

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.

Introduction

KEY WORDS

herpesviruses,
laboratory diagnosis,
diagnostic procedures

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 polymerase 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, experienced laboratory personnel should be consulted.

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

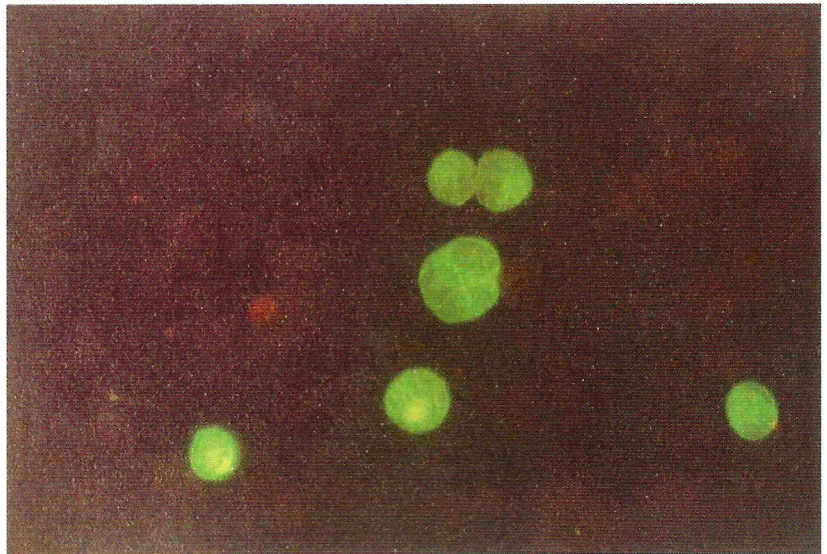
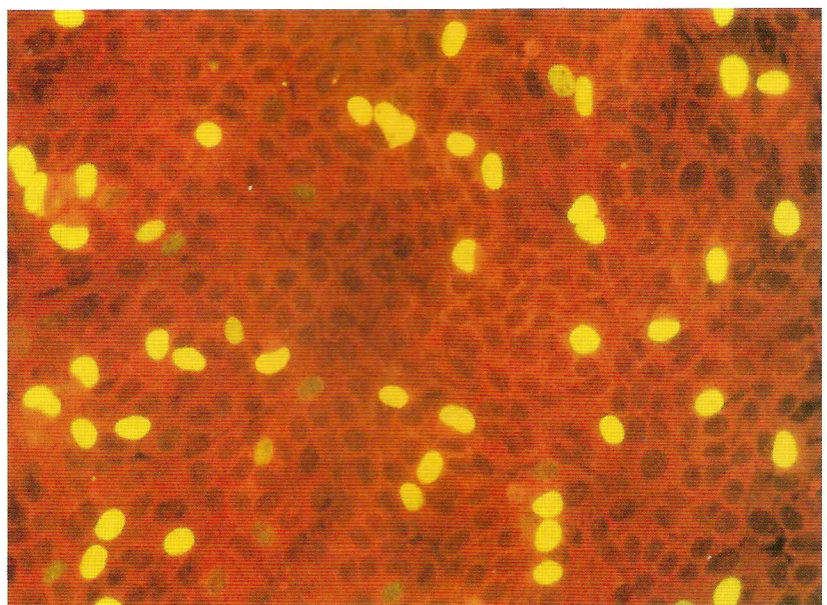


Figure 1. Immunodetection of herpesvirus antigen in skin lesion.

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").



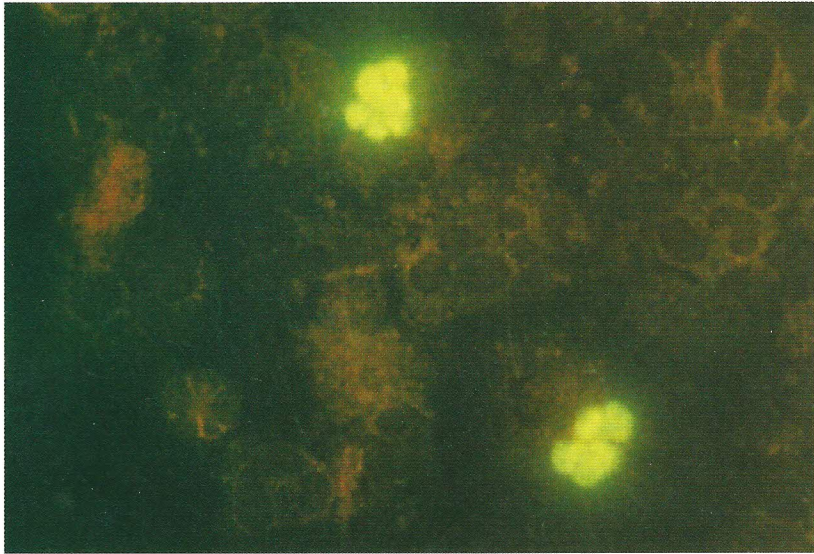
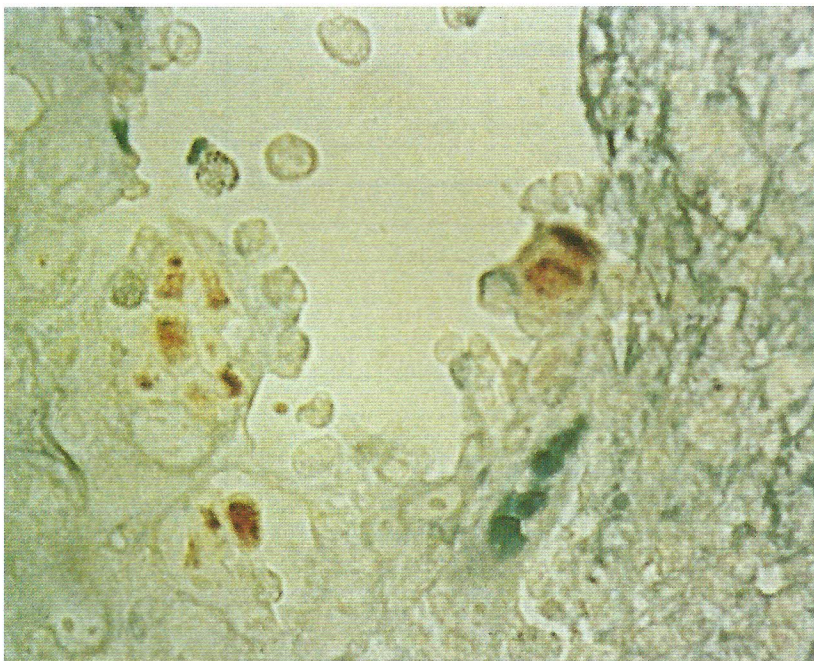


Figure 3. Cytomegalovirus pp65 antigen detected in polymorphonuclear cells.

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

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



for detection of viral DNA in CSF (4).

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
VZV	serum	IgM, IgG detection
	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	
CMV	serum	IgM, IgG detection
	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
HHV-6		antigen detection
	serum	IgM, IgG detection
	blood, CSF, urine	isolation of virus
HHV-7, HHV-8		PCR
	serum	IgM, IgG detection
	blood	PCR

