Laboratory diagnosis of herpesviruses

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SUMMARY

Most of herpesviruses infect children in early infancy. After the primary infection the virus establishes a latent infection and persists in different tissues for life. Clinical reactivations of virus tend to be mild except in immunocompromized persons in who reactivated virus may cause serious complications.

The detection of herpesvirus as the etiological agent is recommended before administration of antiviral therapy. Basic approaches to laboratory diagnosis are direct examination of clinical material for infectious virus, viral antigens, detection of viral nucleic acid and detection of specific antibodies in patient sera.

KEY WORDS

herpes-viruses, laboratory diagnosis, diagnostic procedures

Introduction

After primary infection herpesviruses establish a latency and persist in host's cells for life. Herpes simplex virus (HSV) and Varicella zoster virus (VZV) are latent in ganglia, Cytomegalovirus (CMV) in salivary glands, kidney tissue and in macrophages, while Epstein-Barr virus (EBV) and other lymphotropic herpesviruses (herpesvirus 6, 7 and 8) find its final target in lymphocytes (1). Very often herpesviruses reactivate due to several reasons. When reactivations are clinically expressed they are usually mild. The importance of viruses in the herpesvirus family has increased extensively with advances of transplantation medicine, with the epidemic of HIV infection and with the classification of herpesviruses as sexually transmitted agents (2).

When viral reactivations are clinically expressed, they are usually mild and respond to antiviral therapy. Several factors can lead to reactivation of herpesviruses. Various modes of immunosuppression might induce reactivation of herpesviruses and provoke disseminated disease.

There is a wide spectrum of laboratory tests for the detection of herpesviruses. In each case we have to choose the proper diagnostic procedure that can clearly show the suspected etiological relationship of the virus to the clinical state and that can also detect the active virus when the reactivation is suspected. While some primary herpesvirus infections are so clinically well defined that laboratory diagnosis is not obligatory, in most cases we should confirm the herpesvirus etiology in order to insure administration of the proper therapy. Monoclonal antibodies help to detect viral antigens

directly in clinical material. Using the methods of molecular biology it is possible to detect even a very few viral DNA copies, absolutely confirming the presence of virus but not always indicating whether the virus is present in active form. Asymptomatic persons can occasionally shed herpesviruses (e.g. CMV, HSV). When for example CMV is found in the secretions of a neonate it is evident that congenital or perinatal infection has occurred. By contrast, finding CMV in the urine or in saliva of an asymptomatic individual is of no clinical or diagnostic value (3).

Isolation of herpesviruses in cell culture is the "Gold standard" method for detection of virus, being regularly performed for HSV, VZV, CMV and HHV-6. For EBV, HHV-7 and HHV-8 the isolation has not been introduced in the diagnostic procedures. Isolation of herpesviruses from the appropriate clinical material directly proves the infecting particles, but is a time-consuming, expensive and technically difficult procedure. Immunodetection of herpesvirus antigen is a rapid method with a specificity and sensitivity of over 90%. For detection of herpesvirus DNA, methods include in situ hybridization (ISH) and polymerize chain reaction (PCR). ISH has a very high specificity, but quite a low sensitivity, while PCR is a quick method with high degree of confidence for both the specificity and sensitivity. In many instances electron microscopy (EM) can solve the problem of herpesvirus etiology provided that the appropriate clinical material is collected, such as vesicle fluid for HSV and VZV or urine for CMV. In these materials the viral titer is high enough to allow direct visualization of the virus. Table 1 summarizes diagnostic procedures being used with different clinical specimens for all herpesviruses. If problems arise related to the collection of specimen or to the diagnostic procedures, experienxced laboratory personnel should be consulted.

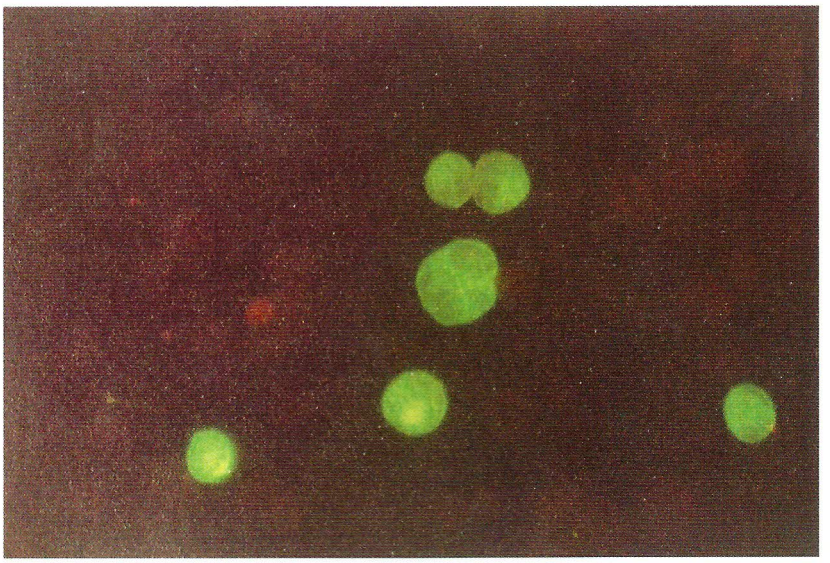


Figure 1. Immunodetection of herpesvirus antigen in skin lesion.

