

NATIONAL STANDARD METHOD

# ISOLATION OF HUMAN HERPES VIRUSES (EXCLUDING HERPES GENITALIS)

VSOP 23

Issued by Standards Unit, Department for Evaluations, Standards and Training  
**Centre for Infections**



UK Clinical Virology Network



Association of Medical Microbiologists  
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## ISOLATION OF HUMAN HERPES VIRUSES (EXCLUDING HERPES GENITALIS)

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# STATUS OF NATIONAL STANDARD METHODS

National Standard Method, 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.

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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).

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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).

The reader is informed that all taxonomy in this document was correct at time of issue.

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# AMENDMENT PROCEDURE

<b>Controlled document reference</b>	<b>VSOP 23</b>
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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
3/ 09.11.09	2	3	15	<b>References</b>	Reviewed and updated

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# ISOLATION OF HUMAN HERPES VIRUSES (EXCLUDING HERPES GENITALIS)

## SCOPE OF THE DOCUMENT

This National Standard Methods (NSM) will outline the methods used to isolate and identify HSV (isolated from non-genital sites), VZV and CMV utilizing two cell culture techniques. The investigation of HSV from genital sites (herpes genitalis) is outlined in [VSOP 17-Isolation of herpes simplex virus associated with herpes genitalis](#).

## INTRODUCTION

### Background<sup>2</sup>

The Family *Herpesviridae* comprises over 100 viruses that have been isolated from a wide range of vertebrates. The Family *Herpesviridae* is sub-divided into three subfamilies or genera; the *Alpha*, *Beta* and *Gammaherpesviridae* depending upon the host range, duration of the reproductive cycle, cytopathology and the characteristics of latent infection. To date, eight members of the Family have been identified as causing an infection in humans, of which four can be isolated and identified employing conventional monolayer cell culture techniques, namely:

- Herpes simplex virus type 1 (HSV1)
- Herpes simplex virus type 2 (HSV2)
- Varicella-zoster virus (VZV)
- Cytomegalovirus (CMV)

### Herpes Simplex virus (HSV)

HSV is a member of the genus *Alphaherpesviridae*. It is a virus that infects mucosal epithelium and can occur at a variety of sites on the body<sup>3</sup>.

During primary infection the virus enters the sensory nerve endings and is transported to the dorsal root ganglion, specific to the affected epithelium, where latent infection is established. It is from this latent virus that reactivation or recurrence of the infection or the asymptomatic shedding of virus can occur.

HSV1 has traditionally been ascribed to infections "above the belt" but this distinction is less clear and infections with HSV1 can commonly be found infecting all sites. Classically HSV1 causes oral herpes (cold sores), but can be associated with infection of the eye<sup>4</sup> (keratoconjunctivitis and corneal ulceration) and with genital infections, where, in the immunocompetent person, the infection remains localised.

HSV can infect the central nervous system (CNS) to manifest itself as herpes encephalitis. This infection can result from either a primary HSV infection or as a result of reactivation of the virus. The requirement for a speedy diagnosis of herpes encephalitis to enable the prompt administration of specific antiviral drugs is essential. The detection of viral DNA in the cerebrospinal fluid (CSF) utilising the Polymerase Chain Reaction (PCR), rather than the isolation of the virus using cell culture techniques, is the method of choice in diagnosing this manifestation<sup>5,6</sup>.

In the neonate, HSV can present as a generalised infection affecting multiple organs and is often fatal<sup>7</sup>. It has been noted that neonates born to mothers who have acquired a primary HSV infection close to term, have a higher incidence of infection<sup>8</sup>, probably due to the higher viral load associated with primary infection as opposed to an infection associated with the reactivation of the virus.

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The development of antiviral drugs effective against HSV infections has highlighted the requirement for diagnostic methods that are accurate, relatively quick, allows typing of isolates and, if required, allows the determination of antiviral susceptibility. The isolation of HSV in monolayer cell culture fulfils these criteria.

### **Varicella-Zoster virus (VZV)<sup>9</sup>**

VZV is a member of the genus *Alphaherpesviridae*. The primary infection with this virus is varicella (chickenpox) which is usually seen as a childhood illness. After an incubation period ranging from 10 - 21 days the symptoms present as a febrile illness with a macular rash rapidly developing into fluid filled vesicles that crust after a few days and then heal. The lesions characteristically appear in "crops" so that all stages of the rash can be present at the same time and the distribution of the rash over the body is centripetal. The severity of the disease increases in the adult when complications such as pneumonia and encephalitis can occur, especially in patients who are immunocompromised. VZV infection in pregnancy either presents a risk to the developing foetus, *in utero*, or if the infection is contracted close to term, as a severe neonatal infection with symptoms developing 7 - 10 days after birth.

