis, as well as cases of HIV, measles, mumps, rabies and viral haemorrhagic fevers should be reported to the HPA Communicable Disease Surveillance Centre, Colindale. Guidance on reporting can be found in the May 2001 document "Reporting to the Communicable Disease Surveillance Centre. A Reference for Laboratories". This can be accessed via the internet at: [www.hpa.org.uk/infections/about/surveillance/CDSC\\_Reporting\\_doc.pdf](http://www.hpa.org.uk/infections/about/surveillance/CDSC_Reporting_doc.pdf).

Acute encephalitis, acute viral meningitis, as well as measles, mumps, rabies, and viral haemorrhagic fevers are all notifiable diseases under the Public Health (Infectious Diseases) Regulations 1988 and should be notified to the Proper Officer, usually the local CCDC.

## **CONTACT**

Any queries relating to this Guidance Note should be made to:

Dr Ken Mutton, Consultant Virologist, Manchester Royal Infirmary  
Sam Gillanders, Head of Standards Unit, Evaluations & Standards Laboratory.

# REFERENCES

1. Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient-identifiable information. London. December 1997.
2. McKinney RE, Jr., Katz SL, Wilfert CM. Chronic enteroviral meningoencephalitis in agammaglobulinemic patients. *Rev Infect Dis* 1987;9:334-56.
3. Rotbart HA, Kinsella JP, Wasserman RL. Persistent enterovirus infection in culture-negative meningoencephalitis: demonstration by enzymatic RNA amplification. *J Infect Dis* 1990;161:787-91.
4. Dunn JJ, Romero JR, Wasserman R, Rotbart HA. Stable enterovirus 5' nontranslated region over a 7-year period in a patient with agammaglobulinemia and chronic infection. *J Infect Dis* 2000;182:298-301.
5. Schacker T, Collier AC, Hughes J, Shea T, Corey L. Clinical and epidemiologic features of primary HIV infection. *Ann Intern Med* 1996;125:257-64.
6. Tedder DG, Ashley R, Tyler KL, Levin MJ. Herpes simplex virus infection as a cause of benign recurrent lymphocytic meningitis. *Ann Intern Med* 1994;121:334-8.
7. Henquell C, Chambon M, Bailly JL, Alcaraz S, De Champs C, Archimbaud C, et al. Prospective analysis of 61 cases of enteroviral meningitis: interest of systematic genome detection in cerebrospinal fluid irrespective of cytologic examination results. *J Clin Virol* 2001;21:29-35.
8. Studahl M, Bergstrom T, Hagberg L. Acute viral encephalitis in adults--a prospective study. *Scand J Infect Dis* 1998;30:215-20.
9. Valero N, Henriquez R, Hernandez C, Pomedá O, Romero M, Urdaneta F, et al. Viral agents in patients with infectious processes of the central nervous system]. *Invest Clin* 2001;42:255-67.
10. Srey VH, Sadones H, Ong S, Mam M, Yim C, Sor S, et al. Etiology of encephalitis syndrome among hospitalized children and adults in Takeo, Cambodia, 1999-2000. *Am J Trop Med Hyg* 2002;66:200-7.
11. Henderson DA, Inglesby TV, Bartlett JG, Ascher MS, Eitzen E, Jahrling PB, et al. Smallpox as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. *JAMA* 1999;281:2127-37.
12. Frey TK. Neurological aspects of rubella virus infection. *Intervirology* 1997;40:167-75.
13. Pfister HW, Lorenzl S, Yousry T. Neuroradiographic manifestations of encephalitis. *N Engl J Med* 1997;337:1393-4.
14. McJunkin JE, los Reyes EC, Irazuzta JE, Caceres MJ, Khan RR, Minnich LL, et al. La Crosse encephalitis in children. *N Engl J Med* 2001;344:801-7.
15. De T, X, Heron B, Lebon P, Ponsot G, Rozenberg F. Limits of early diagnosis of herpes simplex encephalitis in children: a retrospective study of 38 cases. *Clin Infect Dis* 2003;36:1335-9.
16. Klapper PE, Cleator GM, Dennett C, Lewis AG. Diagnosis of herpes encephalitis via Southern blotting of cerebrospinal fluid DNA amplified by polymerase chain reaction. *J Med Virol* 1990;32:261-4.
17. Jeffery KJ, Read SJ, Peto TE, Mayon-White RT, Bangham CR. Diagnosis of viral infections of the central nervous system: clinical interpretation of PCR results. *Lancet* 1997;349:313-7.

## THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 22 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

18. Cinque P, Bossolasco S, Lundkvist A. Molecular analysis of cerebrospinal fluid in viral diseases of the central nervous system. *J Clin Virol* 2003;26:1-28.
19. Yoshikawa T, Asano Y. Central nervous system complications in human herpesvirus-6 infection. *Brain Dev* 2000;22:307-14.
20. Norrby E, Kristensson K. Measles virus in the brain. *Brain Res Bull* 1997;44:213-20.
21. Kojaoghlanian T, Flomenberg P, Horwitz MS. The impact of adenovirus infection on the immunocompromised host. *Rev Med Virol* 2003;13:155-71.
22. Zagardo MT, Shanholtz CB, Zoarski GH, Rothman MI. Rhombencephalitis caused by adenovirus: MR imaging appearance. *AJNR Am J Neuroradiol* 1998;19:1901-3.
23. Sakata H, Taketazu G, Nagaya K, Shirai M, Sugai R, Ikegami K, et al. Outbreak of severe infection due to adenovirus type 7 in a paediatric ward in Japan. *J Hosp Infect* 1998;39:207-11.
24. Straussberg R, Harel L, Levy Y, Amir J. A syndrome of transient encephalopathy associated with adenovirus infection. *Pediatrics* 2001;107:E69.
25. Kojaoghlanian T, Flomenberg P, Horwitz MS. The impact of adenovirus infection on the immunocompromised host. *Rev Med Virol* 2003;13:155-71.
26. Lankester AC, van Tol MJ, Claas EC, Vossen JM, Kroes AC. Quantification of adenovirus DNA in plasma for management of infection in stem cell graft recipients. *Clin Infect Dis* 2002;34:864-7.
27. Arribas JR, Storch GA, Clifford DB, Tselis AC. Cytomegalovirus encephalitis. *Ann Intern Med* 1996;125:577-87.
28. Ribalta T, Martinez AJ, Jares P, Muntane J, Miquel R, Claramonte X, et al. Presence of occult cytomegalovirus infection in the brain after orthotopic liver transplantation. An autopsy study of 83 cases. *Virchows Arch* 2002;440:166-71.
29. Devetag FC, Boscarolo L. Cytomegalovirus meningoencephalitis with paroxysmal course in immunocompetent adults: a new nosographical entity. Clinical, diagnostic and therapeutic correlations, and pathogenetic hypothesis. *Eur Neurol* 2000;44:242-7.
30. Bestetti A, Pierotti C, Terreni M, Zappa A, Vago L, Lazzarin A, et al. Comparison of three nucleic acid amplification assays of cerebrospinal fluid for diagnosis of cytomegalovirus encephalitis. *J Clin Microbiol* 2001;39:1148-51.
31. Solomon T, Dung NM, Vaughn DW, Kneen R, Thao LT, Raengsakulrach B, et al. Neurological manifestations of dengue infection. *Lancet* 2000;355:1053-9.
32. Vazquez S, Lemos G, Pupo M, Ganzon O, Palenzuela D, Indart A, et al. Diagnosis of dengue virus infection by the visual and simple AuBioDOT immunoglobulin M capture system. *Clin Diagn Lab Immunol* 2003;10:1074-7.
33. Teichmann D, Gobels K, Niedrig M, Sim-Brandenburg JW, Lage-Stehr J, Grobusch MP. Virus isolation for diagnosing dengue virus infections in returning travelers. *Eur J Clin Microbiol Infect Dis* 2003;22:697-700.
34. Beaman MH, Wesselingh SL. 4: Acute community-acquired meningitis and encephalitis. *Med J Aust* 2002;176:389-96.
35. Minor PD, Muir P. Enteroviruses. In: Zuckerman AJ, Banatvala JE, Pattison JR, Griffiths PD, Schoub BD, editors. *Principles and Practice of Clinical Virology*. 5<sup>th</sup> ed. Chichester: John Wiley & Sons Ltd; 2004. p. 467-89.

**THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS**

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 23 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

36. Huang CC, Liu CC, Chang YC, Chen CY, Wang ST, Yeh TF. Neurologic complications in children with enterovirus 71 infection. *N Engl J Med* 1999;341:936-42.
37. Ho M, Chen ER, Hsu KH, Twu SJ, Chen KT, Tsai SF, et al. An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. *N Engl J Med* 1999;341:929-35.
38. Gilbert GL, Dickson KE, Waters MJ, Kennett ML, Land SA, Sneddon M. Outbreak of enterovirus 71 infection in Victoria, Australia, with a high incidence of neurologic involvement. *Pediatr Infect Dis J* 1988;7:484-8.
39. Stanway G, Joki-Korpela P, Hyypia T. Human parechoviruses--biology and clinical significance. *Rev Med Virol* 2000;10:57-69.
40. Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarek EB, et al. Development and evaluation of a 'real-time' RT-PCR for the detection of enterovirus and parechovirus RNA in CSF and throat swab samples. *J Med Virol* 2002;67:555-62.
41. Yerly S, Gervaix A, Simonet V, Caflisch M, Perrin L, Wunderli W. Rapid and sensitive detection of enteroviruses in specimens from patients with aseptic meningitis. *J Clin Microbiol* 1996;34:199-201.
42. Lina B, Pozzetto B, Andreoletti L, Beguier E, Bourlet T, Dussaix E, et al. Multicenter evaluating of a commercially available PCR assay for diagnosing enterovirus infection in a panel of cerebrospinal fluid specimens. *J Clin Microbiol* 1996;34:3002-6.
43. Cohen JL. Epstein-Barr virus infection. *N Engl J Med* 2000;343:481-92.
44. Hausler M, Ramaekers VT, Doenges M, Schweizer K, Ritter K, Schaade L. Neurological complications of acute and persistent Epstein-Barr virus infection in paediatric patients. *J Med Virol* 2002;68:253-63.
45. Weinberg A, Li S, Palmer M, Tyler KL. Quantitative CSF PCR in Epstein-Barr virus infections of the central nervous system. *Ann Neurol* 2002;52:543-8.
46. Arvin A, Whitley RJ. Herpes simplex virus infections. In: Remington JS, Klein JO, editors. *Infectious Diseases of the Fetus and Newborn Infant*. 5<sup>th</sup> ed. Philadelphia: WB Saunders Company; 2001. p. 425-46.
47. Corey L, Whitley RJ, Stone EF, Mohan K. Difference between herpes simplex virus type 1 and type 2 neonatal encephalitis in neurological outcome. *Lancet* 1988;1:1-4.
48. Kurtz J, Anslow P. Infantile herpes simplex encephalitis: diagnostic features and differentiation from non-accidental injury. *J Infect* 2003;46:12-6.
49. O'Sullivan CE, Aksamit AJ, Harrington JR, Harmsen WS, Mitchell PS, Patel R. Clinical spectrum and laboratory characteristics associated with detection of herpes simplex virus DNA in cerebrospinal fluid. *Mayo Clin Proc* 2003;78:1347-52.
50. Mommeja-Marin H, Lafaurie M, Scieux C, Galicier L, Oksenhendler E, Molina JM. Herpes simplex virus type 2 as a cause of severe meningitis in immunocompromised adults. *Clin Infect Dis* 2003;37:1527-33.
51. Dennett C, Cleator GM, Klapper PE. HSV-1 and HSV-2 in herpes simplex encephalitis: a study of sixty-four cases in the United Kingdom. *J Med Virol* 1997;53:1-3.
52. Cleator GM, Klapper PE. The herpesviridae. In: Zuckerman AJ, Banatvala JE, Pattison JR, Griffiths PD, Schoub BD, editors. *Principles and Practice of Clinical Virology*. 5<sup>th</sup> ed. Chichester: John Wiley & Sons Ltd; 2004. p. 23-6.

**THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS**

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 24 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

53. Raschilas F, Wolff M, Delatour F, Chaffaut C, De Broucker T, Chevret S, et al. Outcome of and prognostic factors for herpes simplex encephalitis in adult patients: results of a multicenter study. *Clin Infect Dis* 2002;35:254-60.
54. Cinque P, Cleator GM, Weber T, Monteyne P, Sindic CJ, van Loon AM. The role of laboratory investigation in the diagnosis and management of patients with suspected herpes simplex encephalitis: a consensus report. The EU Concerted Action on Virus Meningitis and Encephalitis. *J Neurol Neurosurg Psychiatry* 1996;61:339-45.
55. Weil AA, Glaser CA, Amad Z, Forghani B. Patients with suspected herpes simplex encephalitis: rethinking an initial negative polymerase chain reaction result. *Clin Infect Dis* 2002;34:1154-7.
56. Domingues RB, Lakeman FD, Mayo MS, Whitley RJ. Application of competitive PCR to cerebrospinal fluid samples from patients with herpes simplex encephalitis. *J Clin Microbiol* 1998;36:2229-34.
57. Redington JJ, Tyler KL. Viral infections of the nervous system, 2002: update on diagnosis and treatment. *Arch Neurol* 2002;59:712-8.
58. Kimberlin DW. Advances in the treatment of neonatal herpes simplex infections. *Rev Med Virol* 2001;11:157-63.
59. Malm G, Forsgren M. Neonatal herpes simplex virus infections: HSV DNA in cerebrospinal fluid and serum. *Arch Dis Child Fetal Neonatal Ed* 1999;81:F24-F29.
60. Lewensohn-Fuchs I, Osterwall P, Forsgren M, Malm G. Detection of herpes simplex virus DNA in dried blood spots making a retrospective diagnosis possible. *J Clin Virol* 2003;26:39-48.
61. . Fatal Cercopithecine herpesvirus 1 (B virus) infection following a mucocutaneous exposure and interim recommendations for worker protection. *MMWR Morb Mortal Wkly Rep* 1998;47:1073-6, 1083.
62. Whitley RJ, Gnann JW. Viral encephalitis: familiar infections and emerging pathogens. *Lancet* 2002;359:507-13.
63. Perelygina L, Patrusheva I, Manes N, Wildes MJ, Krug P, Hilliard JK. Quantitative real-time PCR for detection of monkey B virus (Cercopithecine herpesvirus 1) in clinical samples. *J Virol Methods* 2003;109:245-51.
64. Hall CB, Long CE, Schnabel KC, Caserta MT, McIntyre KM, Costanzo MA, et al. Human herpesvirus-6 infection in children. A prospective study of complications and reactivation. *N Engl J Med* 1994;331:432-8.
65. McCullers JA, Lakeman FD, Whitley RJ. Human herpesvirus 6 is associated with focal encephalitis. *Clin Infect Dis* 1995;21:571-6.
66. Caserta MT, Mock DJ, Dewhurst S. Human herpesvirus 6. *Clin Infect Dis* 2001;33:829-33.
67. Singh N, Paterson DL. Encephalitis caused by human herpesvirus-6 in transplant recipients: relevance of a novel neurotropic virus. *Transplantation* 2000;69:2474-9.
68. McCarthy M. Newer viral encephalitides. *Neurologist* 2003;9:189-99.
69. Cuomo L, Trivedi P, Cardillo MR, Gagliardi FM, Vecchione A, Caruso R, et al. Human herpesvirus 6 infection in neoplastic and normal brain tissue. *J Med Virol* 2001;63:45-51.
70. Bell JE. The neuropathology of adult HIV infection. *Rev Neurol (Paris)* 1998;154:816-29.

**THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS**

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 25 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

71. Bell JE, Brettle RP, Chiswick A, Simmonds P. HIV encephalitis, proviral load and dementia in drug users and homosexuals with AIDS. Effect of neocortical involvement. *Brain* 1998;121:2043-52.
72. Masliah E, DeTeresa RM, Mallory ME, Hansen LA. Changes in pathological findings at autopsy in AIDS cases for the last 15 years. *AIDS* 2000;14:69-74.
73. Cinque P, Scarpellini P, Vago L, Linde A, Lazzarin A. Diagnosis of central nervous system complications in HIV-infected patients: cerebrospinal fluid analysis by the polymerase chain reaction. *AIDS* 1997;11:1-17.
74. McCullers JA, Facchini S, Chesney PJ, Webster RG. Influenza B virus encephalitis. *Clin Infect Dis* 1999;28:898-900.
75. Morishima T, Togashi T, Yokota S, Okuno Y, Miyazaki C, Tashiro M, et al. Encephalitis and encephalopathy associated with an influenza epidemic in Japan. *Clin Infect Dis* 2002;35:512-7.
76. Studahl M. Influenza virus and CNS manifestations. *J Clin Virol* 2003;28:225-32.
77. Hanna JN, Ritchie SA, Phillips DA, Shield J, Bailey MC, Mackenzie JS, et al. An outbreak of Japanese encephalitis in the Torres Strait, Australia, 1995. *Med J Aust* 1996;165:256-60.
78. Tiroumourougane SV, Raghava P, Srinivasan S. Japanese viral encephalitis. *Postgrad Med J* 2002;78:205-15.
79. Pandey B, Yamamoto A, Morita K, Kurosawa Y, Rai S, Adhikari S, et al. Serodiagnosis of Japanese encephalitis among Nepalese patients by the particle agglutination assay. *Epidemiol Infect* 2003;131:881-5.
80. Berger JR, Pall L, Lanska D, Whiteman M. Progressive multifocal leukoencephalopathy in patients with HIV infection. *J Neurovirol* 1998;4:59-68.
81. Gibson PE, Knowles WA, Hand JF, Brown DW. Detection of JC virus DNA in the cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy. *J Med Virol* 1993;39:278-81.
82. Weber T, Klapper PE, Cleator GM, Bodemer M, Luke W, Knowles W, et al. Polymerase chain reaction for detection of JC virus DNA in cerebrospinal fluid: a quality control study. European Union Concerted Action on Viral Meningitis and Encephalitis. *J Virol Methods* 1997;69:231-7.
83. Sindic CJ, Van Antwerpen MP, Goffette S. Clinical relevance of polymerase chain reaction (PCR) assays and antigen-driven immunoblots for the diagnosis of neurological infectious diseases. *Brain Res Bull* 2003;61:299-308.
84. Davidson MM, Williams H, Macleod JA. Louping ill in man: a forgotten disease. *J Infect* 1991;23:241-9.
85. Howard CR. Arenaviruses. In: Zuckerman AJ, Banatvala JE, Pattison JR, Griffiths PD, Schoub BD, editors. *Principles and Practice of Clinical Virology*. 5<sup>th</sup> ed. Chichester: John Wiley & Sons Ltd; 2004. p. 589-609.
86. Park JY, Peters CJ, Rollin PE, Ksiazek TG, Gray B, Waites KB, et al. Development of a reverse transcription-polymerase chain reaction assay for diagnosis of lymphocytic choriomeningitis virus infection and its use in a prospective surveillance study. *J Med Virol* 1997;51:107-14.
87. Hosoya M, Shigeta S, Mori S, Tomoda A, Shiraishi S, Miike T, et al. High-dose intravenous ribavirin therapy for subacute sclerosing panencephalitis. *Antimicrob Agents Chemother* 2001;45:943-5.

**THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS**

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 26 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