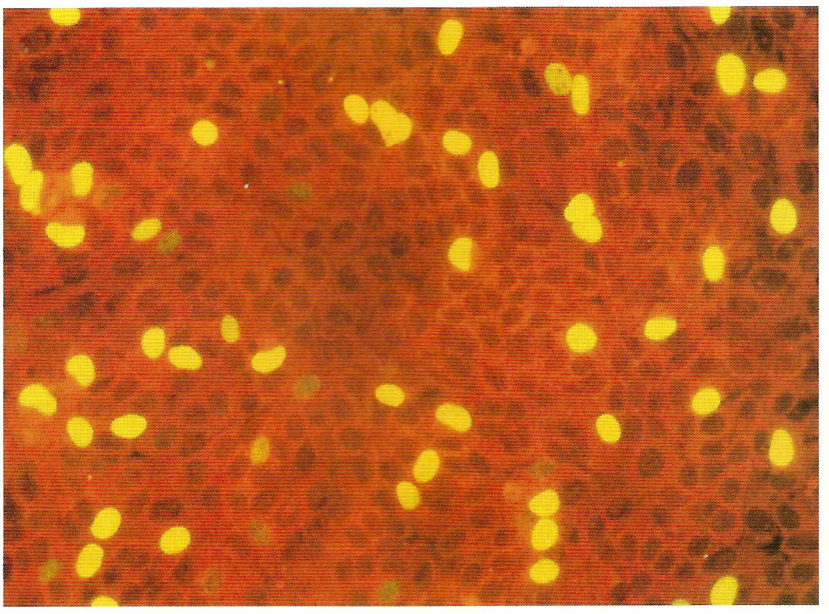
Herpes simplex virus 1, herpes simplex virus 2 (HSV-1, HSV-2)

Clinical material: vesicle fluid, cerebrospinal fluid (CSF), swabs taken from skin and mucous membrane lesions, upper respiratory tract material, conjunctival swabs, blood, serum, tissue sections. The specimen from vesicles is taken with a cotton swab by rubbing it vigorously to collect enough fluid for inoculation into cell culture. For antigen detection it is important to collect epithelial cells. For both cell culture and antigen detection the specimen is put in transport medium and transported to the laboratory within 24 to 48 hours.

Direct detection of virus: EM can be used to visualize the virus in the vesicle fluid. To prepare the clinical specimen for EM, the largest vesicle should be opened and a special grid placed upon the vesicle fluid. The grid should be transported to the laboratory as soon as possible. Although this is the fastest method for diagnosing HSV, EM does not allow one to distinguish between type 1 and type 2 virus. Isolation of virus in cell culture remains the best method to detect infectious virus, although it takes 4 to 7 days to obtain results. From the practical point of view this technique may be less beneficial for the patient, but it is a valuable resource in problematic cases.

Immunodetection of virus antigens: using monoclonal

Figure 2. Cytomegalovirus early antigen detection in cultured cells ("shell vials").