VZV establishes life-long infection of the sensory root ganglion. However, reactivation of the virus takes the form of a unilateral rash, usually limited to a single dermatome. This manifestation of the virus is termed herpes zoster (commonly known as shingles). The condition is most widely seen as a rash in the thoracic region and occasionally the cranial region where eye involvement may occur. Shingles occurs more frequently in the elderly and the immunocompromised. In patients who are immunocompromised, the rash may be associated with a disseminated infection, infecting a wide range of organs.

### **Cytomegalovirus (CMV)<sup>10</sup>**

CMV is a member of the genus *Betaherpesviridae* and demonstrates many characteristics of the genera. It displays marked host cell tropism, is cell associated and replicates more slowly than members of the *Alphaherpesviridae* such as HSV or VZV.

Following a primary infection, as with other members of the *Herpesviridae*, a state of persistent infection or viral latency occurs and virus can be recovered for extended periods from various body fluids such as saliva, urine, semen and breast milk.

In individuals who are immunocompetent, CMV rarely causes a symptomatic infection. It has however been associated with heterophile antibody negative cases of infectious mononucleosis following large scale administration of blood products after major surgery or trauma. Infection with CMV during pregnancy may be asymptomatic but can cause an infection *in utero* giving rise to congenital CMV infection<sup>11</sup>.

CMV infection, in patients who are immunocompromised, either acquired as in AIDS or induced as in allograft recipients, can produce serious consequences accounting for a high degree of morbidity and mortality in these groups. In the latter group infection can arise either as a result of reactivation of endogenous virus or as infection or re-infection by virus present in the transplanted organ. In either instance accurate, speedy diagnosis of the CMV infection is essential to enable anti-viral therapy to be instigated. To this end diagnosis of CMV infection in the patient who is immunocompromised is best effected by the demonstration of viral DNA utilising a PCR technique<sup>12</sup> which is outlined in [VSOP 35-Investigation of Cytomegalovirus infection by Roche Lightcycler PCR](#).

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# 1 SAFETY CONSIDERATIONS<sup>13-23</sup>

## 1.1 SPECIMEN COLLECTION

Appropriate hazard labelling according to local policy.

## 1.2 SPECIMEN TRANSPORT AND STORAGE

Compliance with current postal and courier transportation regulations is essential.

A suitable virus transport system must be used and the specimen should be placed in a sealed plastic bag, separately from the request form.

## 1.3 SPECIMEN PROCESSING

- These viruses are classified as a Hazard Group 2 pathogen; refer to current guidance on the safe handling of Hazard Group 2 organisms
- Pregnant women may be at risk of passing the infection to the foetus. Therefore, staff with no known history of these infections who are, or may be, pregnant should be made aware of the risks involved
- Laboratory procedures that may give rise to infectious aerosols must be conducted in a Class 1 microbiological safety cabinet (MSC)
- It is strongly recommended that disposable gloves are worn when inoculating samples or manipulating cell cultures suspected of containing these viruses.

The above guidance should be supplemented with local COSHH and risk assessments.

# 2 SPECIMEN COLLECTION

## 2.1 TYPE OF SPECIMENS

HSV	Vesicle swabs Lesions swabs Ulcer swabs	Conjunctival swab Throat swabs Mouth swabs
CMV	Mouth washing Throat gargle Bronchoalveolar lavage	Urine Heparinised blood Induced sputum
VZV	Vesicle fluids Vesicle swabs Lesions swabs	Lesion swabs Crusts from lesions Scrapings from lesions

## 2.2 OPTIMAL TIMING OF SPECIMEN COLLECTION

Swabs or samples should be taken as soon as possible after symptoms appear. Scraping or swabs taken from the base of active lesions should be put immediately into Virus Transport Medium (VTM). Repeat sampling is not usually necessary except for suspected cases of CMV. In this case viral excretion or shedding may be transient and continue some considerable time after a primary infection and it may therefore be necessary to examine a number of samples in order to isolate the virus.

All specimens should be taken before anti-viral chemotherapy is commenced.

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## 3 SPECIMEN TRANSPORT AND STORAGE

### 3.1 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

Samples should be placed in a suitable VTM immediately after collection.