88. Tipples GA, Hamkar R, Mohktari-Azad T, Gray M, Parkyn G, Head C, et al. Assessment of immunoglobulin M enzyme immunoassays for diagnosis of measles. *J Clin Microbiol* 2003;41:4790-2.
89. van Binnendijk RS, van den HS, van den KH, Kohl RH, Woonink F, Berbers GA, et al. Evaluation of serological and virological tests in the diagnosis of clinical and subclinical measles virus infections during an outbreak of measles in The Netherlands. *J Infect Dis* 2003;188:898-903.
90. Jenson HB, Leach CT. Mumps. In: Jenson HB, Baltimore RS, editors. *Pediatric Infectious Diseases Principles and Practice*. 2<sup>nd</sup> ed. Philadelphia: WB Saunders; 2002. p. 420-5.
91. Koskiniemi M, Donner M, Pettay O. Clinical appearance and outcome in mumps encephalitis in children. *Acta Paediatr Scand* 1983;72:603-9.
92. Reina J, Ballesteros F, Ruiz dG, Munar M, Mari M. Comparison between indirect immunofluorescence assay and shell vial culture for detection of mumps virus from clinical samples. *J Clin Microbiol* 2003;41:5186-7.
93. Jin L, Vyse A, Brown DW. The role of RT-PCR assay of oral fluid for diagnosis and surveillance of measles, mumps and rubella. *Bull World Health Organ* 2002;80:76-7.
94. Nagai T, Nakayama T. Mumps vaccine virus genome is present in throat swabs obtained from uncomplicated healthy recipients. *Vaccine* 2001;19:1353-5.
95. Cordova SP, Smith DW, Broom AK, Lindsay MD, Dowse GK, Beers MY. Murray Valley encephalitis in Western Australia in 2000, with evidence of southerly spread. *Commun Dis Intell* 2000;24:368-72.
96. National arbovirus and malaria surveillance website.  
<http://www.health.gov.au/internet/wcms/Publishing.nsf/Content/health-arbovirus-pdf-fsmurrayvalley.htm>.
97. Chua KB, Koh CL, Hooi PS, Wee KF, Khong JH, Chua BH, et al. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect* 2002;4:145-51.
98. Lam SK, Chua KB. Nipah virus encephalitis outbreak in Malaysia. *Clin Infect Dis* 2002;34 Suppl 2:S48-S51.
99. Goh KJ, Tan CT, Chew NK, Tan PS, Kamarulzaman A, Sarji SA, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med* 2000;342:1229-35.
100. Chong HT, Kamarulzaman A, Tan CT, Goh KJ, Thayaparan T, Kunjapan SR, et al. Treatment of acute Nipah encephalitis with ribavirin. *Ann Neurol* 2001;49:810-3.
101. McCarthy M. Newer viral encephalitides. *Neurologist* 2003;9:189-99.
102. Barah F, Vallely PJ, Chiswick ML, Cleator GM, Kerr JR. Association of human parvovirus B19 infection with acute meningoencephalitis. *Lancet* 2001;358:729-30.
103. Cassinotti P, Schultze D, Schlageter P, Chevili S, Siegl G. Persistent human parvovirus B19 infection following an acute infection with meningitis in an immunocompetent patient. *Eur J Clin Microbiol Infect Dis* 1993;12:701-4.
104. Druschky K, Walloch J, Heckmann J, Schmidt B, Stefan H, Neundorfer B. Chronic parvovirus B-19 meningoencephalitis with additional detection of Epstein-Barr virus DNA in the cerebrospinal fluid of an immunocompetent patient. *J Neurovirol* 2000;6:418-22.

**THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS**

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 27 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