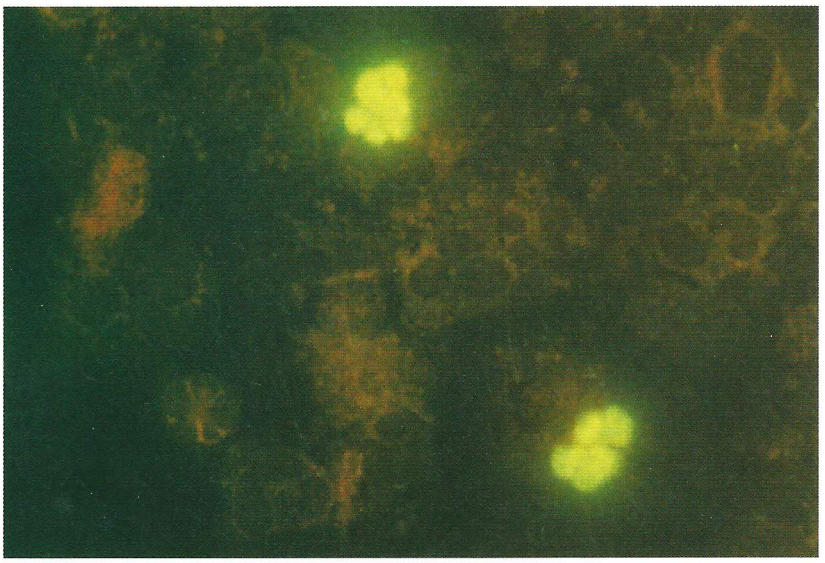
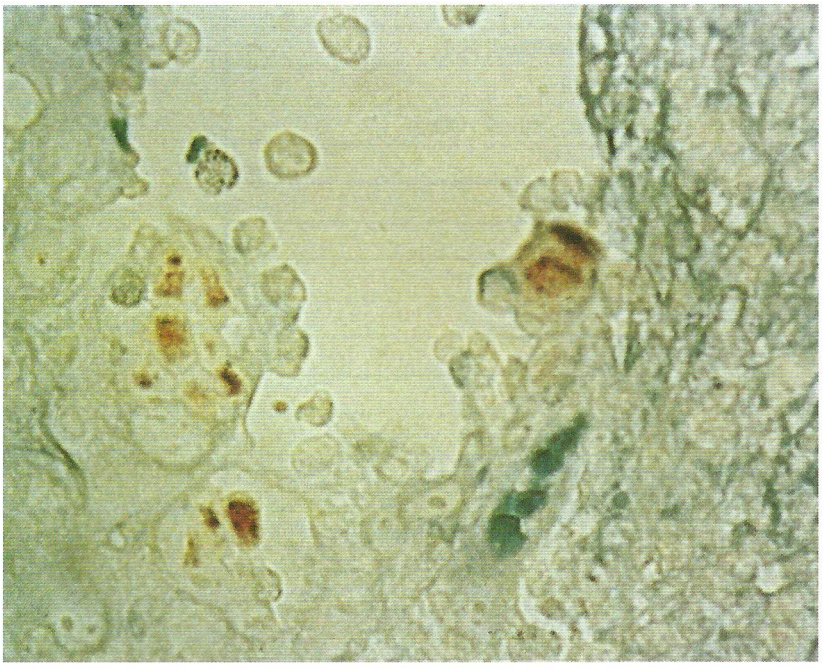


Figure 3. Cytomegalovirus pp65 antigen detected in polymorphonuclear cells.

antibodies it is possible to detect specific viral proteins in 1 to 2 hours after specimen collection. It is important to collect infected cells for the antigens to be clearly identified (Figure 1).

Methods including molecular biology: by ISH it is possible to identify viral DNA in cervical smears (Figure 4) and tissue sections; additional methods based on hybridization are being developed. PCR is a useful tool for detection of viral DNA in CSF (4).

Figure 4. Stained nuclei of cervical cells indicate the presence of herpesvirus DNA (in situ hybridization).



Serology: IgM antibodies appear 3 to 10 days after the initial infection and persist 6 to 8 weeks. During virus reactivation they may reappear and their presence in CSF has an important diagnostic impact. IgG antibodies can be found 7 to 14 days after infection, reaching the highest level 2 weeks later. Seroconversion or 4-fold rise in IgG titer indicate very recent infection. During virus reactivation the level of IgG antibodies increases (3).

Varicella - zoster virus (VZV)

Clinical material: vesicle fluid, blood, CSF, swabs of lesions, upper respiratory tract material, serum, and tissue sections.

Direct detection of virus: virus can be quickly identified by EM examination of vesicle fluid. The infective virus can be isolated in human diploid cell culture. Typical cytopathic effect (CPE) is observed within 3 days to 3 weeks.

Antigen detection: by direct examination of infected cells, results can be obtained in 1 to 2 hours. It is of great importance in diagnosing zoster in immunocompromized patients.

Detection of virus DNA: ISH and PCR. PCR is very important in establishing the etiology of encephalitis in children.

Serology: in primary infection IgM antibodies appear 2 to 5 days after the appearance of clinical manifestations. In zoster cases IgM antibodies appear 8 to 10 days after the appearance of skin lesions. IgG antibodies are found 4 to 6 days after the rash, reaching the highest level after 3 weeks. Some months later they decline and a stable antibody titer results, which persists for life (5).

Epstein-Barr virus (EBV)

Clinical material: blood, CSF, throat washing, nasopharyngeal swab, bioptic material, tissue sections, serum.

Direct detection of virus: EBV is difficult to cultivate in vitro; so the isolation of virus is not a regular diagnostic procedure. Even EM cannot successfully detect the virus, due to the small number of viruses.

Antigen detection: the detection of viral antigens in infected cells is not highly significant while also asymptomatic persons occasionally shed virus in upper respiratory tract secretions.