When there is a delay in processing samples should be refrigerated; if the delay may exceed 24 hrs, samples may be frozen at -70°C or lower and thawed prior to processing. Repeated freezing and thawing should be avoided.

### 3.2 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be processed as soon as possible after collection.

Where used, transportation systems should ensure that specimens arrive in the laboratory within 24 hrs.

## 4 SPECIMEN PROCESSING

### 4.1 TEST SELECTION

Under circumstances where a rapid result is required electron microscopy (EM), immunofluorescence (IF) or a commercial antigen detection assay may be used to provide a provisional interim report, but this result should ideally be confirmed by PCR or culture. For CMV it is also worth considering using a “shell vial” technique.

### 4.2 MICROSCOPY TECHNIQUE

Grids for EM and slides prepared for IF microscopy should preferably be prepared from material taken directly from the lesion. Refer to [VSOP 13 - Investigation of clinical specimens by electron microscopy using the floatation \(direct\) method](#).

### 4.3 CULTURE AND INVESTIGATION

#### Pre-Treatment of specimen

N/A

#### Specimen processing

Specimens should be vortexed or vigorously agitated to release the cellular material into the transport medium. To avoid the release of aerosols the specimen should not be opened immediately after this process.

Specimens should be centrifuged, in order to concentrate the cellular material in the sample, prior to inoculation. To avoid the release of aerosols the specimen should not be opened immediately after centrifugation. If immediate processing of the sample is necessary the specimen should be opened in a class 1 MSC.

If vesicle crusts are received for virus isolation, these may be prepared by grinding the crusts in a Griffiths tube and resuspending the material in VTM. Before inoculating the cell cultures it may be necessary to clarify the VTM by centrifugation. It should be noted that any procedures that potentially produce aerosols must be performed in a class 1 MSC.

#### Isolation and Cytopathic effect

##### HSV<sup>2,24</sup>

Many of the cell lines in routine use for virus isolation are susceptible to infection with HSV. Of these, the semi-continuous human diploid fibroblast cell line MRC-5, has been shown to be the most susceptible to infection and to produce a cytopathic effect (CPE) more rapidly than other cell lines. Of the established cell lines the Vero cell line, derived from African Green Monkey Kidney, is a suitable alternative.

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Inoculate 100 µL – 200 µL of the VTM containing the clinical material into each of two cell culture tubes containing the selected cell line(s). The cultures should be incubated at 35°C – 37°C, with or without rolling, for seven to ten days. For rapid results, cultures should be examined daily for the appearance of the characteristic CPE associated with HSV infection. Alternatively, laboratories may examine cell cultures after 24 hrs and 48 hrs then every other working day.

Blind passage of the cell cultures should not normally be required in order to produce the appearance of a CPE and should only be necessary if the cell cultures become contaminated with bacteria or fungi, or display degeneracy as a result of the clinical material.

### **VZV<sup>9</sup>**

VZV is capable of replicating in many of the mammalian cell lines in routine use, however *in vitro* optimal replication is best effected in a semi-continuous human diploid fibroblast cell lines such as MRC-5.

Inoculate 100 µL – 200 µL of the VTM containing the clinical material into each of two cell culture tubes containing the selected cell line(s). The cultures should be incubated at 35°C - 37°C with or without rolling. If the clinical material contains VZV the appearance of a CPE can take up to 21 days or sometimes longer.

VZV is a highly cell associated virus and consequently viral particles are not released into the culture medium. The consequence of this is that the CPE takes on the nature of small foci of "ballooning" cells. It is important that the cultures are observed on a daily basis, as it is quite possible for the infected cells to drop off the cell culture vessel (tube or flask) and the resulting gap in the cell sheet to be overgrown with uninfected fibroblasts.

Because of the cell associated nature of the virus, "blind" passaging of the culture or serial propagation of the virus requires that the cells be disrupted by a physical method. The cell monolayer should be removed from the surface of the culture vessel by scraping the cell sheet with an appropriate sterile instrument or by trypsinisation and the resultant cell suspension disrupted by either a freezing and thawing cycle or by sonication.

### **CMV**

CMV displays a marked host cell tropism and so can only be isolated in cells of human origin. The cell line of choice for the isolation of CMV in cell culture is the semi-continuous human diploid fibroblast cell line, MRC-5.