105. Rupprecht CE, Hanlon CA, Hemachudha T. Rabies re-examined. *Lancet Infect Dis* 2002;2:327-43.
106. de Mattos CA., de Mattos CC, Rupprecht CE. Rhabdoviruses. In: Knipe DM, Howley PM, editors. *Fields Virology*. 4<sup>th</sup> ed. Vol 1. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1245-77.
107. Black EM, Lowings JP, Smith J, Heaton PR, McElhinney LM. A rapid RT-PCR method to differentiate six established genotypes of rabies and rabies-related viruses using TaqMan technology. *J Virol Methods* 2002;105:25-35.
108. Smith J, McElhinney L, Parsons G. Case report: Rapid ante-mortem diagnosis of a human case of rabies imported into the UK from the Philippines. *J Med Virol* 2002;69:150-5.
109. Lynch M, Lee B, Azimi P, Gentsch J, Glaser C, Gilliam S, et al. Rotavirus and central nervous system symptoms: cause or contaminant? Case reports and review. *Clin Infect Dis* 2001;33:932-8.
110. Laursen K, Knudsen JD. Tick-borne encephalitis: a retrospective study of clinical cases in Bornholm, Denmark. *Scand J Infect Dis* 2003;35:354-7.
111. Dumpis U, Crook D, Oksi J. Tick-borne encephalitis. *Clin Infect Dis* 1999;28:882-90.
112. Niedrig M, Vaisviliene D, Teichmann A, Klockmann U, Biel SS. Comparison of six different commercial IgG-ELISA kits for the detection of TBEV-antibodies. *J Clin Virol* 2001;20:179-82.
113. Dobler G, Treib J, Kiessig ST, Blohn WV, Frosner G, Haass A. Diagnosis of tick-borne encephalitis: evaluation of sera with borderline titers with the TBE-ELISA. *Infection* 1996;24:405-6.
114. Jaaskelainen A, Han X, Niedrig M, Vaheri A, Vapalahti O. Diagnosis of tick-borne encephalitis by a mu-capture immunoglobulin M-enzyme immunoassay based on secreted recombinant antigen produced in insect cells. *J Clin Microbiol* 2003;41:4336-42.
115. Treib J, Woessner R, Dobler G, Fernandez A, Holzer G, Schimrigk K. Clinical value of specific intrathecal production of antibodies. *Acta Virol* 1997;41:27-30.
116. Koskiniemi M, Rantalaiho T, Piiparinen H, von Bonsdorff CH, Farkkila M, Jarvinen A, et al. Infections of the central nervous system of suspected viral origin: a collaborative study from Finland. *J Neurovirol* 2001;7:400-8.
117. Gilden DH, Kleinschmidt-DeMasters BK, LaGuardia JJ, Mahalingam R, Cohrs RJ. Neurologic complications of the reactivation of varicella-zoster virus. *N Engl J Med* 2000;342:635-45.
118. Koskiniemi M, Piiparinen H, Rantalaiho T, Eranko P, Farkkila M, Raiha K, et al. Acute central nervous system complications in varicella zoster virus infections. *J Clin Virol* 2002;25:293-301.
119. Furuta Y, Ohtani F, Sawa H, Fukuda S, Inuyama Y. Quantitation of varicella-zoster virus DNA in patients with Ramsay Hunt syndrome and zoster sine herpette. *J Clin Microbiol* 2001;39:2856-9.
120. Hubalek Z, Halouzka J. West Nile fever--a reemerging mosquito-borne viral disease in Europe. *Emerg Infect Dis* 1999;5:643-50.
121. Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. *Ann Intern Med* 2002;137:173-9.
122. Chowers MY, Lang R, Nassar F, Ben David D, Giladi M, Rubinshtein E, et al. Clinical characteristics of the West Nile fever outbreak, Israel, 2000. *Emerg Infect Dis* 2001;7:675-8.

**THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS**

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 28 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

123. Durand B, Chevalier V, Pouillot R, Labie J, Marendat I, Murgue B, et al. West Nile virus outbreak in horses, southern France, 2000: results of a serosurvey. *Emerg Infect Dis* 2002;8:777-82.
124. Centers for Disease Control and Prevention. West Nile virus infection in organ donor and transplant recipients--Georgia and Florida, 2002. *MMWR Morb Mortal Wkly Rep* 2002;51:790.
125. Martin DA, Muth DA, Brown T, Johnson AJ, Karabatsos N, Roehrig JT. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. *J Clin Microbiol* 2000;38:1823-6.
126. Martin DA, Biggerstaff BJ, Allen B, Johnson AJ, Lanciotti RS, Roehrig JT. Use of immunoglobulin m cross-reactions in differential diagnosis of human flaviviral encephalitis infections in the United States. *Clin Diagn Lab Immunol* 2002;9:544-9.
127. PHLS C. Reporting to the PHLS Communicable Disease Surveillance Centre: a reference for laboratories. May. 2001.

**THE INVESTIGATION OF VIRAL ENCEPHALITIS AND MENINGITIS**

Issue no: 2.1 Issue date: 22.08.05 Issued by: Standards Unit, Evaluations & Standards Laboratory Page 29 of 29

Reference no: QSOP 48i2.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

[www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk)

Email: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)