Detection of virus DNA: EBV DNA can be detected in bioptic material, in tissue sections, in blood. Both ISH

Table 1. Suggested diagnostic procedures for detection of herpesviruses in biologic materials.

Agent	Specimen of choice	Diagnostic procedure
HSV-1, HSV-2	vesicle fluid	EM
		isolation of virus
	blood	isolation of virus
	CSF	PCR
		isolation of virus
	lesion swab	antigen detection
		isolation of virus
		ISH
	semen	isolation of virus
	upper and lower respiratory tract material	antigen detection, isolation of virus
	tissue sections	ISH, PCR
	serum	IgM, IgG detection
VZV	vesicle fluid	EM
		Isolation of virus
	CSF	PCR
		Isolation of virus
	lesion swab	Antigen detection
		Isolation of virus
	upper and lower respiratory tract material	Antigen detection, isolation of virus
	serum	IgM, IgG detection
CMV	urine	Isolation of virus (shell vial)
		Antigen detection
	CSF	PCR
		Isolation of virus
	throat swab or washing	Antigen detection
		Isolation of virus (shell vial)
	blood	Antigen detection
		Isolation of virus (shell vial)
	plasma	PCR
	serum	IgM, IgG detection
EBV	serum	IgM, IgG detection
	throat swab or washing	Antigen detection
	blood, CSF	PCR
	bioptic material	ISH
		PCR
		antigen detection
HHV-6	serum	IgM, IgG detection
	blood, CSF, urine	isolation of virus
		PCR
HHV-7, HHV-8	serum	IgM, IgG detection
	blood	PCR

and PCR can be used. PCR is a convenient method for detecting EBV encephalitis.

Serology: this is the best way to follow the EBV infection. In adult patients heterophilic antibodies can be measured with a satisfactory level of sensitivity in Paul-Bunnell reaction. Specific antibodies to early antigen (EA), viral capsid antigen (VCA) and to nuclear antigen (EBNA) are measured to define the stage of infection. The presence of IgM and IgG antibodies specific to EA always indicates both acute infection and reactivation of the virus. The presence of IgM antibodies specific to VCA indicates acute infection, while the presence of IgG antibodies specific to VCA together with anti-EBNA, indicate past infection (6).

Cytomegalovirus (CMV)

Clinical material: urine, upper and lower respiratory tract material, blood, CSF, cervical swab, amniotic fluid, bioptic material, tissue sections, serum.

Direct detection of virus: EM is a successful method for detection of virus in urine. Isolation of virus in primary cell culture results in typical CPE, but is time-consuming (up to 3 weeks).

Detection of viral antigens: viral antigens are detected directly in clinical material or in cultured cells after the inoculation with clinical material. The »Shell vial« method is a modification of the standard cell culture method shortened to 48 hours by the use of monoclonal antibodies to early or immediate early antigen. (Figure 2). The presence of these antigens indicates virus replication. Detection of pp65 antigen (matrix protein) is possible directly in infected blood cells, which indicates antigenemia and is of great value in acute primary infections as well as in the reactivations (Figure 3). Circulating pp65 positive CMV cells (CCMVC) can also be found in CSF and in upper respiratory tract material (7).

Detection of viral DNA: ISH is a useful method for detecting CMV DNA in tissue sections and in cervical material. The detection of CMV DNA in plasma by PCR indicates active replication of the virus and release of virions from the infected cells. PCR result is positive 1 to 2 days prior to the appearance of pp65 antigen.

Serology: measurement of IgM and IgG antibodies is a useful mode of monitoring the stage of CMV infection.

Human herpesvirus 6 (HHV 6)

Clinical material: blood, CSF, tissue sections, bioptic material, serum.

Direct detection of virus: isolation in primary cell culture takes several days. It is difficult to choose the proper clinical material for EM examination.

Immunodetection of viral antigens: it is possible, however, this method was introduced only recently and its practical value has not been well estimated.

Detection of virus DNA: PCR is a suitable method to prove HHV-6 neurotropic activity by the detection of viral DNA in CSF. ISH can be used to detect virus in archival brain tissue sections.

Serology: IgM antibodies appear 2 to 14 days after the initial infection and persist up to 8 weeks. IgG antibodies are detected 7 to 8 days after the infection and persist for life (8).

Human herpesvirus 7 (HHV 7)

This virus seems to be less important in human pathology than previously thought. All the diagnostic procedures that are mentioned for other herpesviruses are potentially usable, yet in practice very few diagnostic laboratories include this virus in their regular diagnostic program (9).

Human herpesvirus 8 (HHV 8)

Diagnostic procedures are still in the preparatory stage. Diagnostic approach is the same as has been mentioned for other herpesviruses. Detection of IgG antibodies seems to be satisfactory for routine detection as well as for prevalence studies.

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