Inoculate 100 µL – 200 µL of the clinical material into each of two MRC-5 cell culture tubes. The cultures should be incubated at 35°C – 37°C with or without rolling for a minimum period of 28 days. In order to keep the cell cultures from deteriorating it is advisable to feed the cultures with fresh maintenance media every 7 days.

CMV is a cell associated virus and consequently viral particles are not released into the culture medium. The consequence of this is that the CPE is characterised by the appearance of foci containing large refractile cells. Because of the slow replication of CMV, cell cultures should be observed for up to 28 days before being discarded as negative. Due to the cell associated nature of the virus, "blind" passaging of the cultures or serial propagation of the virus requires that the cells be disrupted by a physical method. The cell monolayer should be removed from the surface of the culture vessel (tube or flask) by scraping the cell sheet with an appropriate sterile instrument or by trypsinisation and the resultant cell suspension disrupted by either a freezing and thawing cycle or by sonication.

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## 4.4 IDENTIFICATION

### Within the laboratory

The CPE produced by HSV, CMV and VZV can be considered characteristic, especially by an experienced person, however it should be confirmed by either direct or indirect IF utilising a monoclonal antibody.

Sero-typing of HSV isolates is recommended on all new isolates using either direct or indirect IF with type specific monoclonal antibodies.

### Referral to Reference Laboratories

Referral is necessary for:

- Typing of isolates
- Antiviral susceptibility testing (available at the Antiviral Susceptibility Reference Laboratory, Birmingham Laboratory, Health Protection Agency, West Midlands).

## 5 QUALITY ASSURANCE

A quality system should be in place to ensure that appropriate internal and external quality assessment and quality control procedures are maintained<sup>25</sup>.

It is essential that laboratories have evidence of adequate validation of methods, equipment and commercial and in-house test procedures demonstrating that they are fit for purpose<sup>26</sup>.

## 6 LIMITATIONS

Successful isolation of organisms depends on correct specimen collection, transport, storage and processing; the quality and range of cell lines used and the use of correct conditions for culture and the provision of adequate/suitable clinical information.

Only cell lines proved to be susceptible to human herpes viruses should be used and susceptibility should be checked on acquisition and at regular intervals. Cells retrieved from liquid nitrogen storage should be checked for sensitivity before use.

The procedure(s) in these documents aim to describe good microbiological standard methods for the specimen types specified. Other procedures may be required and professional interpretation by qualified staff is essential. Please note that knowledge of infectious diseases changes constantly and although this NSM is under regular review it may not include emerging pathogens.

The above guidance should be supplemented with local assessment of limitations.

## 7 REPORTING PROCEDURE

Specimens that produce a characteristic CPE that is subsequently confirmed by IF utilising a specific monoclonal antibody should be reported as:

eg "Cytomegalovirus isolated"

Specimens not displaying a characteristic CPE after a minimum of 28 days incubation should be reported as:

"Virus not isolated"

HSV positive results should be reported as "Herpes simplex virus type ... isolated".

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## 8 REPORTING TO THE HPA<sup>27</sup>

All clinically significant identifications of HSV from neurological or genital sites or cases of meningitis, encephalitis and deaths require reporting to the Cfl.

All clinically significant identifications of CMV require reporting to the Cfl.

All clinically significant identifications of VZV from neonates or pregnant women and cases of meningitis, encephalitis, pneumonia and deaths require reporting to the Cfl.

Chickenpox is not a notifiable disease in England and Wales and therefore does not require reporting to the local CCDC. It is a notifiable disease in Scotland and should be reported to the Scottish Centre for Infection and Environmental Health (SCIEH).

HSV and CMV are not notifiable diseases and therefore do not require reporting to the local CCDC.

## 9 RELEVANT NATIONAL STANDARD METHODS

For additional details on specific areas of diagnosis refer to the relevant NSMs available through the Department for Evaluations, Standards and Training web page ([www.hpa-standardmethods.org.uk](http://www.hpa-standardmethods.org.uk)).

Other documents that may be of relevance to this NSM are:

[VSOP 13 - Investigation of clinical specimens by electron microscopy using the floatation \(direct\) method](#)

[VSOP 17-Isolation of herpes simplex virus associated with herpes genitalis.](#)

[VSOP 35-Investigation of Cytomegalovirus infection by Roche Lightcycler PCR](#)

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The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

For further information please contact us at:

Standards Unit  
Department for Evaluations, Standards and Training  
Centre for Infections  
Health Protection Agency  
Colindale, London  
NW9 5EQ

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

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